Pharmacology, Biochemistry and Behavior 91 (2008) 170–175

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Persistent depressive state after chronic stress in rats is accompanied by HPA axis dysregulation and reduced prefrontal dopaminergic neurotransmission

Kazushige Mizoguchi [a,*], Hirotaka Shoji [a], Ryuji Ikeda [a], Yayoi Tanaka [a], Takeshi Tabira [b]

[a] Department of Geriatric Medicine, National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, 36-3 Gengo, Morioka, Obu, Aichi 474-8522, Japan
[b] National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, 36-3 Gengo, Morioka, Obu, Aichi 474-8522, Japan

ARTICLE INFO

Article history:
Received 5 February 2008
Received in revised form 26 June 2008
Accepted 7 July 2008
Available online 13 July 2008

Keywords:
Chronic stress
Persistency
Hypothalamo-pituitary-adrenal axis
Dopamine
Prefrontal cortex
Depression
Rotarod performance
Rat

ABSTRACT

Exposure to stress is thought to play an important role in the etiology of depression. Dysregulation of the hypothalamo-pituitary-adrenal (HPA) axis characterized by glucocorticoid negative feedback resistance is frequently observed in human depressives. Additionally, dysfunctions of the dopaminergic and serotonergic systems in the prefrontal cortex (PFC) are thought to be involved in the development of a depressive state. In rats, chronic stress induces a behaviorally depressive state, concomitant with dysregulation of the HPA axis and reductions in dopaminergic and serotonergic transmissions in the PFC. Considering that dysregulation of the HPA axis is associated with relapse and persistency of depression, it is possible that the chronic stress-induced depressive state persists during long-term rest after its exposure. In the present study, we examined this possibility in rats and found that the behaviorally depressive state in the rotarod test, negative feedback resistance in the dexamethasone suppression test, and a decrease in the extracellular concentration of dopamine but not serotonin in the PFC persisted for 3 months following a 4-week stress session. These results suggest that dysregulation of the HPA system and reduced dopaminergic transmission in the PFC underlies persistent behavioral depression following chronic stress.

1. Introduction

Exposure to stress is thought to precipitate or exacerbate several neuropsychiatric disorders including depression (Mazure, 1995). In a stressful situation, secretion of glucocorticoid hormones is facilitated by activation of the hypothalamo-pituitary-adrenal (HPA) axis (Miller et al., 1992). Several reports have demonstrated that dysregulation of the HPA axis, presenting as negative feedback resistance to cortisol secretion in the dexamethasone (DEX) suppression test (DST), is observed in approximate half of all human depressives (Carroll et al., 1981; Kalin et al., 1982; Holsboer, 1983; Arana et al., 1985). Although glucocorticoid secretion is negatively regulated by glucocorticoids at the level of the anterior pituitary gland (Miller et al., 1992), several brain regions, such as the hypothalamus, hippocampus, and prefrontal cortex (PFC), which have abundant glucocorticoid receptors, are also involved (Feldman and Conforti, 1985; Magarinos et al., 1987; Kovács and Makara, 1988; Diorio et al., 1993; Feldman and Weidenfeld, 1999; Mizoguchi et al., 2003).

On the other hand, based on observations from clinical, neuropsychological, and neuroimaging studies, dysfunction of the PFC has been suspected to be accountable for some depressive symptoms (Cummings, 1992; Deutch, 1993; Fibiger, 1995). Dolan et al. (1994) have provided evidence that neuropsychological symptoms in depression are associated with profound hypometabolism, particularly involving the medial PFC. Drevets et al. (1997, 2000) have demonstrated that both bipolar and unipolar depressives are identified by decreases in cerebral blood flow and the rate of glucose metabolism in the PFC. Although many types of neurotransmitters are thought to be associated with PFC dysfunction, dopamine (DA) and serotonin (5-HT) have an important role. For example, agents that enhance dopaminergic transmission, e.g., bupropion, have been used successfully as antidepressants (Calabrese and Markovitz, 1991). Several other antidepressants, fluoxetine, clomipramine, imipramine, and desipramine, also increase extracellular concentrations of DA and 5-HT in the rat PFC (Tanda et al., 1994; Matsumoto et al., 1999). These findings strongly suggest that modulations by DA and 5-HT of the neural processes within the PFC are involved in the pathophysiology of depression.

