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Pharmacological stimulation of Hypoxia Inducible Factor-1α facilitates the corticosterone response to a mild acute stressor

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Abstract

While both glucocorticoids (the principal output of the hypothalamic-pituitary-adrenal axis) and oxidative stress have been implicated in outcomes due to an excessive or prolonged stress response, the precise mechanisms linking these two systems remain poorly elucidated. One potential mediator between the hypothalamic-pituitary-adrenal axis and oxidative stress is the Hypoxia Inducible Factor-1 (HIF-1) pathway. HIF-1 is an oxygen-responsive transcription factor with diverse effects including changes in cellular metabolism. The experiments in this manuscript sought to determine if pharmacological stimulation of HIF-1α via administration of dimethyloxalylglycine (DMOG) would facilitate the corticosterone response to a mild acute stressor. DMOG administration significantly increased plasma corticosterone five minutes after an acute airpuff without changing baseline plasma corticosterone or plasma corticosterone level two hours post-startle. DMOG administration also reduced hippocampal gene expression of the pro-translocation co-chaperone for the glucocorticoid receptor, FKBP4, two hours after airpuff startle. At this same two-hour time point, hippocampal expression of FKBP5, an anti-translocation co-chaperone of glucocorticoid receptor, in the DMOG-treated group was also positively correlated with plasma corticosterone levels. These data indicate that there is significant crosstalk between the hypothalamic-pituitary-axis and the HIF-1 pathway and extend the current knowledge of glucocorticoid and hypoxia interactions in an ethologically relevant stress model.

Keywords

Hypoxia inducible factor 1α; dimethyloxalylglycine; airpuff startle; stress; FKBP4; FKBP5

1. Introduction

Transcription factors control gene expression in all organ systems. The profound influence of transcription factors accounts for the pervasive effects of glucocorticoids and other

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steroid hormones on physiology and behavior given that a subset of steroid receptors are transcription factors [2]. Glucocorticoids are the main effector of the hypothalamic-pituitary-adrenal (HPA) axis, the slower acting branch of the stress response. Activation of the HPA axis is eventually reduced through negative feedback via stimulation of glucocorticoid receptors (GR) within the hippocampus, hypothalamus, and anterior pituitary [20, 23]. Binding of glucocorticoids and activation of the GR regulate transcription of numerous target genes containing glucocorticoid responsive elements (GREs) in their promoter regions. Further titrating the activation and translocation of the glucocorticoid receptor are two of its cochaperones: FK506 binding protein 4 (FKBP4), found to increase transcriptional activity, and FK506 binding protein 5 (FKBP5), found to reduce nuclear translocation [1, 33]. Another mode of action whereby glucocorticoids modulate expression of target genes containing no GREs is through “transcriptional crosstalk” with other transcription factors [30, 34].

Hypoxia inducible factor-1 (HIF-1) is a transcription factor that regulates overlapping target genes with GR and may mediate the link between oxidative stress and the HPA axis. This heterodimeric transcription factor is composed of α and β subunits, of which the HIF-1β subunit is constitutively expressed [7, 16, 25]. Under normoxic conditions, the HIF-1α subunit is maintained at low levels by oxygen-dependent prolyl hydroxylases that modify HIF-1α for binding by von Hippel-Lindau tumor suppressor protein and proteasomal degradation. Under hypoxic conditions, HIF-1α is stabilized, and target gene transcription is enhanced, including transcription of HIF-1α as well as transcription of vascular factors, glycolytic factors, and immune factors [25]. We have previously demonstrated that targets of HIF are altered following stressor exposure [11, 18]. HIF-1α can be pharmacologically manipulated as well by administration of dimethyloxalylglycine (DMOG), an ester of N-oxalylglycine that inhibits prolyl-4-hydroxylases and stabilizes HIF-1α expression [9]. HIF activity is affected as well, as DMOG administration increases hypoxia-response-element (HRE) reporter activity in cell lines [8, 12], and hypoxia and DMOG-induced changes in gene expression are blocked by RNA interference of HIF-1α [15].

