



PERGAMON

Psychoneuroendocrinology 28 (2003) 481–500

www.elsevier.com/locate/psyneuen

PNEC

Inescapable shock induces resistance to the effects of dexamethasone

K.A. O'Connor *, J.D. Johnson, S.E. Hammack, L.M. Brooks,
R.L. Spencer, L.R. Watkins, S.F. Maier

*Department of Psychology & Center for Neuroscience, University of Colorado, Boulder,
CO 80309-0345, USA*

Received 23 January 2001; received in revised form 3 October 2001; accepted 15 April 2002

Abstract

Administration of bacterial endotoxin (lipopolysaccharide; LPS) elevates proinflammatory cytokines, such as interleukin-1 β (IL-1 β) and IL-6, and activates the hypothalamic-pituitary-adrenal (HPA) axis. Corticosterone (CORT), the glucocorticoid (GC) effector hormone of the HPA axis in rats, inhibits both proinflammatory cytokine production/release and activity of the HPA axis itself. Exposure to chronic or repeated stressors often induces resistance to the effects of GCs. The following experiments were conducted to test the hypothesis that an acute stressor, inescapable tailshock (IS), alters responsivity of the HPA axis and proinflammatory cytokine system to dexamethasone (DEX), a synthetic GC. First, we examined the ability of various doses of DEX to suppress proinflammatory cytokine and HPA activity in response to LPS challenge 24 h after either home cage (HCC) or IS treatment. Upon finding resistance to DEX in IS animals, we examined the duration of the altered response to DEX by testing animals 1, 4 and 21 days after IS. To test whether IS animals were selectively resistant to the suppressive effects of DEX on the response to LPS, the ability of DEX to suppress HPA activity in response to a non-inflammatory stressor, exposure to an elevated "pedestal", was assessed. Again, DEX resistance was observed in IS animals. Finally, we examined whether changes in the responsivity to DEX were dependent upon the controllability of the stressor. The induction of DEX resistance was independent of the degree of behavioral control that the animal had over the stressor. Thus, a single session of IS induces DEX resistance of both HPA axis and cytokine responses measured *in vivo*.

© 2003 Elsevier Science Ltd. All rights reserved.

* Corresponding author. Tel.: +1-303-492-8892; fax: +1-303-492-2967.

E-mail address: koconnor@psych.colorado.edu (K.A. O'Connor).

Keywords: Stress; Lipopolysaccharide; Proinflammatory cytokines; Glucocorticoid

1. Introduction

Exposure to an intense acute stressor, such as inescapable tailshock (IS), often produces physiological changes that persist long after the termination of the acute event. For example, elevated basal corticosterone (CORT) levels and reduced corticosteroid binding globulin levels can be observed for 48–96 h following a single session of IS (Fleshner et al., 1995). Furthermore, IS has been shown to sensitize, or prime, both the pituitary-adrenal axis and the proinflammatory cytokine response to subsequent challenges (Johnson et al., 2002a,b). IS also produces an acute phase response, characterized by decreases in negative acute phase proteins and increases in positive acute phase proteins and core body temperature (Deak et al., 1997). The mechanism(s) by which IS produces these effects is unknown. Interestingly, glucocorticoid (GC) hormones negatively regulate aspects of the acute phase response, proinflammatory cytokine production/release and activity of the hypothalamic-pituitary-adrenal (HPA) axis itself (Lee et al., 1988; Morrow et al., 1993; Johnson et al., 1996; Pezeshki et al., 1996). Thus, a diminution of the negative feedback effects of GCs could explain several behavioral and physiological changes observed after IS, including the acute phase response, CORT basal shift, and sensitization of the proinflammatory cytokine and HPA axis response.

The cascade of events following peripheral administration of lipopolysaccharide (LPS; a component of the cell wall of gram negative bacteria) illustrates the negative feedback function of GCs. LPS induces an innate immune response marked by increased levels of circulating proinflammatory cytokines, such as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF), and IL-6 (Janeway and Travers, 1994). These pleiotropic molecules mediate many of the behavioral, physiological, and hormonal consequences of LPS administration. For example, proinflammatory cytokines have been implicated as mediators of HPA axis activation by LPS (Besedovsky et al., 1991; Derijk et al., 1991). As noted, GCs (CORT in the rat), the effector hormones of the HPA axis, act to inhibit both proinflammatory cytokine production/release and HPA axis activity, thereby limiting these responses and avoiding deleterious consequences that may occur if these systems operate unabated (Ramachandra et al., 1992; Buchman, 2001).

