FK506 Binding Protein 5 Shapes Stress Responsiveness: Modulation of Neuroendocrine Reactivity and Coping Behavior

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Background: The Hsp90 cochaperone FK506 binding protein 5 (FKBP5) is an established regulator of the glucocorticoid receptor (GR), and numerous genetic studies have linked it to stress-related diseases such as major depression or posttraumatic stress disorder. However, translational studies including genetic animal models are lacking.

Methods: Mice deficient of FKBP5 were generated and analyzed in comparison with wildtype littermates. They were subjected to several test paradigms characterizing their emotionality, stress reactivity, and coping behavior as well as hypothalamus-pituitary-adrenal axis function and regulation. Moreover, protein expression of GR and FKBP5 was determined in different brain structures 8 days after stress exposure. The combined dexamethasone/corticotropin-releasing hormone test was performed both in mice and healthy human subjects of different FKBP5 genotypes. The GR function was evaluated by reporter gene assays.

Results: Under basal conditions, deletion of FKBP5 did not change exploratory drive, locomotor activity, anxiety-related behavior, stress-coping, or depression-like behavior. After exposure to different acute stressors of sufficient intensity, however, it led to a more active coping behavior. Moreover, loss of FKBP5 decreased hypothalamus-pituitary-adrenal axis reactivity and GR expression changes in response to stressors. In mice and humans, the FKBP5 genotype also determined the outcome of the dexamethasone/corticotropin-releasing hormone test.

Conclusions: This study in mice and humans presents FKBP5 as a decisive factor for the physiological stress response, shaping neuroendocrine reactivity as well as coping behavior. This lends strong support to the concept emerging from human studies of FKBP5 as important factor governing gene–environment interactions relevant for the etiology of affective disorders.

Key Words: Dex/CRH test, emotionality, FKBP51, HPA axis, stress-coping behavior, stress reactivity

In the recent years, polymorphisms in the FK506 binding protein 5 (FKBP5), also referred to as FKBP51, have emerged as one of the most important and intriguing associations with stress-related phenotypes and diseases such as major depression and posttraumatic stress disorder (1–7). FKBP5 was originally identified as component of the progesterone receptor-chaperone heterocomplex (8). Its role in stress regulation was first conjectured upon discovery of elevated FKBP5 levels in squirrel monkeys (9,10). These New World primates exhibit glucocorticoid resistance together with markedly elevated levels of plasma cortisol but lack signs of detrimental glucocorticoid excess (11–13). The decreased hormone-binding affinity of their glucocorticoid receptor (GR) (12) was later largely attributed to the inhibitory action of FKBP5 on GR (14–17).

FKBP5 possesses peptidylprolyl isomerase activity (18) and features a domain for interaction with the central chaperone heat shock protein (Hsp) 90. Although interaction with Hsp90 is essential for its inhibitory action on GR, peptidylprolyl isomerase activity is dispensable (16). Thus, according to the current mechanistic concept, FKBP5 competes with other proteins for access to the Hsp90-GR heterocomplex, thereby interfering with the action of GR-stimulatory factors, in dependency of its expression levels (19).

The well-documented influence of FKBP5 on GR function served as rationale for its inclusion as one of the candidates in the gene association study that first associated *FKBP5* polymorphisms with response to antidepressant treatment (1). Over decades, ample evidence accumulated for an essential role of GR function in stress-related psychiatric disorders such as major depression and posttraumatic stress disorder (20–22). In general, elevated levels of glucocorticoids in response to stressful life events constitute a healthy adaptive reaction, provided this response is balanced and transient. The hypothalamus-pituitary-adrenal (HPA) axis is a key control system to balance hormonal and behavioral responses to stressors. A hallmark of HPA axis regulation is the negative feedback exerted by glucocorticoids via GR on the secretion of stress hormones (20). Substantial evidence suggests that this attenuation of HPA axis activity, which is an integral part of the adaptive response to stressors and challenges, is often impaired in patients suffering from major depression (23,24). For example, major depression has been repeatedly shown to be associated with elevated levels of circulating glucocorticoids, decreased responsiveness to dexa-

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methasone (Dex) suppression and increased adrenocortical response to stimulation with corticotropin-releasing hormone (CRH) in the combined Dex/CRH test (20,24). Although the exact physiological consequences of prolonged glucocorticoid elevation are not fully understood (25), it is intriguing that successful treatment of depression mostly goes along with a normalization of HPA axis reactivity. Moreover, remitted individuals with incompletely attenuated HPA axis overdrive have a higher risk of relapse (26). These and other observations led to the formulation of the corticosteroid receptor hypothesis of depression, which stipulates a link between corticosteroid receptor dysfunction and major depression (23).

This hypothesis is further supported by animal experiments (27). For example, mice with impaired GR function due to transgenic antisense RNA expression exhibit neuroendocrine characteristics similar to those observed in major depression, including a hyperactive HPA axis (28). Antidepressant treatment counteracted these alterations (29). In addition, acquired deficit of forebrain GR produces depression-like phenotypes in behavioral and physiological stress reactivity, which were normalized by antidepressant treatment (30).