Although the roles of HIF in cancer biology and ischemia have been well characterized [7, 26], the potential role of HIF in stress-induced changes in physiology and behavior has not been considered previously. Given that HIF and GR share targets and have the biological potential for interaction, we tested the hypothesis that pharmacological stimulation of the HIF pathway facilitates the HPA axis response to an acute stressor. In order to test this hypothesis, we administered DMOG or saline to adult male rats with previously implanted jugular catheters and subsequently exposed these rats to an acute airpuff startle (APS). We also assessed hippocampal gene and protein expression of the glucocorticoid receptor (gene name *Nr3c1)* as well as the negative regulator FKBP5 and the positive regulator FKBP4. This study revealed that pharmacological stimulation of the HIF pathway with DMOG potently upregulated corticosterone output following an acute stressor and that these changes in plasma corticosterone correlated with changes in hippocampal FKBP5 protein expression.

2. Materials and methods

2.1 Animal husbandry

Two-month old male Wistar rats (n=18) were obtained from Charles River (Wilmington, MA) and housed on a 14:10 reverse light:dark cycle in a facility controlled for humidity (60%) and temperature (20 °C-23 °C). Rodent diet 5001 chow (Purina Mills, Richmond, IN) and water were maintained *ad libitum* throughout the study. Rats were pair-housed and allowed to acclimate to the housing facility for one week prior to surgery. Post-surgery, rats were individually housed. All experiments were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) of Emory University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 Surgeries

Jugular catheter surgeries were performed on all rats by an expert surgeon as described in Thrivikraman et al [28]. In brief, rats were dosed with a ketamine/xylazine/acepromazine cocktail (25:5:1 mg/kg), and surgery was performed 30 minutes later. The right jugular vein was isolated by blunt dissection, and a catheter consisting of polyethylene (PE-50) in silastic tubing was introduced and secured with ligatures. Sterile saline was flushed through the catheter and a slow withdrawal of blood attempted to ensure patency. The polyethylene end of the catheter was then exteriorized through an incision at the nape of the neck, flushed with sterile saline, and subsequently filled with gentamicin. All surgical incisions were swabbed with betadine and closed, and rats were allowed to recover individually-housed in clean cages for three days. Rats were observed daily and monitored for signs of pain and distress. All rats were individually-housed at this point and subjected to this same stress prior to subsequent treatment. Though individual-housing could act as a potential stressor that might interact with dimethyloxalylglycine to influence the corticosterone response to an additional stress (airpuff startle), individual-housing is necessary to prevent rats from removing or impairing another's jugular catheter and was used in both treatment groups. Use of the catheters was deemed preferable in light of the “3R Model” guiding the use of laboratory animals [24] to sacrificing multiple pair-housed animals at each time point.

2.3 Dimethyloxalyl glycine and airpuff startle (APS)

On the third day post-surgery, rats were administered 200 mg/kg dimethyloxallyl glycine (DMOG) (Cayman Chemical, Ann Arbor, MI; n=8) or an equivalent volume of saline (n=10) by intraperitoneal (i.p.) injection. Intraperitoneal injection was considered preferable to gavage given the known lasting effects of gavage on heart rate, blood pressure, and corticosterone [29]. Eight hours later, rats were administered 100 mg/kg DMOG or saline, and eight hours after the second dose, rats were administered a final dose of 200 mg/kg DMOG or saline. This procedure was chosen based on the schedule and dosing used in Nagel, et al. [17], in which DMOG administration prior to middle cerebral artery occlusion, an ischemic stroke model known to be influenced by the HPA axis [27], was neuroprotective. Three hours after the final DMOG administration, a baseline blood draw was taken. Unlike gavage, plasma corticosterone levels return to baseline within 90 minutes of saline injection [5], thus this three-hour waiting period was deemed appropriate for a

baseline blood draw. While possible that prior injection stress could alter the response to APS, this injection stress was equally administered across groups.

After the baseline blood draw, APS was administered to all rats. The APS consisted of three blocks of puffs applied with 1 min intervals between each block, with each block consisting of three one-second air blasts over a five-minute window. Blood sampling occurred over 120 minutes, taking sequential 300 ul samples at seven time points following the APS: 5, 10, 15 30, 45, 60, and 120 minutes. An equal volume of heparinized saline was infused after each sample to account for lost blood volume. After the final blood sampling, rats were decapitated, and brains were collected, flash frozen, and later dissected. Blood was centrifuged at 1500 rcf for 20 minutes at 4°C, serum was collected, and it was then frozen until used for radioimmunoassay.