The effects of GCs are mediated by two intracellular receptors: the mineralocorticoid receptor (MR; or Type I receptor) and the glucocorticoid receptor (GR; or Type II receptor). Dexamethasone (DEX), a synthetic glucocorticoid, has been widely used to study GC effects on the functioning of the immune system and HPA axis. Controversy has surrounded the site at which DEX inhibits HPA axis activity (see Cole et al., 2000 for a review). Of relevance to the present experiments, low doses of DEX appear to exert their effects by selective activation of GR on peripheral (e.g. pituitary and spleen), rather than brain tissues (Miller et al., 1992; Cole et al., 2000).

Several studies have shown that a subset of patients with affective disorders, most

notably depression, is resistant to DEX (Murphy et al., 1991; Ribeiro et al., 1993; Modell et al., 1997). That is, the negative feedback effects of DEX are less pronounced in some patients with affective disorders. Furthermore, GC resistance appears in subsets of AIDS, Alzheimer's, asthma and leukemia patients, as well as in healthy elderly volunteers (Oxenkrug et al., 1983; Martignoni et al., 1990; Norbiato et al., 1992; Kaspers et al., 1994; Barnes et al., 1995). In fact, it has been postulated that resistance to the negative feedback effects of endogenous GCs could play a role in the origin and pathogenesis of many of these disorders (Nasman et al., 1995; DeRijk and Sternberg, 1997; Leung et al., 1998; Norbiato et al., 1998; Miller et al., 1999; Holsboer, 2000).

Experimentally, repeated or chronic stressor exposure has been shown to produce a state of functional GC resistance in which the effects of GC administration are absent or blunted in repeatedly stressed animals. Exposure to footshock on 2 consecutive days renders the CORT response to subsequent ether exposure on day 3 resistant to the suppressive effects of DEX (Greenberg et al., 1989). Additionally, after 5 consecutive days of LPS administration, adrenal activity in response to photic stimuli on day 7 is resistant to DEX suppression (Weidenfeld and Yirmiya, 1996). Furthermore, 2 consecutive days of social defeat block the suppressive effects of DEX on pituitary-adrenal activity induced by corticotropin releasing hormone (CRH) 7 and 14 days later (Buwalda et al., 1999). Recently, repeated social reorganization of mice has been reported to abolish the suppressive effects of CORT on splenocyte proliferation to a mitogen *in vitro* (Sheridan et al., 2000; Stark et al., 2001). In sum, at least 1 day following the final exposure to certain repeated stressors, components of both the HPA axis and immune system have been shown to be resistant to the effects of GCs.

IS produces several physiological changes that resemble effects of repeated stressor exposure. For example, elevated basal CORT levels and reduced corticosteroid binding globulin levels, which occur following IS (Fleshner et al., 1995), have also been reported following repeated exposure to stressors. The present experiments address the possibility that IS also produces GC resistance, another change reminiscent of repeated stressor exposure. As already noted, such GC resistance could explain many of the persistent effects of IS.

The following experiments were conducted to test the hypothesis that an acute stressor, IS, alters responsiveness of the HPA axis and proinflammatory cytokine system to DEX *in vivo*. First, we examined the ability of various doses of DEX to suppress proinflammatory cytokine and HPA activity in response to LPS 24 h after IS exposure. Upon finding a resistance to DEX in IS animals, we examined the duration of the altered response to DEX by testing animals 1, 4 and 21 days after IS. To examine whether IS animals were selectively resistant to the suppressive effects of DEX on the response to LPS, the ability of DEX to suppress HPA activity in response to exposure to an elevated "pedestal" was assessed. Finally, we examined whether these changes in the responsiveness to DEX were dependent upon the controllability of the stressor.

2. Materials and methods

2.1. Subjects

Adult male viral-free Harlan Sprague Dawley rats (Indianapolis, IN; 275–375 gm) were individually housed in hanging metal cages (24.5 cm × 19 cm × 17.5 cm) with standard rat chow and water available *ad libitum*. The colony room was maintained at 25°C on a 12-h light-dark cycle (lights on 0700). Rats were given at least 2 weeks to acclimatise to the colony prior to experimentation. All rats were briefly handled on the 3 days prior to experimentation. Care and use of the animals were in accordance with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

2.2. Inescapable tailshock treatment

The IS procedure was identical to that previously reported (Nguyen et al., 1998). Briefly, rats were restrained in Plexiglas tubes (23.4 cm long and 7 cm in diameter) and modified fuse-clip electrodes coated with electrode paste were securely fastened to their tails. Each rat received 100 5-s tail shocks (1.6 mA) once per min on a variable intertrial interval ranging from 30 to 90 s. Upon stressor termination, rats were returned to their home cages.