A large number of animal studies indicate that exposure to acute or chronic stress can alter the activity of the neuroendocrine and neurotransmitter systems that affect behavior. Exposure to stress in rats or mice sometimes induces several emotional or psychic states including anxiety, anhedonia, enhanced fear, and depression (D'Aquila et al., 1994; Bekris et al., 2005; Bergström et al., 2008; Wood et al., 2008). In particular, the mesoprefrontal dopaminergic system shows vulnerability to acute stress (Abercrombie et al., 1989), and chronic stress results in reduced dopaminergic transmission in the PFC, which causes a

* Corresponding author. Tel.: +81 562 46 2311x5553; fax: +81 562 48 2373.
E-mail address: k-mizo@nils.go.jp (K. Mizoguchi).

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doi:10.1016/j.pbb.2008.07.002

cognitive deficit (Mizoguchi et al., 2000) and a depressive state that is evaluated in the rotarod test (Mizoguchi et al., 2002a). Chronic stress also reduces serotonergic transmission in the PFC (Mizoguchi et al., 2002a). Interestingly, these chronically stressed rats show dysregulation of the HPA system, which is characterized by DEX-mediated negative feedback resistance to corticosterone (CORT) secretion (Mizoguchi et al., 2001). Both animal and human studies have suggested that some depressive symptoms can be attributed to dysregulation of the HPA system (Hatotani et al., 1977; Kuroda et al., 1992; Steckler et al., 1999). Moreover, in clinical studies, normalization of the HPA function following chronic treatment with an antidepressant drug seems to be a prerequisite for stable remission of depressive psychopathology, i.e., persistent cortisol non-suppression in the DST after clinical recovery can predict symptomatic relapse in depression and the normalized HPA function is critical for relief of the clinical symptomatology of this disorder (Charles et al., 1989; Stokes, 1995; O'Toole et al., 1997). Thus, dysregulation of the HPA system is thought to be related to the relapse and persistency of depression for a long time.

From these findings, it is possible that depressive state-related abnormalities occurring in chronically stressed rats persist for a long time after chronic stress exposure. The present study was designed to clarify this possibility. Thus, we examined the influences of long-term rest for 3 months on four chronic stress-induced abnormalities, i.e., a behaviorally depressive state, dysregulation of the HPA system, and reductions in dopaminergic and serotonergic transmissions in the PFC.

In the present study, we selected the rotarod test to evaluate the behaviorally depressive state, because this test can detect the antidepressive action of several antidepressants, such as desipramine and trazodone. Thus, Morimoto and Kito (1994) have indicated that these antidepressants increase the riding time on the rotating rod, but psychostimulant caffeine and anxiolytic diazepam do not. This test also shows higher sensitivity to these antidepressants than the forced swimming test. In addition, we have shown that chronic stress in rats decreases the riding time and treatment with trazodone reverses this decrease, suggesting that this test can also detect the depressive state (Mizoguchi et al., 2002a). Furthermore, this test involves no habituation or adaptation to water used for stress exposure, which may cause problems in measuring the duration of immobility in the forced swimming test. However, it is well known that the riding time is also influenced by relaxation or weakness of the muscles or motor dysfunction, and these motoric deficits decrease the riding time. Therefore, we also checked the clinging time in the traction test to assess muscle strength (Kuribara et al., 1977; Mizoguchi et al., 2002a). If the animals tested show a decrease in the riding time concomitant with a decrease in the clinging time, the decreased riding time is thought to be caused mainly by motoric deficits; however, if the decreased riding time is not accompanied by the decreased clinging time, the decreased riding time is not thought to be due to motoric deficits.

2. Materials and methods

2.1. Animals

All animal experiments were performed in accordance with our institutional guidelines after obtaining permission from the Laboratory Animal Committee. Naive adult male Wistar rats (Japan Clea, Tokyo, Japan) weighing 300–350 g were used. They were housed four per cage in a temperature (22±2 °C), humidity (55±10%) and light (12-h light/dark schedule; lights on at 7:00 a.m. and off at 7:00 p.m.) -controlled environment and were fed laboratory food and water *ad libitum*.

Fifty-six rats were used in the present study. They were divided into the following two experimental groups: chronically stressed group and naive non-stressed (control) group (n=28, respectively). Twenty rats in each group were used for the behavioral and endocrinological studies and the remaining rats in each group (n=8) were used for the microdialysis study.