2.4 Corticosterone ELISA

To assess serum corticosterone, the ImmunChem Double Antibody ^{125}I-radioimmunoassay (MD Biomedicals, Orangeburg, NY) was used. Samples were run in duplicate in accordance with kit instructions, precipitates counted in a gamma counter, reads normalized to non-specific binding, and concentrations calculated from a standard curve using a four-parameter logistic model.

2.5 Quantitative real-time polymerase chain reaction

Gene expression of the glucocorticoid receptor (GR) and its co-chaperones FK506 binding protein 5 (FKBP5), FK506 binding protein 4 (FKBP4), and peptidyl prolylisomerase D (PPID) were assessed in left hippocampal tissue via real time polymerase chain reaction. Primers for *Nr3c1, Fkbp4, Fkbp5* and *Ppid,* as well as housekeeping genes *Gapdh*, *Hprt1*, *Prl3a1,* and *Actb* were designed using Primer3Plus. Tissue was homogenized in *mir*Vana PARIS cell disruption buffer (Ambion, Carlsbad, CA) using a Tissue Lyser II (Qiagen, Boston, MA). Reverse transcription was performed with the High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA), and concentrations of cDNA were quantified with Quant-IT Pico Green dsDNA Assay Kit (Invitrogen, Eugene, OR). Quantitative real-time PCR was performed using Thermo Absolute Blue SYBR Green ROX (ThermoScientific) on the 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Gene expression changes are expressed as $2^{-\Delta\Delta Ct}$ relative to the geometric mean of the housekeeping genes.

2.6 Immunoblotting

Expression of GR, FKBP5, and FKBP4 was quantified in the left hippocampus only by immunoblot. Tissue was homogenized in mirVana PARIS RNA and Native Protein Purification Kit (Ambion Life Technologies, Grand Island NY) cell disruption buffer in a Tissue Lyser II, from which RNA and protein were purified. Protein concentrations were determined by BCA analysis (ThermoScientific, Rockford, IL). For each region, 30 μg of protein homogenate was loaded in a BioRad Mini-Protein TGX gel (BioRad, 4-15%) and separated by gel electrophoresis. Protein was transferred onto a PVDF membrane (Millipore, Billerica, MA) and blocked in 7.5% non-fat dried milk in Tris-buffered-saline and Tween 20 for 1 hour. Membranes were incubated with rabbit anti-GR (1:1000, Santa Cruz, sc-1004),

mouse anti-FKBP4 (1:20,000, Abcam, ab59460), or goat anti-FKBP5 (1:1000, ThermoScientific, pa1-020) overnight at 4°C. After washing, membranes were incubated with horseradish peroxidase labeled anti-rabbit (1:5000, Santa Cruz, sc-2004), anti-mouse (1:5000, Santa Cruz, sc-2005), or anti-goat (1:5000, Bethyl, a-50-201p) antibodies for one hour at room temperature. Membranes were visualized using chemiluminescence (SuperSignal West Fempto, ThermoScientific, Waltham, MA). Expression of β-actin was determined as a control. Membranes were incubated with a monoclonal anti-β-actin antibody (1:50,000, Sigma Aldrich, St. Louis, MO, A3854) for 30 minutes at room temperature before being visualized with chemiluminescence as above. Between each different primary antibody, membranes were stripped for twenty minutes at room temperature with Restore Western Blot Stripping Buffer (Pierce Biotechnology, Rockford, IL) before being washed and blocked again.

2.7 Statistical analyses

All statistical analyses were run in Graph Pad 6.0. For the corticosterone RIA, the sample concentrations were calculated from a standard curve fit to a four-parameter logistic model and compared by a one-way repeated measures ANOVA with α=0.05. For the real-time PCR, $2^{-\Delta\Delta Ct}$ values were compared with a student's t-test. For the immunoblots, the percent optical density for each sample was calculated using ImageJ (NIH, Bethesda, MD) and normalized to the percent optical density of β-actin for that sample, and the two groups (treated and untreated) were compared by a two-tailed t-test with α=0.05. Normalized values were correlated to plasma corticosterone using Pearson's correlations.