2.3. Drug treatment

Dexamethasone. DEX (Sigma) dissolved in propylene glycol was injected s.c. at doses ranging from 10–50 µg/kg. Control injections were equivolume (1 ml/kg) vehicle. DEX was administered 90 min prior to LPS administration or elevated pedestal exposure.

Lipopolysaccharide. LPS (*Escherichia coli*, 0111:B4; Sigma, Lot #17H4041) dissolved in sterile, endotoxin-free 0.9% saline vehicle was injected intraperitoneally at a dose of 10 µg/kg. Control injections were equivolume (1 ml/kg) vehicle.

2.4. Sample collection

For blood samples taken from the tail, a small nick was made in a lateral tail vein and the tail was caudally stroked until approximately 300 µl of blood was acquired in a microfuge tube. The entire sampling procedure (i.e. from the time that the cage was touched until collection was complete) was less than 2 min in order to minimize the stress to the animals. Sera were separated from whole blood by centrifugation and stored at –20°C until assayed.

For large blood samples (>300 µl), unanesthetized animals were decapitated. Approximately 10 ml of trunk blood were collected in a conical tube and stored on crushed ice until centrifugation. For collection of blood in which ACTH was to be measured, trunk blood was also collected in 10 ml conical tubes with 0.117 ml of 15% (K₃) ethylenedinitrilo tetraacetic acid (EDTA; Vacutainer). Sera were extracted

and stored at -20°C until assayed. For samples in which ACTH was to be measured, plasma was extracted not more than 1 h after collection and stored at -70°C until assayed.

2.5. *Limulus Amebocyte Lysate (LAL) assay*

Serum gram-negative bacterial endotoxin levels were measured by the LAL assay (BioWhittaker QCL-1000). The assay was performed according to the manufacturer's instructions. Briefly, samples diluted in LAL reagent water were mixed with LAL proenzyme. Endotoxin catalyzes the conversion of proenzyme to enzyme. After a brief incubation, substrate was added to each well. Finally, a 25% acetic acid solution was added to each well to stop the enzyme-substrate reaction. Absorbances were measured at 410 nm. In appropriate experiments, the LAL assay was used to verify LPS injections. Animals injected with LPS, but lacking detectable serum endotoxin levels were dropped from subsequent analyses.

2.6. *Adrenocorticotropin Hormone (ACTH) assay*

Plasma levels of ACTH were determined by radioimmunoassay. Plasma samples (50 μl) and ACTH standards (10–1000 pg/ml) were incubated overnight at 4°C with antiserum (rabbit antibody Rb7; courtesy of Dr. William Engeland, University of Minnesota) and 100 μl of [^{125}I] ACTH. Goat anti-rabbit IgG (Calbiochem) and normal rabbit serum were added and allowed to incubate for 30 min before the antibody-bound ACTH was separated from free ACTH with a PBS buffer containing 5% PEG. The bound fraction was counted with a gamma counter (Wallac, 1470 Wizard).

2.7. *Corticosterone (CORT) assay*

Serum CORT was measured by radioimmunoassay. Serum samples (20 μl) were diluted in 0.01M phosphate buffered saline (PBS; 1 ml) and heat inactivated for 1 h at 70°C . Samples and corticosterone standards (25–2000 pg/tube) were incubated overnight with antiserum (B3-163, Endocrine Sciences) and [$^3\text{-H}$] corticosterone (20 000 cpm/tube). Antibody-bound steroid was then separated from free steroid with 500 μl dextran-coated activated charcoal in 0.01M PBS. The bound fraction was mixed with scintillation cocktail and counted with a liquid scintillation analyzer (Packard, 1600TR).

2.8. *Interleukin-1 β and Interleukin-6 ELISAs*

IL-1 β and IL-6 levels were measured from serum using commercially available ELISA kits (R&D Systems). The assays were performed according to the manufacturer's instructions. The assays utilize goat anti-rat polyclonal antibody. Absorbances were read at 450 nm.

2.9. Statistics

In general, 2-way ANOVAs were performed on data collected from trunk blood samples. Repeated measures ANOVAs were used to analyze data collected from repeated tail-bleed samples. Post hoc tests were performed, when appropriate, by the Student–Newman–Keuls method.

