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p11 IS UP-REGULATED IN THE FOREBRAIN OF STRESSED RATS BY GLUCOCORTICOID ACTING VIA TWO SPECIFIC GLUCOCORTICOID RESPONSE ELEMENTS IN THE p11 PROMOTER

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Abstract—Posttraumatic stress disorder (PTSD) is one of the most common psychiatric disorders. Despite the extensive study of the neurobiological correlates of this disorder, the underlying mechanisms of PTSD are still poorly understood. Recently, a study demonstrated that dexamethasone (Dex), a synthetic glucocorticoid, can up-regulate p11, known as S100A10-protein which is down-regulated in patients with depression, (Yao et al., 1999; Huang et al., 2003) a common comorbid disorder in PTSD. These observations led to our hypothesis that traumatic stress may alter expression of p11 mediated through a glucocorticoid receptor. Here, we demonstrate that inescapable tail shock increased both prefrontal cortical p11 mRNA levels and plasma corticosterone levels in rats. We also found that Dex up-regulated p11 expression in SH-SY5Y cells through glucocorticoid response elements (GREs) within the p11 promoter. This response was attenuated by either RU486, a glucocorticoid receptor (GR) antagonist or mutating two of three glucocorticoid response elements (GRE2 and GRE3) in the p11 promoter. Finally, we showed that p11 mRNA levels were increased in postmortem prefrontal cortical tissue (area 46) of patients with PTSD. The data obtained from our work in a rat model of inescapable tail shock, a p11-transfected cell line and postmortem brain tissue from PTSD patients outline a possible mechanism by which p11 is regulated by glucocorticoids elevated by traumatic stress. Published by Elsevier Ltd on behalf of IBRO.

Key words: posttraumatic stress disorder, glucocorticoid response elements, glucocorticoid receptor, p11, prefrontal cortex.

Posttraumatic stress disorder (PTSD) is a psychiatric disorder that occurs after life threatening traumatic events, such as military combat, natural disasters, terrorist incidents, serious accidents, or violent personal assaults (Ursano, 2002; Grieger et al., 2004). PTSD is a disabling condition associated with marked deficits in social, occupational, and familial function (Howard and Hopwood, 2003). About 7.8% of the American population have experienced PTSD during their lifetime (Kessler et al., 1995). Recently, the significance of PTSD has dramatically increased because of the high prevalence of PTSD in military personnel. Studies have shown rates of PTSD in returning combat troops from the Iraq war of 15.6–17.1% (Hoge et al., 2004).

Several studies have implicated the hypothalamic–pituitary–adrenal axis (HPA axis) as the key circuit in the pathogenic processes underlying PTSD. Over the last decade, many studies have shown abnormal HPA axis activity in PTSD, but these studies do not always report changes in the same direction (Yehuda et al., 1995b; Lindauer et al., 2006). Both higher and lower concentrations of circulating glucocorticoids in PTSD patients have been reported. For example, Holocaust survivors with PTSD have low urinary cortisol excretion (Yehuda et al., 1995b). High early morning salivary cortisol levels have been reported in police officers with PTSD (Lindauer et al., 2006). Bereaved children suffering the death of a parent following the September 11, 2001, terrorist attacks had higher morning and 4:00 pm baseline cortisol concentrations than non-bereaved children (Pfeffer et al., 2006). Thus, the different stressors, the different methods used, the different patient populations recruited, and the different stages of the disorder examined in the various studies have been suggested as explanations for these diverse results.

In animal studies, traumatic stress induces a plasma glucocorticoid elevation (Vogel and Jensh, 1988), which regulates stress-related behavior (de Quervain et al., 1998; Adamec et al., 2006) and gene expression (Liberzon and Young, 1997; Roseboom et al., 2006). Acute restraint stress increases 5-HT7 receptor mRNA expression in the rat hippocampus (Yau et al., 2001). Postnatal handling increases the expression of cAMP-inducible transcription factors in the rat hippocampus (Meaney et al., 2000). Recently, a study demonstrated that dexamethasone (Dex) can up-regulate p11, an S-100 calcium-binding protein (Yao et al., 1999; Huang et al., 2003), which was down-regulated in patients with depression (Svenningsson et al., 2006), a common comorbid disorder in PTSD. These

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Abbreviations: CAT, chloramphenicol acetyltransferase; ChIP, chromatin immunoprecipitation; Dex, dexamethasone; GR, glucocorticoid receptor, prefrontal cortex; HPA, hypothalamic–pituitary–adrenal; mut, mutant p11 promoter CAT construct; PTSD, posttraumatic stress disorder; GR, glucocorticoid receptor; PFC, prefrontal cortex.

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observations led to our hypothesis that traumatic stress may alter the expression of p11 in the brain and that this alteration may be mediated by glucocorticoid receptors. To test this hypothesis, first, we conducted an experiment that mimicked traumatic stress in rats by exposing them to inescapable tail-shock, and examined p11 expression in the prefrontal cortex (PFC) and plasma levels of corticosterone. Then, we studied possible molecular mechanisms of glucocorticoid regulation of p11 induction in SH-SY5Y cells at both gene and protein levels by real-time PCR, Western blot, GRE mutation and chromatin immunoprecipitation (ChIP). Finally, we determined whether there is any change in the p11 expression levels in the postmortem PFC of patients with PTSD. The results of our studies suggest that the mechanism by which glucocorticoid regulates p11 expression occurs through GR in the promoter of p11, and substantiate an important role of glucocorticoids in traumatic stress and PTSD.