3. Results

3.1 DMOG enhances plasma corticosterone response to APS

APS significantly changed plasma corticosterone across time (Figure 1; $F_{7,77}$=4.6; p<0.01), and administration of DMOG significantly elevated plasma corticosterone relative to saline-treated animals ($F_{1,77}$=24; p<0.01). Holm-Sidak post-hoc analysis of the effect of DMOG at each time point after APS revealed that DMOG-treated animals had significantly higher corticosterone levels at the five-minute and 15-minute time points (adjusted p<0.01 and p=0.05, respectively), which began to return to saline-treated levels by the 30-minute time point (adjusted p>0.05). In addition, the area under the curve (AUC) for mean plasma corticosterone levels over time for the DMOG treated animals was 1.99 fold higher than the AUC for the saline-treated animals (DMOG AUC: 9227; Saline AUC: 4643). However, baseline plasma corticosterone levels were neither significantly different in post-hoc testing (adjusted p>0.05) nor in a separate t-test (t_{11}=1.384, t=0.19).

3.2 DMOG decreases hippocampal FKBP4 gene expression after APS

DMOG administration significantly reduced gene expression of the positive glucocorticoid receptor co-chaperone, *Fkbp4*, in the left hippocampus two hours after exposure to APS (t_{16}=2.780; p<0.01; figure 2a). Gene expression of the receptor itself, *Nr3c1* (t_{16}=0.1.461; p=0.16) and its co-chaperones *Fkbp5* (t_{16}=0.3995; p>0.05) and *Ppid* (t_{16}=1.467; p>0.05) were unchanged by DMOG administration prior to APS (data not shown).

3.3 Hippocampal FKBP5 expression correlates with plasma corticosterone after APS

Protein expression of hippocampal FKBP5 positively correlated with plasma corticosterone 60 minutes ($r(16)=0.55$, $p< 0.05$), 90 minutes ($r(16)=0.54$, $p<0.05$), and 120 minutes ($r(16)=0.60$, $p<0.05$) after APS startle (figure 2b). When subsequently analyzing by treatment group, no correlation was found at any time point for the saline treated groups ($p>0.05$ for all time points), but a positive correlation remained between FKBP5 expression and plasma corticosterone for the DMOG treated group at 120 minutes ($r(6)=0.80$, $p<0.05$). However, no overall differences between DMOG- or saline-treated animals were observed in hippocampal protein expression of the glucocorticoid receptor ($t_{16}=1.249$; $p>0.05$), FKBP4 ($t_{16}=0.7872$; $p>0.05$), or FKBP5 ($t_{16}=1.278$; $p>0.05$) two hours after APS (figure 2c).

4. Discussion

Administration of a prolyl-4-hydroxylase inhibitor (DMOG), a pharmacological stabilizer of HIF-1α, resulted in increased plasma corticosterone following acute air puff startle (APS). These data demonstrate that significant crosstalk exists between the HIF pathway and the HPA axis, specifically in response to acute stress given that baseline corticosterone concentrations were unaffected. The changes in the glucocorticoid co-chaperone *Fkbp4* and relationship between plasma corticosterone and FKBP5 in DMOG-treated animals also indicate additional crosstalk between the HIF-pathway and the HPA axis. These data shed light on the potential link between oxidative stress and the HPA axis, and they may have implications for conditions that stimulate primary change in one of these two pathways such as inflammatory conditions [21, 31], tumorigenesis [6, 32], angiogenesis [19], and vascular injury [13, 18].

While the effects of pharmacological manipulation of HIF-1α during an acute psychophysiological stress such as APS have not previously been reported, the effects of hypoxia and hypoxic preconditioning have been assessed. Neonatal hypoxia has been shown to elicit an exaggerated corticosterone response to ACTH injection in conjunction with an elevation in adrenal proteins involved in steroidogenesis but not adrenal HIF [22]. The response to hypoxia is developmentally dependent, shifting from an ACTH and cAMP-independent mechanism to an ACTH and cAMP-dependent mechanism between postnatal days two and eight [3, 10]. This literature is consistent with effects demonstrated here, namely, that pharmacological stimulation of HIF-1α to mimic hypoxic conditions will facilitate a corticosterone response (figure 1). The data presented herein are novel in that this is the first presentation of data that demonstrate that HIF-1 stabilization can facilitate the corticosterone response to a minor psychophysiological stressor and that this facilitation can be mediated pharmacologically.