3. Results

3.1. Experiments 1a and 1b—methods

Effects of IS on DEX-induced suppression of HPA activity and proinflammatory cytokine release in response to LPS. Experiments 1a and 1b were conducted identically with the exception of the dose of DEX employed. On day 1 of the experiments, rats either remained in their home cages as controls (HCC) or were exposed to IS. 24 h following HCC or IS treatment, animals were injected s.c. with DEX or vehicle (Veh). 90 min following DEX or Veh injection, all rats were injected i.p. with 10 µg/kg LPS. 2 h following LPS injection, rats were decapitated. Plasma from trunk blood was assayed for IL-1β, IL-6, ACTH, and CORT. In Experiment 1a, 39 rats (n=5–8/group) were randomly assigned to HCC-Veh, HCC-25 µg/kg DEX, HCC-50 µg/kg DEX, IS-Veh, IS-25 µg/kg DEX, or IS-50 µg/kg DEX groups. In Experiment 1b, 28 rats (n=6–8/group) were randomly assigned to HCC-Veh, HCC-10 µg/kg DEX, IS-Veh, or IS-10 µg/kg DEX groups.

3.2. Experiment 1a—results

Effects of IS on DEX-induced suppression of HPA activity and proinflammatory cytokine release in response to LPS. The results of Experiment 1a are shown in Figs. 1(a,b) and 2(a,b). DEX suppressed ACTH [$F(2,33) = 13.327, p < 0.0001$], IL-1β [$F(2,33) = 9.385, p < 0.001$], and IL-6 [$F(2,33) = 23.571, p < 0.0001$] responses to LPS. IS treatment did not alter the suppressive effects of DEX on any of these responses. Both the 25 and 50 µg/kg doses of DEX reduced the ACTH and IL-6 response to LPS. However, only the 50 µg/kg dose of DEX reduced the IL-1β response to LPS. Interestingly, while DEX maximally suppressed the CORT response to LPS in HCC rats, the drug was less effective in IS rats. ANOVA revealed significant effects of Stress [$F(1,33) = 14.572, p < 0.001$], Drug [$F(2,33) = 78.441, p < 0.0001$], and Stress × Drug interaction [$F(2,33) = 5.16, p < 0.05$]. The CORT response to LPS did not differ between HCC and IS treated animals that received vehicle or 50 µg/kg DEX injection. However, IS treatment impaired the ability of 25 µg/kg DEX to suppress the CORT response to LPS.

3.3. Experiment 1b—results

Effects of IS DEX-induced suppression of HPA activity and proinflammatory cytokine release in response to LPS. Since the doses of DEX used in Experiment 1a

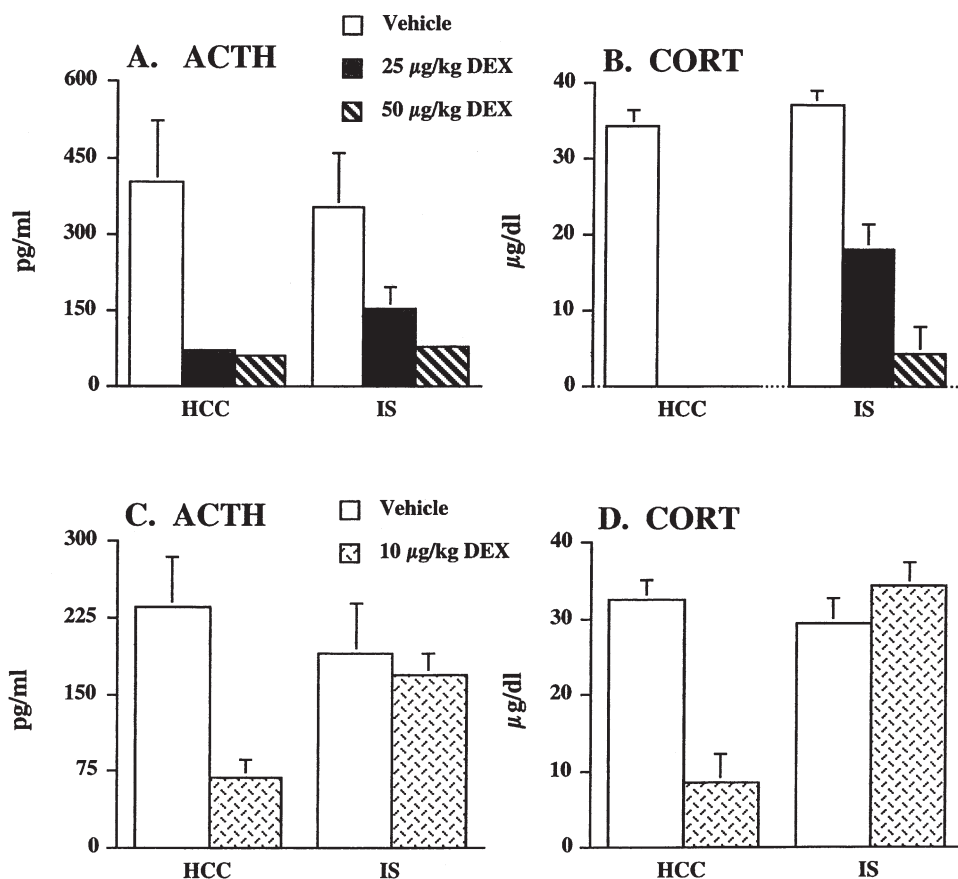


Fig. 1. ACTH (A and C) and CORT (B and D) levels (means \pm SEM) in response to LPS in high (A and B) and low (C and D) dose DEX pretreated home cage control (HCC) or inescapably shocked (IS) rats.

