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# Effects of repeated tianeptine treatment on CRF mRNA expression in non-stressed and chronic mild stress-exposed rats

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**Abstract**

Accumulating evidence suggests that dysregulation of corticotropin-releasing factor (CRF) may play a role in depression and that this dysregulation may be corrected by antidepressant drug treatment. Here, we examined whether chronic mild stress (CMS) alters CRF mRNA levels in stress-related brain areas including the bed nucleus of the stria terminalis (BNST) and the central nucleus of amygdala (CeA), and whether repeated tianeptine treatment can attenuate CMS-induced changes in CRF mRNA levels. Male rats were exposed to CMS for 19 days, and control animals were subjected to brief handling. Both groups were injected daily with tianeptine or saline. CMS significantly increased CRF mRNA levels in the dorsal BNST (dBNST), but not in other areas. Repeated tianeptine treatment prevented the CMS-induced increase in CRF mRNA levels in the dBNST, and reduced CRF mRNA levels in dBNST in non-stressed controls. Moreover, repeated tianeptine treatment significantly decreased CRF mRNA levels in the ventral BNST and CeA of non-stressed controls as well as CMS-exposed rats. These results show that CMS induces a rather selective increase of CRF mRNA in the dBNST. In addition, these results suggest that repeated tianeptine treatment diminishes the basal activity of CRF neurons and reduces their sensitivity to stress.


*Keywords:* Tianeptine; Stress; Corticotropin-releasing factor; Bed nucleus of the stria terminalis; Amygdala; Depression; Anxiety

## 1. Introduction

Corticotropin-releasing factor (CRF) was first described as a hypothalamic neuropeptide that stimulates the secretion of adrenocorticotropin (ACTH) (Vale et al., 1981). In addition to the paraventricular nucleus of hypothalamus (PVN), CRF is found in stress-related brain areas such as the bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), and Barrington's nucleus (Imaki et al., 1991; Morin et al., 1999). CRF functions as a neurotransmitter in the integration of behavioral and autonomic responses to stress (Koob, 1999). As stress is known to be a predisposing factor for major depression, it is possible that dysregulation of the CRF system may contribute to the pathology of depression (Nemeroff et al., 1984). For example, increased concentrations of CRF in cerebrospinal fluid (CSF) have been reported in depressed patients (Banki et al., 1987). Moreover, the normalization of elevated CRF concentrations in CSF has been reported after successful treatment of depression by fluoxetine (De Bellis et al., 1993) or electroconvulsive shock (Nemeroff et al., 1991).

Tianeptine is a serotonin reuptake enhancer (Mennini et al., 1987), and has an antidepressant efficacy similar to that of selective serotonin reuptake inhibitors (SSRIs) (Wilde and Benfield, 1995). Interestingly, chronic administration of tianeptine has been shown to attenuate lipopolysaccharide- or stress-induced increases in plasma ACTH and corticosterone

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levels (Castanon et al., 2003; Delbende et al., 1994). These findings suggest that chronic tianeptine treatment may suppress pituitary–adrenal axis activation in response to stress. In addition, tianeptine attenuated stress-induced reduction of hypothalamic CRF concentration (Delbende et al., 1994), suggesting that tianeptine exerts antidepressant effects by regulating the pituitary–adrenal axis and/or CRF neurotransmission in the hypothalamus.

A caveat of antidepressant research is the use of normal laboratory rats (Nestler et al., 2002). This concern emerges from the observation that antidepressant treatments in humans without depression cause typical side effects rather than antidepressant effects (Nestler et al., 2002). Thus, the elucidation of mechanisms of antidepressants using normal rats may not reflect the mechanism of antidepressant effects in the depressed brain. To take this possibility into account, we injected tianeptine under a chronic stress condition to imitate the clinical feature of depression. Anhedonia, the loss of interest in or ability to derive pleasure from all or most activities, is one of the two core symptoms of depression according to the Diagnostic and Statistical Manual of Mental Disorders, fourth revision (1994). Since a similar state of anhedonia can be induced in rats by chronic mild stress (CMS) procedures (Moreau et al., 1992; Stout et al., 2000), a CMS model has been used as an animal model of depression. Moreover, it has been shown that increased CRF concentration in the BNST was observed in CMS anhedonic rats (Stout et al., 2000). In the present study, we tested whether CMS increases CRF mRNA expression in stress-related brain areas, and whether repeated tianeptine treatment during CMS procedures may inhibit CMS-induced changes in CRF mRNA.