The downregulation of *Fkbp4* expression in DMOG-treated animals (figure 2a) and concomitant relationship between FKBP5 and plasma corticosterone, most notably in DMOG-treated animals (figure 2b), provide evidence for a potential locus of crosstalk between the HIF-1 pathway and the HPA axis. FKBP4 is known to increase transcriptional activity while FKBP5 is known to reduce nuclear translocation and transcriptional activity of the glucocorticoid receptor [1, 33]. This potential reduction in *Fkbp4* can be reconciled to

the exaggerated corticosterone response in the DMOG-treated animals. While we did not observe a corresponding reduction in FKBP4 protein expression, it is possible that the time-lapse between the early facilitation in the corticosterone response (figure 1) and the measurement of the gene and protein expression two hours later may have impaired the resolution of the results. However, the observations that FKBP5 expression correlated with plasma corticosterone at the time when corticosterone levels were returning to baseline and that this correlation remained in post-hoc analyses of the DMOG group alone at the 120 minute time-point, provide some evidence for HIF-1 mediated regulation of the glucocorticoid negative feedback loop.

This crosstalk between the HIF-1 pathway and the HPA axis may be of clinical importance; in adult animals, hypoxia and hypoxic pre-conditioning induce production of reactive oxygen species (ROS) alongside activation of HIF-1. Preconditioning is neuroprotective [4], potentially due to its ability to downregulate the threshold for production of HIF-1α and downstream factors [14]. Given the crosstalk between the HIF-1 pathway and HPA axis demonstrated here, preconditioning regiments may do well to consider the potential effects of acute stress upregulating the threshold for production of HIF-1α and downstream factors.

The results in this manuscript have demonstrated that administration of a prolyl hydroxlase inhibitor facilitates the glucocorticoid response to a mild acute stressor. This facilitated glucocorticoid response occurs in conjunction with a downregulation of the positive GR co-chaperone *Fkbp4* and a correlation between the negative GR co-chaperone FKBP5 expression in the hippocampus and plasma corticosterone. The data presented herein provide a strong foundation for significant interaction between the HIF-1 pathway and the HPA axis that may be relevant for future studies of the stress response, hypoxic conditioning, and neuroprotection.

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References

1. Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology. 2009; 34(Suppl 1):S186–S195. [PubMed: 19560279]
2. Bourke CH, Harrell CS, Neigh GN. Stress-induced sex differences: adaptations mediated by the glucocorticoid receptor. Hormones and behavior. 2012; 62:210–218. [PubMed: 22426413]
3. Bruder ED, Taylor JK, Kamer KJ, Raff H. Development of the ACTH and corticosterone response to acute hypoxia in the neonatal rat. American journal of physiology. Regulatory, integrative and comparative physiology. 2008; 295:R1195–1203.
4. Gidday JM, Fitzgibbons JC, Shah AR, Park TS. Neuroprotection from ischemic brain injury by hypoxic preconditioning in the neonatal rat. Neurosci Lett. 1994; 168:221–224. [PubMed: 8028780]
5. Grota LJ, Bienen T, Felten DL. Corticosterone responses of adult Lewis and Fischer rats. Journal of neuroimmunology. 1997; 74:95–101. [PubMed: 9119985]