2.2. Stress exposure

The procedure for stress exposure usually used in the study of stress-induced gastric lesions (Konturek et al., 1991; Brzozowski et al., 1993), with some modifications, was described previously (Mizoguchi et al., 2000, 2001, 2003). Briefly, the animals were placed in a stress cage [dimensions, 11.8 length×29.1 width×19.5 height (cm)] which was divided into 10 compartments and made of wire net, and immersed for 2 h to the level of the xiphoid process in a water bath maintained at 21 °C by heating and cooling pumps (Coolnit CL-19; Taitec, Tokyo, Japan). The animals were subjected to this stress session once a day for 4 weeks (chronic stress). To examine whether chronic stress-induced abnormalities were observed after long-term rest, animals were allowed a 3-month resting period. In our preliminary experiments, gastric ulcer was not produced by single or chronic exposure. Although relatively severe, this stress is not intense enough to produce gastric ulcer. Control rats were not subjected to the chronic stress session and were housed with no treatment during the chronic stress session and the 3-month post-stress period.

2.3. Rotarod test

The experimental procedure was described elsewhere (Dunhan and Miya, 1957; Commissaris and Rech, 1983; Ahmad and Nicholls, 1990; Mizoguchi et al., 2002a). Briefly, the rat was initially put on the rotating rod (diameter, 10 cm; 7 rpm, Shinano Manufacturing Co., Tokyo, Japan), and rats that immediately fell off (within 10 s) were removed from the experiments. The remaining animals were then subjected to the chronic stress described above. On the day of the experiment (i.e., at the end of the 3-month post-stress period), the rotarod test was performed. Thus, the time (s) that the rat remained on the rod was recorded automatically in each case for up to 180 s. The trial was conducted five times for each rat, and the mean riding time was used as the mean value of each rat. When the riding time was over 180 s, the rat was released from the rod, and the time was recorded as 180 s.

2.4. Traction test

After the end of the rotarod test, the traction test was performed. The experimental procedure of this test was described elsewhere (Kuribara et al., 1977; Mizoguchi et al., 2002a). Briefly, a wire (2 mm diameter; 40 cm long) was set horizontally 50 cm above the base. The rat was forced to grasp the wire with its two forepaws, and the time (s) that it clung to the wire was measured for up to 60 s. The trial was conducted three times for each rat, and the mean clinging time was used as the mean value of each rat. When the clinging time was over 60 s, the rat was released from the wire, and the time was recorded as 60 s.

2.5. Locomotor activity test

After the traction test, the spontaneous locomotor activity of the rat was measured during a 5-min period using Animex apparatus (ANIMEX AUTO, MK-110, Muromachi Kikai Co., Tokyo, Japan), as described previously (Mizoguchi et al., 2002a).

2.6. DST

At the end of all behavioral experiments, the negative feedback ability of the rats was examined in the DST. The DST paradigms were designed to assess spontaneous CORT secretion according to our method described previously (Mizoguchi et al., 2001). Briefly, the rats received a single intraperitoneal injection of either DEX phosphate (DEX-P) dissolved in sterile saline at a dose of 30 μg/kg or its vehicle (1 ml/kg) between 10:00 a.m. and 11:00 a.m. In our previous study, this dose of DEX-P was sufficient to suppress plasma CORT levels in naive non-stressed rats, but not in chronically stressed rats (Mizoguchi et al.,

Table 1
Weights of body and adrenal glands and their ratio at the end of a 3-month post-stress period following a 4-week stress session

	Body (g)	Adrenals (mg)	Ratio (mg/g)
Naive	490.7±6.2	39.2±1.1	0.080±0.002
Stress	467.7±6.1*	41.9±1.2	0.090±0.003**

Each value is the mean±SEM of 20 rats per group. Asterisks indicate a significant difference from the value of naive non-stressed rats; *$p<0.05$; **$p<0.01$.

2001). Four hours later, the animals were sacrificed by decapitation, and trunk blood was collected in a 15-ml polypropylene tube containing 250 µl of 100 mM EDTA-2Na, pH 7.4. The plasma was separated by centrifugation at 1800 ×*g* for 20 min and stored at −20 °C until radioimmunoassay. The weight of the bilateral adrenal glands was measured after decapitation.