were completely suppressive on some responses, a lower dose of DEX was employed in Experiment 1b. 10 µg/kg DEX suppressed the ACTH, CORT, IL-1 β , and IL-6 responses to LPS in HCC treated animals. However, this dose of DEX did not suppress these same responses in IS treated animals (Figs. 1(c,d) and 2(c,d)). This resulted in significant Stress \times Drug interactions for ACTH [$F(1,24) = 4.557, p < 0.05$], CORT [$F(1,24) = 18.604, p < 0.0005$], IL-1 β [$F(1,24) = 6.961, p < 0.05$], and IL-6 [$F(1,24) = 6.253, p < 0.05$]. Post hoc tests indicate that DEX significantly blunted the ACTH, CORT, IL-1 β , and IL-6 responses to LPS in HCC, but not IS treated animals.

3.4. Experiments 2a, 2b and 2c—methods

Timecourse of DEX resistance after IS. In Experiments 2a, 2b and 2c rats were injected s.c. with 10 µg/kg DEX or Veh 1, 4, and 21 days, respectively, following

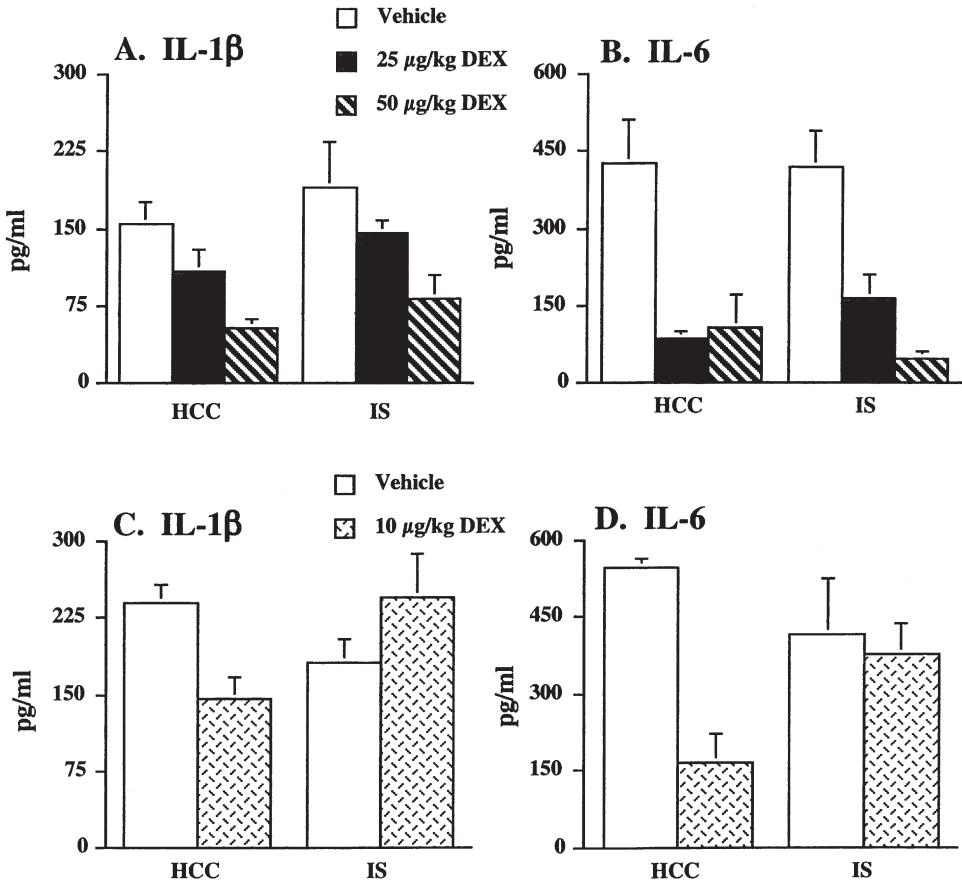


Fig. 2. IL-1 β (A and C) and IL-6 (B and D) levels (means \pm SEM) in response to LPS in high (A and B) and low (C and D) dose DEX pretreated home cage control (HCC) or inescapably shocked (IS) rats.