6. Harris AL. Hypoxia--a key regulatory factor in tumour growth. Nature reviews. Cancer. 2002; 2:38–47. [PubMed: 11902584]
7. Hirota K, Semenza GL. Regulation of angiogenesis by hypoxia-inducible factor 1. Critical Reviews In Oncology/Hematology. 2006; 59:15–26. [PubMed: 16716598]
8. Irwin R, LaPres JJ, Kinser S, McCabe LR. Prolyl-hydroxylase inhibition and HIF activation in osteoblasts promotes an adipocytic phenotype. Journal of cellular biochemistry. 2007; 100:762–772. [PubMed: 17031858]
9. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ. Targeting of HIF- alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science (New York, N.Y.). 2001; 292:468–472.
10. Johnson K, Bruder ED, Raff H. Adrenocortical control in the neonatal rat: ACTH- and cAMP-independent corticosterone production during hypoxia. Physiological reports. 2013; 1:e00054. [PubMed: 24303136]
11. Kelly SD, Harrell CS, Neigh GN. Chronic stress modulates regional cerebral glucose transporter expression in an age-specific and sexually-dimorphic manner. Physiology & behavior. 2014; 126:39–49. [PubMed: 24382486]
12. Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. Science. 2002; 295:858–861. [PubMed: 11823643]
13. Lin F, Pan LH, Ruan L, Qian W, Liang R, Ge WY, Huang B. Differential expression of HIF-1alpha, AQP-1, and VEGF under acute hypoxic conditions in the non-ventilated lung of a one- lung ventilation rat model. Life sciences. 2015
14. Liu J, Narasimhan P, Yu F, Chan PH. Neuroprotection by hypoxic preconditioning involves oxidative stress-mediated expression of hypoxia-inducible factor and erythropoietin. Stroke; a journal of cerebral circulation. 2005; 36:1264–1269.
15. Loboda A, Stachurska A, Dorosz J, Zurawski M, Wegrzyn J, Kozakowska M, Jozkowicz A, Dulak J. HIF-1 attenuates Ref-1 expression in endothelial cells: reversal by siRNA and inhibition of geranylgeranylation. Vascular pharmacology. 2009; 51:133–139. [PubMed: 19524065]
16. Majmundar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. Molecular cell. 2010; 40:294–309. [PubMed: 20965423]
17. Nagel S, Papadakis M, Chen R, Hoyte LC, Brooks KJ, Gallichan D, Sibson NR, Pugh C, Buchan AM. Neuroprotection by dimethyloxalylglycine following permanent and transient focal cerebral ischemia in rats. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2011; 31:132–143.
18. Neigh GN, Karelina K, Zhang N, Glasper ER, Owens MJ, Plotsky PM, Nemeroff CB, Devries AC. Cardiac arrest and cardiopulmonary resuscitation dysregulates the hypothalamic- pituitary-adrenal axis. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2009; 29:1673–1682.
19. Neigh GN, Owens MJ, Taylor WR, Nemeroff CB. Changes in the vascular area fraction of the hippocampus and amygdala are induced by prenatal dexamethasone and/or adult stress. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2010; 30:1100–1104.
20. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends in neurosciences. 2008; 31:464–468. [PubMed: 18675469]
21. Pyter LM, Kelly SD, Harrell CS, Neigh GN. Sex differences in the effects of adolescent stress on adult brain inflammatory markers in rats. Brain, Behavior, And Immunity. 2013; 30:88–94.
22. Raff H, Hong JJ, Oaks MK, Widmaier EP. Adrenocortical responses to ACTH in neonatal rats: effect of hypoxia from birth on corticosterone, StAR, and PBR. American journal of physiology. Regulatory, integrative and comparative physiology. 2003; 284:R78–85.
23. Sapolsky RM, Meaney MJ, McEwen BS. The development of the glucocorticoid receptor system in the rat limbic brain. III. Negative-feedback regulation. Brain research. 1985; 350:169–173. [PubMed: 3986611]
24. Schiffelers MJ, Blaauboer BJ, Hendriksen CF, Bakker WE. Regulatory acceptance and use of 3R models: a multilevel perspective. Altex. 2012; 29:287–300. [PubMed: 22847256]