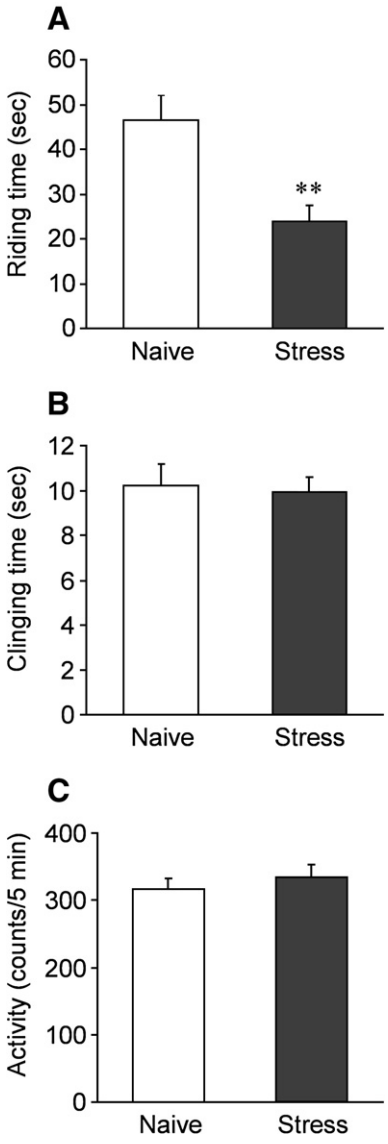

Fig. 1. Behavioral performances; A, rotarod performance; B, traction performance; C, locomotor activity. The behavioral test was performed at a 3-month recovery period following a 4-week stress session. Each column is the mean±SEM of 20 rats per group. **$p<0.01$, a significant difference from naive non-stressed rats.

2.7. CORT radioimmunoassay

The plasma concentration of CORT was measured by radioimmunoassay. The [^{125}I]-labeled CORT (46.3 kBq) double antibody radioimmunoassay kit for rats (Amersham Biosciences, Tokyo, Japan) was used. To displace CORT from corticosteroid-binding globulin in the plasma, the plasma was heated for 30 min at 60 °C. The assay was carried out in duplicate at room temperature, using rabbit anti-CORT serum as the first antibody and donkey anti-rabbit serum coated on magnetizable polymer particles as the second antibody. According to the manufacturer, cross-reactivity is low. The highest cross-reactivity is found with 11-deoxycorticosterone (2.4% in contrast to 100% for CORT).

2.8. Microdialysis

The extracellular concentrations of DA and 5-HT in the PFC were measured using an *in vivo* microdialysis technique in freely moving animals according to our previous reports (Mizoguchi et al., 2000, 2002b, 2004). Briefly, at the end of the 3-month post-stress period, the rats were stereotaxically implanted with a guide cannula (long, 9 mm; outer diameter, 0.8 mm; Bioanalytical Systems, West Lafayette, IN) with a stylet in the PFC (anteroposterior, +3.2; lateral, +1.2; vertical, −2.5 from the bregma) (Paxinos and Watson, 1986) under pentobarbital anesthesia (45 mg/kg, i.p.). The rats were initially treated with Xylocaine (AstraZeneca PLC, London, UK) to minimize pain, and were monitored on a daily basis for signs of distress or infection. Five days after the surgery, the rats were restrained gently while the stylet was removed and replaced with a microdialysis probe (PC-12; tip length, 4 mm; tip diameter, 0.5 mm; Bioanalytical Systems). Using a microinfusion pump, Ringer's solution (in mM: Na, 147; K, 4.0; $CaCl_2$, 3.0) was perfused at a rate of 0.6 µl/min. After an equilibration period of 3 h, the perfusate was collected every 1 h. To examine the response to stimuli, the KCl concentration in Ringer's solution was raised to 100 mM. The concentrations of DA and 5-HT in each perfusate (35 µl) were measured subsequently using an HPLC system (HTEC-500; Eicom, Kyoto, Japan) equipped with a coulometric electrochemical detector (ECD-200; Eicom). A reverse-phase ODS column (CA-5; Eicom) was used with a mobile phase consisting of 82 mM sodium phosphate (pH 6.0), 800 mg/l sodium 1-octanesulfonate, 50 mg/l EDTA and 180 ml/l methanol.