HCC or IS treatment. Ninety min following DEX or Veh injection, a baseline blood sample was taken and 10 μ g/kg LPS was injected i.p. Subsequent blood samples were taken 1, 2 and 3 h after LPS injection. Tailblood samples were processed and assayed for CORT levels. Immediately following the final blood sample, rats were decapitated and trunk blood was collected for cytokine analysis. In Experiment 2a, 27 rats (n=6–7/group) were tested for DEX resistance 1 day following HCC or IS treatment. In Experiment 2b, 26 rats (n=6–8/group) were tested for DEX resistance 4 days following HCC or IS treatment. In Experiment 2c, 32 rats (n=7–9/group) were tested for DEX resistance 21 days following HCC or IS treatment.

3.5. Experiment 2a—results

Timecourse of DEX resistance after IS—1 day post-IS. Consistent with prior results (Fleshner et al., 1995), IS treatment elevated basal CORT levels 24 h later (Fig.

3(a)). This resulted in a significant effect of Stress at the baseline timepoint [$F(1,24) = 18.052, p < 0.001$]. Furthermore, DEX suppressed basal CORT levels, resulting in a significant effect of Drug [$F(1,24) = 9.165, p < 0.01$]. IS treatment did not alter the ability of DEX to suppress basal CORT levels. Repeated measures ANOVA of the post-LPS timepoints revealed a significant Time \times Stress \times Drug interaction [$F(2,48) = 3.215, p < 0.05$]. In HCC-Veh animals, LPS administration elevated CORT levels 2 and 3 h after injection. DEX suppressed this response at both timepoints. As previously reported, IS animals exhibited a more rapid rise in CORT levels such that elevated CORT levels were detectable 1 h after LPS (Johnson et al., 2002a). Furthermore, DEX did not suppress the CORT response to LPS in IS treated animals at any timepoint (Fig. 3(a)). As in Experiment 1, IS altered the ability of DEX to suppress the IL-1 β and IL-6 response to LPS (Fig. 4(a,b)). This resulted in a significant Stress \times Drug interaction for both IL-1 β [$F(1,30) = 4.305, p <$

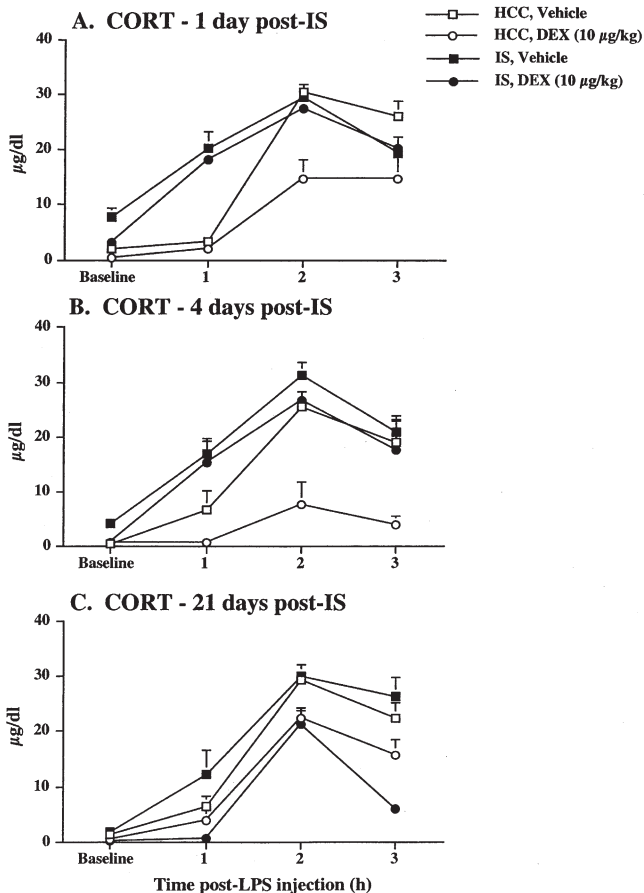


Fig. 3. LPS-induced CORT levels (means \pm SEM) in DEX pretreated home cage control (HCC) or inescapably shocked (IS) rats 1 (A), 4 (B), and 21 (C) d following stressor treatment.