25. Semenza G. Signal transduction to hypoxia-inducible factor 1. Biochem Pharmacol. 2002; 64:993–998. [PubMed: 12213597]
26. Semenza GL. Involvement of hypoxia-inducible factor 1 in human cancer. Internal medicine (Tokyo, Japan). 2002; 41:79–83.
27. Sugo N, Hurn PD, Morahan MB, Hattori K, Traystman RJ, DeVries AC. Social stress exacerbates focal cerebral ischemia in mice. Stroke; a journal of cerebral circulation. 2002; 33:1660–1664.
28. Thrivikraman KV, Huot RL, Plotsky PM. Jugular vein catheterization for repeated blood sampling in the unrestrained conscious rat. Brain Res Brain Res Protoc. 2002; 10:84–94. [PubMed: 12431707]
29. Turner PV, Brabb T, Pekow C, Vasbinder MA. Administration of substances to laboratory animals: routes of administration and factors to consider. Journal of the American Association for Laboratory Animal Science : JAALAS. 2011; 50:600–613. [PubMed: 22330705]
30. Wagner AE, Huck G, Stiehl DP, Jelkmann W, Hellwig-Burgel T. Dexamethasone impairs hypoxia-inducible factor-1 function. Biochemical and biophysical research communications. 2008; 372:336–340. [PubMed: 18501194]
31. Whyte MK, Walmsley SR. The regulation of pulmonary inflammation by the hypoxia-inducible factor-hydroxylase oxygen-sensing pathway. Annals of the American Thoracic Society. 2014; 11(Suppl 5):S271–276. [PubMed: 25525731]
32. Willenberg HS, Haase M, Papewalis C, Schott M, Scherbaum WA, Bornstein SR. Corticotropin-releasing hormone receptor expression on normal and tumorous human adrenocortical cells. Neuroendocrinology. 2005; 82:274–281. [PubMed: 16721033]
33. Wolf IM, Periyasamy S, Hinds T Jr. Yong W, Shou W, Sanchez ER. Targeted ablation reveals a novel role of FKBP52 in gene-specific regulation of glucocorticoid receptor transcriptional activity. The Journal Of Steroid Biochemistry And Molecular Biology. 2009; 113:36–45. [PubMed: 19073255]
34. Zalachoras I, Houtman R, Meijer OC. Understanding stress-effects in the brain via transcriptional signal transduction pathways. Neuroscience. 2013; 242:97–109. [PubMed: 23545270]

Highlights

- Interactions between HIF1α and the HPA axis may facilitate the stress response.
- HIF stimulation via DMOG enhances the corticosterone response to mild stress.
- HIF stimulation alters expression of glucocorticoid cochaperones after stress.
- FKBP4 and FKBP5 may mediate crosstalk between the HIF pathway and the HPA axis.

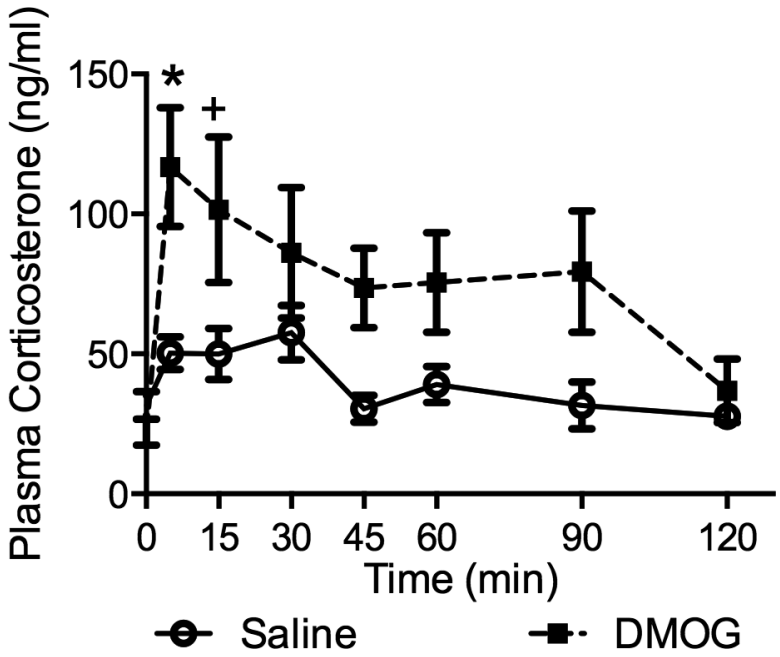

Figure 1. DMOG administration facilitates the corticosterone response to air puff startle

Repeated measures ANOVA of multiple plasma corticosterone samples from rats treated with either dimethyloxalylglycine (DMOG) or saline and then submitted to an air puff startle revealed that DMOG administration significantly increased the corticosterone response to airpuff startle (APS). Holm-sidak post-hoc testing revealed a specific elevation in corticosterone at the 5-minute time point ($p=0.005$), but the difference did not reach significance at the 15-minute time point ($p=0.051$) or at the 90-minute time point ($p=0.075$). Data are shown as mean ± SEM. Significance at $p<0.05$ level is demonstrated with an asterisk; $p=0.051$ is denoted by a plus sign.