Given the established role of GR and its regulatory protein FKBP5 in stress-related disorders and associated endophenotypes, we undertook a molecular, neuroendocrine, and behavioral characterization of *Fkbp5*$^{-/-}$ mice under basal conditions as well as in response to stressors. We considered the latter point particularly important, because the ability of an organism to respond and the way it responds to stressors have been accepted as crucial factors in stress-related disorders (20). Deletion of *Fkbp5* resulted in alterations of HPA axis reactivity and feedback regulation, induced more active coping behavior, and impacted on the expression changes of GR 8 days after stress exposure. In similarity to the findings in mice, *FKBP5* genotypes in humans also altered the outcome of the Dex/CRH test.

Methods and Materials

Details of all experimental procedures are provided in Supplement 1. All work was in accord with accepted standards of humane care and use of experimental animals and approved by the appropriate local authority.

Cell Culture and Reporter Gene Assays

Conditions for cultivating cells, reporter gene assays, and plasmid details have been described previously (19,31–34). Briefly, mouse embryonic fibroblast (MEF) cells derived from knockout (KO) and wildtype (WT) animals were cultured in medium containing steroid-free serum for 24 hours before transfection. Plasmids used were a steroid responsive luciferase reporter and expression vectors for GR and *Fkbp5*. The following day, cells were exposed to Dex and harvested for protein extraction and luciferase activities measured 1 day later.

Experimental Animals and Housing Conditions

All mice were derived from heterozygous matings. Animals homozygous for the *Fkbp5* KO or WT alleles were used for the experiments. The animal housing and experimental rooms were maintained under standard laboratory conditions. Commercial mouse diet and water were available ad libitum. In all experiments, young adult males were used (10–16 weeks of age). At least 2 weeks before each experiment, mice were single-housed and habituated to the experimental room to avoid transportation and dominance hierarchy effects.

Basal Behavioral Phenotyping

Fkbp5$^{-/-}$ and *Fkbp5*$^{+/+}$ littermates were tested in paradigms assessing anxiety-related behavior, exploratory drive, locomotor activity, stress-coping, and depression-like behavior (35). The battery of tests consisted of the open-field test, the elevated plus-maze test, the dark-light box test, and the forced swim test (FST). All tests were performed as described previously (36,37) in the order listed between 9:00 AM and 12:00 AM with an inter-test interval of 48 hours (i.e., 1 day rest between the tests, as recommended for successive behavioral testing) (38).

HPA Axis Function and Regulation

Neuroendocrine stress reactivity (37) was assessed in the same mice that had been characterized in the previously described behavioral test battery by subjecting them to a "stress reactivity test" 1 week after behavioral testing. Briefly, the stress reactivity test comprises a 15-min restraint period and blood sampling immediately before and after stress exposure as well as 75 min thereafter, for determination of corticosterone levels. The test was performed during the first hours of the light phase when corticosterone levels are at the trough of the circadian glucocorticoid rhythm (39,40).

In the Dex/CRH test (41)—assessing HPA axis functions—a blood sample was taken 7 days before the actual test to obtain a basal reference value ("untreated"). On the day of testing, the mice received an IP injection of Dex at 9:00 AM, followed by an injection of CRH at 3:00 PM (.15 mg/kg). Blood samples were collected immediately before CRH injection ("after Dex" value) and 30 min later ("after CRH" value). Two independent Dex/CRH tests were performed with naïve mice, with either a relatively high (2 mg/kg) or low (.05 mg/kg) dose of Dex.

Dex/CRH Test and Genotyping in Human Subjects

Healthy subjects between 20 and 44 years of age (33 men, 32 women; mean age 27.4 years, SD 6.5, all Caucasians from German descent) without any history of psychiatric or severe somatic disorders, verified by standardized clinical interviews, were recruited from webpage advertising and local notice boards. Participants gave written informed consent after all study details were explained. The study protocol was approved by the ethical committee at the Medical Department of the Ludwig-Maximilians-University Munich, Germany. Subjects received 1.5 mg Dex orally at 11 PM and were injected with human CRH (100 μg) at 3 PM the next day. Blood samples for determination of cortisol levels were drawn 1 day before Dex treatment, immediately before CRH injection, and 30 min thereafter.

FKBP5 genotyping was performed with pyrosequencing. The single nucleotide polymorphism rs1360780 was selected, because we found it associated with *FKBP5* expression changes (1). Genotype call rate was 100%, and no deviation from the Hardy-Weinberg-Equilibrium was observed ($p = .358$).

Effects of Acute Stressors on Coping Behavior

In order to investigate the effects of acute stress exposure on the coping behavior of *Fkbp5*$^{-/-}$ and *Fkbp5*$^{+/+}$ mice, several experiments were performed with stressors of different intensity and addressing the response of the animals in the FST 24 hours later.

Tissue Protein Extraction and Immunoblotting

Mice were sacrificed under basal conditions 8 days after the last behavioral test or the Dex/CRH tests, and total protein was extracted from different brain regions. Immunoblots were performed as described with minor modifications (16). Briefly, equal amounts of protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to a membrane, probed with suitable pri-