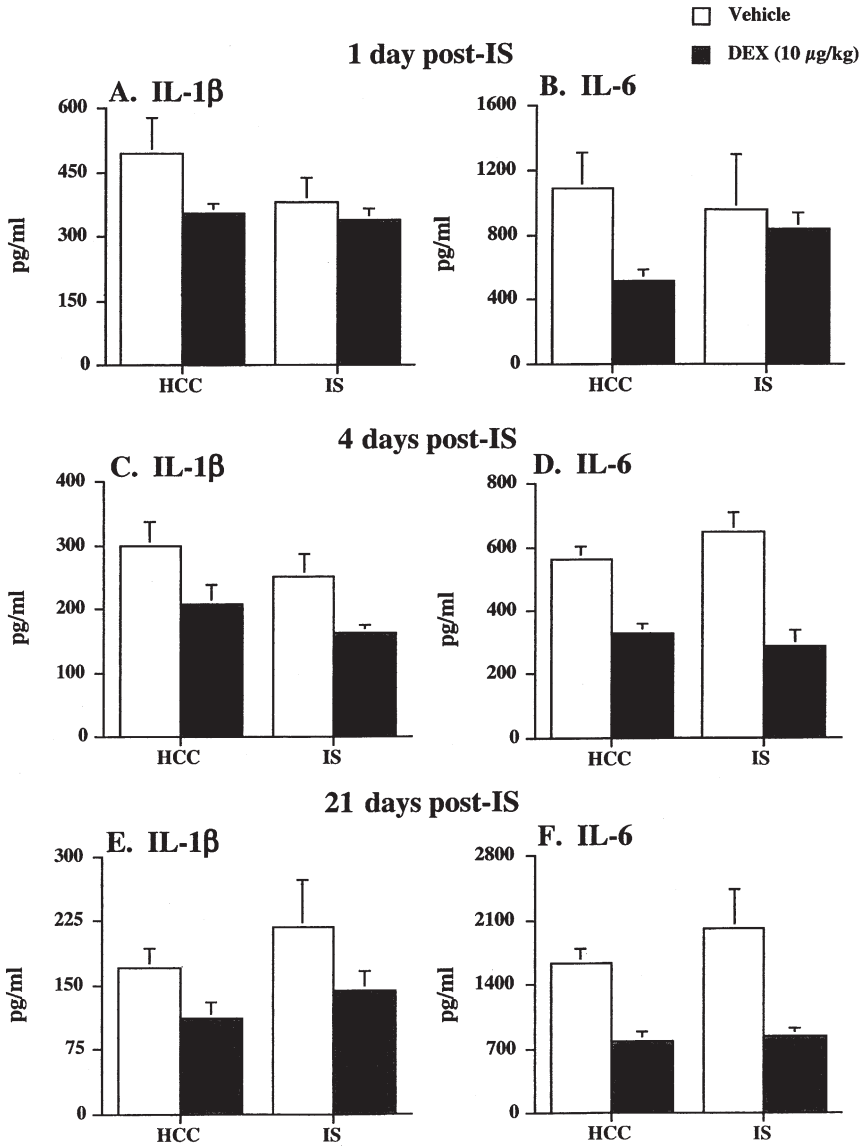


Fig. 4. LPS-induced IL-1 β (A, C and E) and IL-6 (B, D and F) (means \pm SEM) in DEX pretreated home cage control (HCC) or inescapably shocked (IS) rats 1 (A and B), 4 (C and D), and 21 (E and F) d following stressor treatment.

0.05] and IL-6 [$F(1,30) = 7.915, p < 0.01$]. Post hoc tests revealed that DEX significantly suppressed both the IL-1 β and IL-6 responses to LPS in HCC, but not IS animals.

3.6. Experiment 2b—results

Timecourse of DEX resistance after IS—4 days post-IS. Consistent with prior results (Fleshner et al., 1995), basal CORT levels were elevated 4 days after IS treatment (Fig. 3(b)). This resulted in a significant effect of Stress at the baseline timepoint [$F(1,23) = 5.318, p < 0.05$]. Furthermore, DEX suppressed basal CORT levels in IS treated animals, resulting in a significant Stress \times Drug interaction [$F(1,23) = 5.223, p < 0.05$]. The low basal CORT levels observed in HCC-Veh animals precluded interpretation of the effectiveness of DEX in suppressing basal CORT levels in HCC animals. Repeated measures ANOVA of the post-LPS timepoints revealed a significant Stress \times Drug interaction [$F(1,23) = 4.594, p < 0.05$]. In HCC-Veh animals, LPS administration elevated CORT levels. DEX suppressed this response. However, IS altered the ability of DEX to suppress the CORT response to LPS (Fig. 3(b)). Post hoc tests indicate that DEX suppressed the CORT response to LPS in HCC animals, but did not alter the CORT response to LPS in IS animals. DEX suppressed the IL-1 β [$F(1,22) = 7.432, p < 0.05$] and IL-6 [$F(1,23) = 37.152, p < 0.0001$] responses to LPS administration. IS treatment 4 days prior did not alter the ability of DEX to suppress these responses (Fig. 4(c,d)).