2.9. Statistics

The data of the extracellular concentrations of DA and 5-HT were initially analyzed using two-way analysis of variance (ANOVA). The remaining data were analyzed using one-way ANOVA. Individual

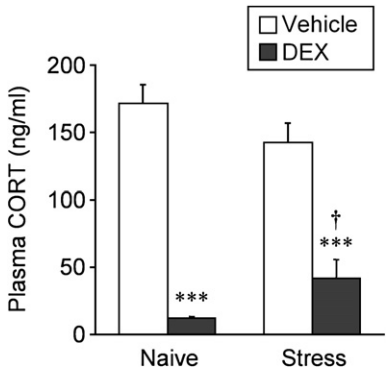

Fig. 2. Plasma CORT levels after DEX-P injection in DST. The test was performed at a 3-month recovery period following a 4-week stress session. Each column is the mean±SEM of 10 rats per group. ***$p<0.001$; a significant difference from vehicle-injected rats in each group; †$p<0.05$, a significant difference from naive non-stressed and DEX-P-injected rats.

between-group comparisons were employed using Fisher's protected least significant difference test or the unpaired *t* test.

3. Results

3.1. Weights of body and adrenals

The weights of the body and adrenal glands at the end of the 3-month post-stress period following the 4-week stress session are shown in Table 1. The body weight of the chronically stressed and rested rats was significantly lighter than that of the naive non-stressed rats [$F(1,38)=6.970$, $p<0.05$]. Although the weight of the adrenal glands did not change between the chronically stressed and rested rats and the naive non-stressed rats, the ratio (adrenal weight/body weight) significantly increased in the chronically stressed and rested rats, compared with the naive non-stressed rats [$F(1,38)=2.591$, $p<0.01$].

3.2. Behavioral performances

The behavioral performances at the end of the 3-month post-stress period following the 4-week stress session are shown in Fig. 1. The riding time on the rotated rod was significantly decreased in the chronically stressed and rested rats, compared with the naive non-stressed rats [$F(1,38)=11.761$, $p<0.01$] (Fig. 1A). The clinging time and the locomotor activity were not affected by the chronic stress and rest, respectively (Fig. 1B and C).

3.3. Negative feedback

The plasma CORT levels following DEX-P injection are shown in Fig. 2. The basal CORT levels (vehicle-injected) did not change between the chronically stressed and rested rats and the naive non-stressed rats. The basal CORT levels in the naive non-stressed rats were significantly suppressed by the DEX-P injection [$F(1,18)=130.621$, $p<0.001$]. Although the basal CORT levels in the chronically stressed and rested rats were also significantly suppressed by the DEX-P injection [$F(1,18)=24.935$, $p<0.001$], these CORT levels were higher than those of the naive non-stressed rats [$F(1,18)=4.521$, $p<0.05$].

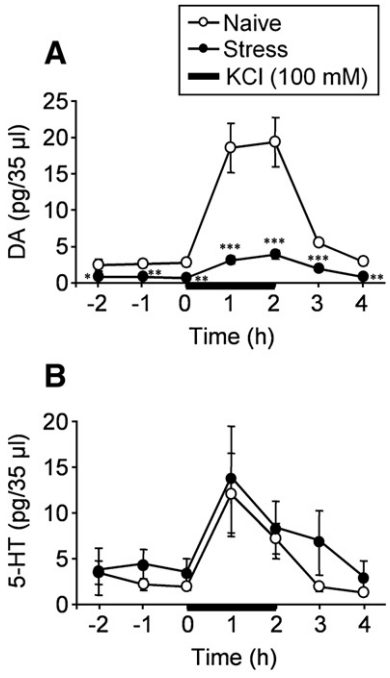

Fig. 3. Extracellular concentrations of DA and 5-HT in the PFC. These concentrations were measured at a 3-month recovery period following a 4-week stress session. Each point is the mean±SEM of 7–8 rats per group. *$p<0.05$, **$p<0.01$, ***$p<0.001$; a significant difference from naive non-stressed rats.

3.4. Extracellular concentrations of DA and 5-HT

The extracellular DA concentrations in the PFC are shown in Fig. 3A. The basal concentration of DA (−2 h to 0 h in Fig. 3A) was significantly decreased in the chronically stressed and rested rats, compared with the naive non-stressed rats [−2 h, $F(1,13)=5.561$, $p<0.05$; −1 h, $F(1,13)=10.497$, $p<0.01$; 0 h, $F(1,13)=9.554$, $p<0.01$]. A marked increase in the DA concentration was observed in the naive non-stressed rats under perfusion with high KCl (100 mM) (1 and 2 h); however, this high KCl-induced increase in the DA concentration was significantly attenuated in the chronically stressed and rested rats [1 h, $F(1,13)=24.102$, $p<0.001$; 2 h, $F(1,13)=23.523$, $p<0.001$]. Two-way ANOVA revealed a significant stress effect [$F(1,13)=37.362$, $p<0.001$], a significant time effect [$F(6,13)=33.794$, $p<0.001$], and a significant stress-by-time interaction effect [$F(6,13)=15.367$, $p<0.001$].

