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Enhancement of Stress Resilience Through Histone Deacetylase 6–Mediated Regulation of Glucocorticoid Receptor Chaperone Dynamics

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ABSTRACT

BACKGROUND: Acetylation of heat shock protein 90 (Hsp90) regulates downstream hormone signaling via the glucocorticoid receptor (GR), but the role of this molecular mechanism in stress homeostasis is poorly understood. We tested whether acetylation of Hsp90 in the brain predicts and modulates the behavioral sequelae of a mouse model of social stress.

METHODS: Mice subjected to chronic social defeat stress were stratified into resilient and vulnerable subpopulations. Hypothalamic-pituitary-adrenal axis function was probed using a dexamethasone/corticotropin-releasing factor test. Measurements of Hsp90 acetylation, Hsp90-GR interactions, and GR translocation were performed in the dorsal raphe nucleus. To manipulate Hsp90 acetylation, we pharmacologically inhibited histone deacetylase 6, a known deacetylase of Hsp90, or overexpressed a point mutant that mimics the hyperacetylated state of Hsp90 at lysine K294.

RESULTS: Lower acetylated Hsp90, higher GR-Hsp90 association, and enhanced GR translocation were observed in dorsal raphe nucleus of vulnerable mice after chronic social defeat stress. Administration of ACY-738, a histone deacetylase 6–selective inhibitor, led to Hsp90 hyperacetylation in brain and in neuronal culture. In cell-based assays, ACY-738 increased the relative association of Hsp90 with FK506 binding protein 51 versus FK506 binding protein 52 and inhibited hormone-induced GR translocation. This effect was replicated by overexpressing the acetylation-mimic point mutant of Hsp90. In vivo, ACY-738 promoted resilience to chronic social defeat stress, and serotonin-selective viral overexpression of the acetylation-mimic mutant of Hsp90 in raphe neurons reproduced the behavioral effect of ACY-738.

CONCLUSIONS: Hyperacetylation of Hsp90 is a predictor and causal molecular determinant of stress resilience in mice. Brain-penetrant histone deacetylase 6 inhibitors increase Hsp90 acetylation and modulate GR chaperone dynamics offering a promising strategy to curtail deleterious socioaffective effects of stress and glucocorticoids.

Keywords: Acetylation, ACY-738, Hsp90, HDAC6, Raphe, Resilience, Serotonin, Social defeat, Stress

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Stressful life events play a significant role in the precipitation of affective disorders, but only a subset of individuals develop lasting psychological sequelae after severe stress (1–4). The glucocorticoid receptor (GR) and several components of its multiprotein chaperone complex have been implicated as a point of convergence for genetic, epigenetic, and environmental factors shaping stress vulnerability (5–9). Converging data from psychiatric genetics and animal studies indicate that mechanisms affecting the stoichiometry and interactions of proteins within the GR chaperone heterocomplex profoundly influence the physiology of the hypothalamic-pituitary-adrenal (HPA) axis (10–12) and stress coping (13–18). However, how molecular regulation of GR chaperone dynamics translates into emergent neurobehavioral traits defining vulnerability or resilience is not well understood.

Heat shock protein 90 (Hsp90) is a core component of the GR chaperone complex. Through direct interactions with the GR and cochaperones (including immunophilins FK506-binding protein 51 [FKBP51] and FK506-binding protein 52 [FKBP52]), Hsp90 affects multiple aspects of the GR response, including hormone binding (19), nuclear mobility (20), DNA binding (21), and clearance (22). Posttranslational modifications of Hsp90 are a potentially key mechanism allowing flexible and circuit-specific downstream regulation of steroid signaling (23,24). Lysine N-acetylation has emerged in recent years as a key biochemical switch regulating protein-protein interactions of Hsp90 with several nuclear receptors (25–28) and cochaperones (29,30). The lysine histone deacetylase 6 (HDAC6) is the best-characterized regulator of Hsp90 acetylation. We and others have previously shown that genetic or pharmacologic inhibition of HDAC6 leading to Hsp90 hyperacetylation reduces the neurobehavioral impact of emotional stressors and exogenous glucocorticoids (31–34). Although HDAC6 is found in tissue homogenates from virtually all GR-expressing brain regions, our previous results indicate that the enzyme shows a striking enrichment in serotonin (5–hydroxytryptamine) neurons of the raphe nucleus (DRN), where we identified down-regulation of HDAC6 messenger

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RNA after stress as an adaptive molecular signature correlating with resilience and antidepressant response (31).