EXPERIMENTAL PROCEDURES

Real-time PCR analysis of p11 gene expression

RNA was extracted from human postmortem brain or cell lysates using TRIzol. cDNA was generated from 5 μg of total RNA for each sample using Superscript III RT (reverse transcriptase) and oligo (dT) primers (Invitrogen, Carlsbad, CA, USA) to exclude that differences in RNA-content could result also from differences in sample weights. Real-time PCR was performed on the generated cDNA product in the iQ5 system using SYBR Green (Bio-Rad, Hercules, CA, USA). The following sequences were used for human p11 mRNA analyses: forward 5′-AAATTCGCTGGGGATAAAGG-3′ and reverse 5′-AGCCCACTTTGCCATCTCTA-3′ primers. The sequences were used for rat p11 mRNA analyses: forward 5′-TGCTCATGGAAAG GGAGTTC-3′ and reverse 5′-CCCCGCCACTAGTGATAGAA-3′ primers. Beta-actin mRNA level was unchanged by Dex exposure and was used as an internal control for normalizing p11 mRNA levels in control and experimental samples. The sequences for beta-actin primers were as described by Applied Biosystems. Dilution curves confirmed the linear dependence of the threshold cycle number on the concentration of template RNAs. Relative quantitation of p11 mRNA in control and experimental samples was obtained using the standard curve method. Statistics were performed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA).

Stress procedures

All rats were handled and weighed before each study began. Animals either remained undisturbed in their home cages as controls or were exposed to inescapable tail shock. The stress protocol involved placing the rats in a Plexiglas restraining tube (23.4 cm long and 7 cm in diameter) and exposing them to 100 inescapable shocks (2.0 mA) for 5 s each, with an average intertrial interval of 60 s. The shocks were applied through electrodes taped to the tail. After stressor termination, all animals were returned to their home cages. The number and strength of the shocks were optimized to yield a model of inescapable stress as measured by changes in behavior and by elevated plasma corticosterone levels. The number of animals used and their suffering were minimized.

Tissue collection

Animals were anesthetized with a brief exposure to Haldane immediately or 48 h after inescapable tail shock. Their brains were quickly removed after decapitation. All dissections were performed on a frosted glass plate placed on top of crushed ice. Brain samples, which included prefrontal frontal cortex, hippocampus, amygdala and cerebellum were quickly frozen on dry ice and stored at −70 °C until time of use.

Plasma corticosterone assay

Rats were anesthetized with a brief exposure to Haldane and killed via rapid decapitation immediately after termination of 3-day inescapable tail shock between 8:00 and 10:00 A.M. After each animal was killed, trunk blood was collected and frozen. Rat plasma corticosterone of non-stressed control and stressed groups was measured using the DSL-10-81100 ACTIVE Rat Corticosterone Enzyme Immunoassay Kit following the manufacturer's protocol (Diagnostic Systems Laboratories, Inc., Webster, TX, USA).

Cell culture

SH-SY5Y cells, a human neuroblastoma cell line, were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in DMEM culture medium obtained from Biosource (Rockville, MD, USA) containing 10% fetal calf serum. Cells were grown in 35 mm tissue culture plates coated with type I collagen (Becton Dickinson, Bedford, MA, USA) in immunocytochemistry experiments. Six-well tissue culture plates coated with type I collagen were used for transfection studies; 75-cm^2 type I collagen-coated flasks were employed for protein expression, real-time PCR studies, and nuclear protein extraction. All experiments were done using cultures that were 90% confluent.

Treatment

Cultured SH-SY5Y cells were incubated with the specified doses of Dex for 24 h for dose-response experiments, or with 100 nM Dex for periods of 24 and 48 h. Steady-state mRNA levels and protein levels were assessed after 24 h of incubation with the specified doses of Dex for dose-response experiments or for 24 and 48 h with a dose of 100 nM. Cells incubated without Dex for the same times were used as a control. For GR translocation study, cells were treated with Dex (100 nM) for 6 h. For promoter studies, cells were incubated with Dex for 6 or 24 h prior to collection. RU486 was added to cell cultures 1 h before incubation with Dex or with medium, and was maintained for the incubation period.

Western blot

Western blot analyses were performed as described elsewhere (Zhang et al., 2003). Protein concentration in the samples was determined by Bio-Rad Protein Concentration Reagent. Equal amounts of total protein (20 μg per lane) were resolved in 10% SDS polyacrylamide gels and blotted onto PVDF membranes for immunoblotting analysis. Protein expression was detected using a 1:500 dilution of mouse anti-p11 monoclonal antibody (BD Transduction Laboratories, Franklin Lakes, NJ, USA) and a 1:1000 dilution of horseradish peroxidase–conjugated goat anti-mouse IgG as a secondary antibody (Bio-Rad Laboratories, Hercules, CA, USA). The density values are presented as means±S.D. from three experiments. The density was used to quantify immunoreactivity in terms of percentage of p11 induction relative to control (non-stressed rats or non-Dex-treated cells).

Immunostaining protocols

To evaluate GR translocation, the effect of Dex on nuclear GR was examined by immunostaining. Immunolabeling of GR was performed in Dulbecco's PBS (Quality Biologicals, Gaithersburg,