The extracellular 5-HT concentrations in the PFC are shown in Fig. 3B. The concentrations did not change between the chronically stressed and rested rats and the naive non-stressed rats under both basal and KCl-stimulated conditions.

4. Discussion

In the present study, a behaviorally depressive state, as well as dysregulation of the HPA system and reduction in dopaminergic, but not serotonergic, transmission in the PFC, was observed after a 3-month post-stress period following a 4-week stress session. These abnormalities are thought to persist for 3 months. In addition, dysregulation of the HPA system for a long period may underlie the depressive state through a hypodopaminergic mechanism in the PFC.

The rotarod test was established to evaluate the pharmacological actions of psychotropic agents, such as skeletal muscle relaxants and anticonvulsants, in the central or peripheral nervous system (Dunhan and Miya, 1957). Subsequently, this test has been shown to be useful to evaluate not only the antidepressive effects of several antidepressants (Morimoto and Kito, 1994), but also the behaviorally depressive state (Mizoguchi et al., 2002a). Thus, the rotarod test has been thought to detect, at least in part, a depressive state.

As shown in Fig. 1, impairment of the rotarod performance was observed in the chronically stressed and rested rats, and this is not thought to be due to muscle relaxation and motor dysfunction, because chronic stress and rest did not affect the clinging time and locomotor activity. In our previous study (Mizoguchi et al., 2002a), similar behavioral impairment was observed at the end of a 10-day post-stress period following the same chronic stress session as in the present study, and was ameliorated by treatment with an antidepressant, suggesting that the rotarod impairment observed in the chronically stressed rats involves a behaviorally depressive state (Mizoguchi et al., 2002a). Therefore, the present results suggest that the behaviorally depressive state persists during the 3-month post-stress period. However, because it is well known that chronic stress also induces other states, such as anxiety or enhanced fear (D'Aquila et al., 1994; Bekris et al., 2005; Bergström et al., 2008; Wood et al., 2008), the involvement of these states in the rotarod impairment would not be ruled out.

In clinical studies, several reports have shown that persistent cortisol non-suppression in the DST after clinical recovery can predict symptomatic relapse in depression and normalization of HPA function is critical for relief of the clinical symptomatology of this disorder, indicating that dysregulation of the HPA system is thought to be related to the relapse and persistency of depression for a long period (Charles et al., 1989; Stokes, 1995; O'Toole et al., 1997). As a better diagnostic utility, it has been reported that the DEX/CRH test, which combines the DST with a corticotropin-releasing hormone (CRH) challenge, proved to be more reliable to show HPA axis dysfunction in depressive patients (Heuser

et al., 1994; Zobel et al., 2001; Watson et al., 2006; Appelhof et al., 2006; Aubry et al., 2007). Since the behaviorally depressive state was observed in the rats rested for 3 months after the chronic stress session, as shown in Fig. 1, we next examined the function of the HPA axis using the DST. As shown in Fig. 2, attenuation of negative feedback was observed when DEX was injected systemically into the chronically stressed and rested rats. In our previous study (Mizoguchi et al., 2001), we indicated that HPA dysregulation occurring at the end of a 10-day post-stress period following the same chronic stress session as the present study reflected the consequence of the cumulative stress effects, but was not an acute stress response; therefore, chronic stress-induced dysregulation of the HPA axis, in addition to the behaviorally depressive state, is thought to persist during the 3-month post-stress period.

Stress-related neuropeptides, such as CRH and vasopressin, may be involved in the HPA dysregulation observed after the 3-month post-stress period. Clinical studies have repeatedly shown that depressed patients exhibit increases in CRH and vasopressin concentrations in the spinal fluid (Nemeroff et al., 1984), and these increases normalize after clinical recovery (Amsterdam et al., 1988; Nemeroff et al., 1991). In animals, exposure to chronic stress induced by inescapable tail shock (O'Connor et al., 2004), psychological procedures (Scott and Dinan, 1998), or maternal deprivation (Ladd et al., 1996) increases the basal level of mRNA expression or protein concentration of CRH or vasopressin in the hypothalamus of rats, concomitant with dysregulation of the HPA axis. These findings suggest an important role of CRH and vasopressin in the regulation of the HPA system.