The present study was designed to extend our analysis of HDAC6-dependent regulation of GR chaperone dynamics in stress homeostasis and resilience. We asked whether Hsp90 acetylation is regulated by chronic social defeat stress (CSDS), a murine model of traumatic stress, and tested whether this molecular mark predicts the behavioral and neuroendocrine impact of CSDS in individual mice. We next used novel pharmacologic and viral-mediated approaches to test the causal influence of Hsp90 acetylation in resilience. Finally, we used tissue culture to examine how Hsp90 acetylation influences GR chaperone dynamics and hormone-induced GR nuclear trafficking in serotonin neurons. Data in this report lend support to the hypothesis that HDAC6-dependent regulation of GR chaperone dynamics is a critical mechanism mediating emotional adaptations during psychosocial stress and is a viable target for pro-resilient pharmacologic interventions.

METHODS AND MATERIALS

A more detailed description of all methods used is provided in Supplement 1.

Animals

All mice were 8–12 weeks of age, housed on a 12-hour light cycle with food and water available ad libitum. All studies were conducted according to protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee in accordance with institutional guidelines.

Repeated Social Defeat and Social Interaction Testing

The CSDS, social interaction test, and stratification into vulnerable and resilient were completed as previously described (31,36,37), using TopScan video-tracking software (CleverSys, Reston, Virginia).

Corticosterone Measurements

Corticosterone response was measured using a commercial enzyme-linked immunosorbent assay kit (Assaypro LLC, St. Charles, Missouri). Detailed timelines are presented in Figure 1A.

Measurements of Nuclear GR, Hsp90-GR Association, and Hsp90 Acetylation

At 2 weeks after behavioral testing for social avoidance, mice were subjected to an additional 10 min of CSDS exposure. Brains from animals kept in sensory contact with the aggressor were dissected 30 min after the end of the physical interaction. Punches were taken of the DRN using a brain matrix. Nuclear fractions were prepared using a commercially available kit (BioVision, Milpitas, California). Standard Western blotting procedures were used, and blots were analyzed on an Odyssey system (LI-COR Biotechnology, Lincoln, Nebraska) and quantified using ImageJ software (National Institutes of Health, Bethesda, Maryland). The Hsp90-GR complex was immunoprecipitated from DRN punch homogenates using mouse monoclonal HSP 90α/β F-8 Antibody (sc-13119; Santa Cruz Biotechnology, Dallas, Texas).

In Vivo Drug Treatments

The HDAC6 inhibitor ACY-738 (provided by Acetylon Pharmaceuticals, Boston, Massachusetts) was administered at a dose of 5 mg/kg by intraperitoneal (i.p.) injection 10 days before the beginning of the CSDS procedure and continued throughout the stress period for a total of 20 days (Figure 2B). Data from our laboratory indicate that the inhibitory concentration of 50% of ACY-738 for HDAC6 is 1.7 nmol/L, and selectivity over class 1 HDACs is between 60-fold and 120-fold, depending on the isoform. At the dose used in the present study, ACY-738 does not elevate histone acetylation in the brain (34).

Expression Vectors

Human Hsp90 wild-type (WT) and K294A point mutant were obtained from Len Neckers (National Cancer Institute, Rockville, Maryland). Glucocorticoid receptor–green fluorescent protein (GR-GFP) plasmid was obtained from Edwin Sanchez (University of Toledo College of Medicine, Toledo, Ohio). Flag-tagged human FKBP51 and FKBP52 expression vectors were obtained from Theo Rein (Max Planck Institute for Psychiatry, Munich, Germany).

Virus Construction and Stereotactic Surgery

Flag-tagged Hsp90 construct containing a lysine-to-alanine mutation at position 294 (K294A) was cloned into a Cre-dependent AAV2 FLEX backbone (38) under control of the human synapsin promoter (AAV.hSynap.Flex.SV40) and packaged into adeno-associated virus 2.9 viral particles and injected into the DRN of mice expressing Cre recombinase in a serotonin selective manner driven by the Pet1 promoter (35). A timeline of these experiments is presented in Figure 3A.

Immunohistochemistry

Mice were perfused with 4% paraformaldehyde, and brains were processed using standard single or dual immunolabeling methods as previously reported (31).

Cell-Based Assays

RN46A-B14 cells, an immortalized rat raphe cell line, were used for all tissue culture assays. For GR translocation, cells were treated with vehicle (.75% dimethyl sulfoxide), 2.5 μmol/L Tubastatin A (39), or 2.5 μmol/L ACY-738 (34) for 1 hour, followed by 1 μmol/L dexamethsone (Sigma-Aldrich, St. Louis, Missouri) or vehicle (2% ethyl alcohol) for 30 min. Cells were fixed in 4% paraformaldehyde and immunostained for GFP (Aves Labs, Inc, Tigard, Oregon) and then imaged, and ratios of GR-GFP signal inside and outside the nucleus were obtained in a high-throughput manner using the ImageXpress Micro System (Molecular Devices, LLC, Sunnyvale, California).

Data Analysis

All variables were distributed normally and were analyzed using parametric statistics with *t* test or one-way analysis of variance followed by Fisher's protected least significant difference or Tukey post hoc tests where appropriate. Correlations between pairs of variables were examined using linear regressions, and proportions were compared using the Fisher's