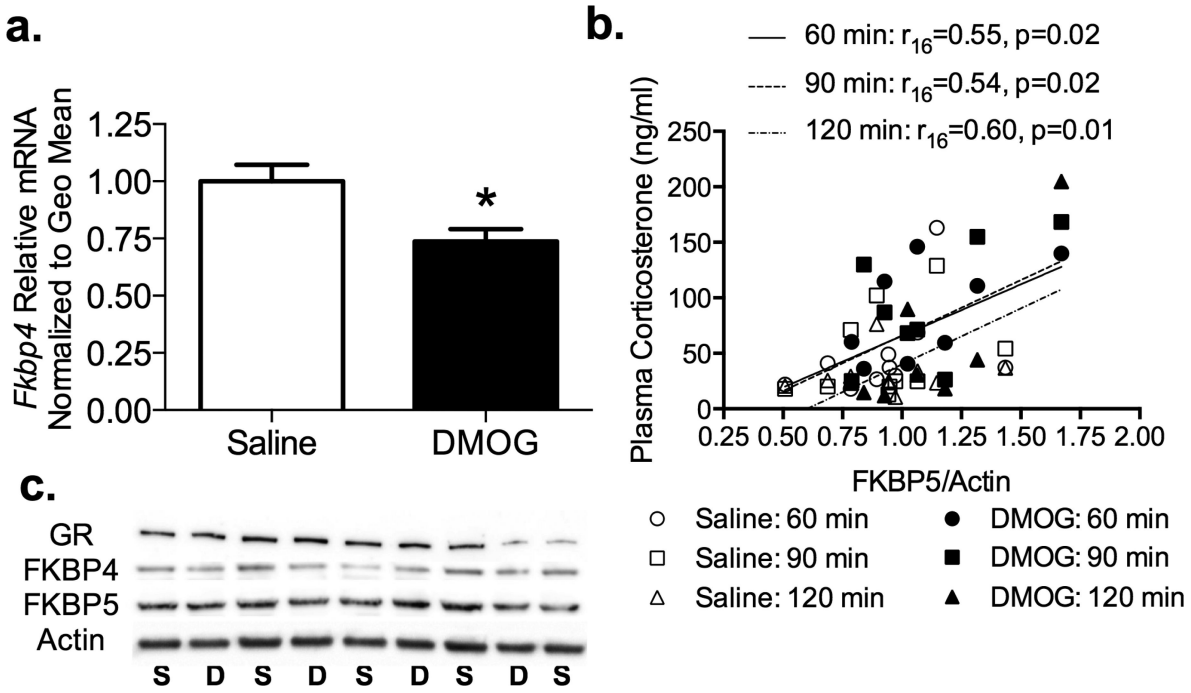

Figure 2. DMOG alters expression of glucocorticoid co-chaperones after airpuff startle

2a. DMOG administration significantly reduced gene expression of the positive glucocorticoid receptor co-chaperone *Fkbp4* in the left hippocampus two hours after exposure to APS ($p<0.05$). **2b**. Protein expression of hippocampal FKBP5 positively correlated with plasma corticosterone 60 minutes, 90 minutes, and 120 minutes after APS ($p<0.05$ for all). No correlation was found at any time point for the saline treated groups alone ($p>0.05$ for all), but a positive correlation remained between FKBP5 expression and plasma corticosterone for the DMOG treated group at 120 minutes. 2c. However, no significant differences in protein expression of GR, FKBP4, or FKBP5 normalized to actin were observed between saline and DMOG treated animals. Representative blots of FKBP5 and actin expression in the hypothalamus of saline and DMOG treated animals after APS; S=saline, D=DMOG.