Since the same chronic stress procedure used in the present study reduces dopaminergic and serotonergic transmissions in the PFC (Mizoguchi et al., 2000, 2002a), we next examined the influences of the 3-month post-stress period on the chronic stress-induced reduction in these transmissions. As shown in Fig. 3A, the extracellular DA concentrations in the PFC decreased under the basal and KCl-stimulated conditions in the chronically stressed and rested rats. This reduced dopaminergic transmission is thought to cause the behavioral depressive state shown in Fig. 1, because the chronic stress-induced depressive state was ameliorated by intra-PFC stimulation with a DA D_1 receptor agonist (Mizoguchi et al., 2002b). However, the extracellular 5-HT concentrations were not changed between the chronically stressed and rested rats and the naive non-stressed rats. These results suggest that dopaminergic neurons show vulnerability to stress, and PFC dopaminergic dysfunction developed by chronic stress persists for a long time, but serotonergic dysfunction returns to the normal level.

The factors that contribute to the chronic stress-induced dopaminergic dysfunction in the PFC and the persistent mechanisms of this dysfunction are unknown, and it is possible that some stress-sensitive neurotransmitters or hormones are involved. For example, gamma-aminobutylic acid (Hegarty and Vogel, 1995), norepinephrine (Gresch et al., 1993), and glutamate (Jedema and Moghaddam, 1994) can modulate the activity of DA neurons. Alternatively, there may be relationship between changes in the glucocorticoid response and DA activity. Thus, glucocorticoids may modulate extracellular DA concentration by acting directly on DA neurons that express glucocorticoid receptors (Härfstrand et al., 1986; Diorio et al., 1993). The administration of glucocorticoids modifies DA metabolism (Versteeg et al., 1983; Rothschild et al., 1985) and increases DA release in the PFC (Imperato et al., 1989). Also, glucocorticoids may decrease DA catabolism by acting as reversible monoamine oxidase inhibitors (Veals et al., 1977) or they may decrease catecholamine reuptake by inhibiting its transporters (Gilad et al., 1987; Arnsten, 2000). Conversely, suppression of endogenous glucocorticoids by adrenalectomy reduces DA release in the nucleus accumbens (Piazza et al., 1996) or PFC (Mizoguchi et al., 2004) with no change in the number of DA neurons in the ventral tegmental area, an originating area of DA neurons in the PFC. Thus, glucocorticoids can positively regulate dopaminergic activity in the PFC. Several reports have shown that the response to exogenous glucocorticoids is reduced in chronically stressed rats. For example, in chronically footshocked rats, the plasma levels of CORT (Haracz et al., 1988) or beta-endorphin (Young et al., 1990) are not decreased by the administration of glucocorticoids. We also found similar attenuated glucocorticoid negative feedback in the chronically stressed rats, and this attenuation is caused partially by reduction in glucocorticoid-induced actions via down-regulation of glucocorticoid receptors in the PFC (Mizoguchi et al., 2003). Considering the finding that attenuation of negative feedback was observed after the 3-month post-stress period (Fig. 2), the reduced glucocorticoid actions may be involved in the long-term dopaminergic dysfunction in the PFC (Fig. 3A), which in turn causes the behaviorally depressive state (Fig. 1). Correlating with this theory, Boyle et al. (2005) have demonstrated that mice showing impaired glucocorticoid receptor function in the forebrain develop behavioral abnormality that mimics major depression in humans.

Alternatively, degeneration of nerve terminals or neuronal atrophy of DA neurons may occur in the PFC. This hypothesis is supported by previous studies showing that chronic stress induced neuronal atrophy in the hippocampus (Mizoguchi et al., 1992; Watanabe et al., 1992).

In conclusion, although the involvement of anxiety or enhanced fear is unclear, the present study suggests that the behaviorally depressive state persists during the 3-month post-stress period following chronic stress exposure, concomitant with dysregulation of the HPA system and reduction in dopaminergic transmission in the PFC. These results suggest that dysregulation of the HPA system may develop the depressive state through a hypodopaminergic mechanism in the PFC for a long period.

Acknowledgments

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (18590663), a Research Grant for Longevity Sciences (18C-8) from the Ministry of Health, Labor and Welfare of Japan, and Takeda Science Foundation.

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