## 2. Materials and methods

### 2.1. Animals

Adult male Sprague–Dawley rats (Orient, Seoul, South Korea) weighing 190–210 g at the beginning of the CMS exposure were used. Rats were handled daily for 7 days to habituate them to the laboratory environment. Rats were housed three per cage in a humidified room with a 12-h light/dark cycle (lights on at 6:00 AM), and food and water were available ad libitum except during food or water deprivation periods during CMS procedures. All procedures used in this study were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

### 2.2. Stress paradigm

For CMS, rats were exposed to a variable stress regimen for 19 days as previously described (Moreau et al., 1992; Stout et al., 2000). In brief, the stressor included repeated 1 h periods of confinement in a small cage ($24 \times 10 \times 9$ cm), one period of continuous overnight illumination, one overnight period of food and water deprivation followed by 2 h access to restricted food (3 g per cage), one overnight period of water deprivation followed by 1 h exposure to an empty bottle, and one overnight period of group housing (6 rats per cage) in a soiled cage (200 ml water in sawdust bedding). Animals were also maintained on a reversed light/dark cycle from Friday evening to Monday morning. The last procedure of CMS consisted of reversed light/dark cycle Friday evening for 12 h. Lights in the animal room were not turned off on Saturday morning after the final CMS procedure was completed. All perfusions were conducted between 11:00 and 14:00 h in order to reduce possible circadian changes in CRF peptide levels. Non-stressed control rats were housed in a separate room and were briefly handled each day without any deliberately stressing procedures.

### 2.3. Antidepressant administration

The animals were divided into four groups: saline-treated non-stressed controls (CON + SAL, $n = 9$), saline-treated CMS-exposed group (CMS + SAL, $n = 9$), tianeptine-treated non-stressed controls (CON + TIA, $n = 9$) and tianeptine-treated CMS-exposed group (CMS + TIA, $n = 9$). Tianeptine was dissolved in 0.9% saline, which was used as the vehicle. Tianeptine (10 mg/kg) was injected intraperitoneally (i.p.) 30 min before restraint stress each morning (08:30 h) during the 19-day CMS period.

### 2.4. Body weight, food and water intake measurements

For measurement of food and water intake, a different batch of animals was exposed to CMS. Rats were housed individually to determine daily food and water intake. The food hopper and water bottle were removed and weighed at 08:30 h. At this time, rats were removed from their cages, weighed, and then injected with saline or tianeptine. A fresh supply of preweighed food and water was returned 1 h later. Daily food intake (g/day) was calculated by subtracting the weight of the hopper from its initial weight. Daily water intake (ml/day) was determined by subtracting the volume of the bottle from its initial volume. Cages were carefully monitored for evidence of food spillage or grinding. Cumulative food and water intake were calculated from daily food and water intake, which included food and water intake during food or water deprivation periods in CMS-exposed rats. However, mean daily food and water intake were averaged from daily food and water intake except during food and water deprivation periods in CMS-exposed rats as well as in non-stressed rats.

### 2.5. Histological procedures

Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg i.p.) and were perfused intracardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PPB, pH 7.2). The brains were fixed in situ for 1 h, removed, post-fixed in PPB for 2 h, and finally placed in 20% sucrose/PPB overnight at 4 °C. Serial coronal sections (30 μm) of whole brain were obtained using a freezing microtome and were stored in cryoprotectant solution [30% RNase free sucrose, 30% ethylene glycol, and 1% polyvinyl pyrrolidone (PVP-40) in 100 mM sodium phosphate buffer, pH 7.4] at −20 °C.

### 2.6. Probe labeling

The CRF riboprobe was constructed from a 1.2-kb EcoRI fragment of full-length rat CRF cDNA (a gift from K. Mayo, Northwestern University, Evanston, IL, USA) subcloned into a pBluescript II-SK+ plasmid. Transcription reactions were performed using an Ambion MAXIscript kit (Austin, TX, USA) with SP6 RNA polymerases according to the manufacturer's instructions. After transcription and removal of the cDNA template with 2 U of DNase, the cRNA probes were recovered using NucTrap minicolumns (Stratagene, La Jolla, CA, USA).

### 2.7. In situ hybridization

Brain sections were permeabilized with proteinase K (1 μg/ml, 37 °C, 30 min), treated with acetic anhydride in 0.1 M triethanolamine (pH 8.0), washed in 2 × SSC (pH 7.0), and were then transferred into 500 μl of hybridization solution in 24-well culture plates. The hybridization solution was comprised of 50% formamide, 0.01% polyvinyl pyrrolidone, 0.01% Ficoll, 0.01% bovine serum albumin, 50 μg/ml denatured salmon sperm DNA, 250 μg/ml yeast tRNA, 40 mM dithiothreitol (DTT), 10% dextran sulfate, and $^{35}$S-labeled CRF cRNA probes at $1 \times 10^7$ cpm/ml. Sections were hybridized with the CRF riboprobe for 18 h at 55 °C in the hybridization solution. After overnight