3.7. Experiment 2c—results

Timecourse of DEX resistance after IS—21 days post-IS. Basal CORT levels were not different between HCC and IS animals 21 days following treatment (Fig. 3(c)). This is consistent with the results of Fleshner et al. (1995), which show that the CORT basal shift persisted for up to 4 days after IS. DEX suppressed basal CORT levels, resulting in a significant effect of Drug [$F(1,28) = 7.794, p < 0.01$]. IS treatment did not alter the ability of DEX to suppress basal CORT levels. Repeated measures ANOVA of the post-LPS timepoints revealed significant effects of Drug [$F(1,28) = 22.903, p < 0.0001$] and Time [$F(2,56) = 123.864, p < 0.0001$]. CORT levels were elevated 2 and 3 h after LPS injection. IS treatment did not alter this response. Furthermore, DEX suppressed the CORT response to LPS and IS treatment did not alter this effect (Fig. 3(c)). DEX suppressed the IL-1 β [$F(1,28) = 6.758, p < 0.05$] and IL-6 [$F(1,23) = 22.04, p < 0.0001$] responses to LPS administration. IS treatment 21 days prior did not alter the ability of DEX to suppress these responses (Fig. 4(e,f)).

3.8. Experiment 3—methods

Effects of IS on serum DEX levels. On day 1, 13 rats (n=6–7/group) were exposed to HCC or IS treatment. 24 h later, a baseline blood sample was taken and 10 μ g/kg DEX was administered s.c. Subsequent blood samples were taken 30, 90, 150 and 210 min after DEX injection. Serum DEX levels were assessed using a commercially available DEX forensic kit (Neogen Corp.). The assay was performed according to the manufacturer's instructions. DEX (Sigma) was used to create a standard curve.

3.9. Experiment 3—results

Effects of IS on plasma DEX levels. Blood levels of DEX 30, 90, 150 and 210 min after injection are shown in Fig. 5. Measurable blood levels were present 30 min after injection and increased to the 210 min timepoint. This resulted in a significant effect of Time [$F(3,33) = 144.228, p < 0.0001$]. Repeated measures ANOVA also revealed a significant Stress \times Time interaction [$F(3,33) = 5.347, p < 0.0041$]. Post hoc tests indicate that IS treated animals had higher blood levels of DEX 210 min after injection. IS treatment did not alter blood levels of DEX at any other timepoint.

3.10. Experiment 4—methods

Effects of IS on the CORT response to ACTH challenge. 24 h following HCC or IS treatment, 32 animals ($n=4-6$ /group) were administered 100 $\mu\text{g}/\text{kg}$ DEX to suppress endogenous ACTH levels. 90 min following DEX administration, baseline blood samples were taken and animals were injected i.p. with 0.25, 0.8, or 2.5 IU/kg ACTH (Sigma) dissolved in sterile saline. Subsequent blood samples were taken 15, 30, 45 and 60 min post ACTH injection. Blood samples were processed and assayed for CORT levels.

3.11. Experiment 4—results

Effects of IS on the CORT response to ACTH challenge. CORT levels were assessed 15, 30, 45 and 60 min after ACTH administration (Fig. 6). ACTH administration elevated CORT levels [$F(2,26) = 6.636, p < 0.01$]. Post hoc analyses indicate

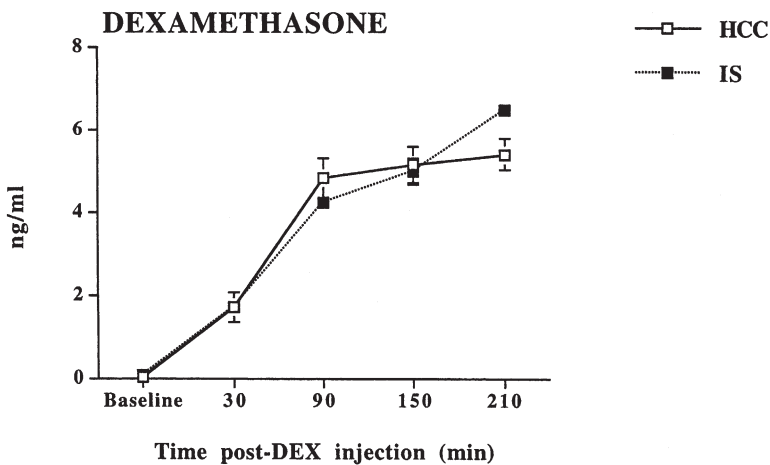


Fig. 5. Plasma DEX levels (means \pm SEM) following DEX injection in home cage control (HCC) or inescapably shocked (IS) rats 1 d following stressor treatment.

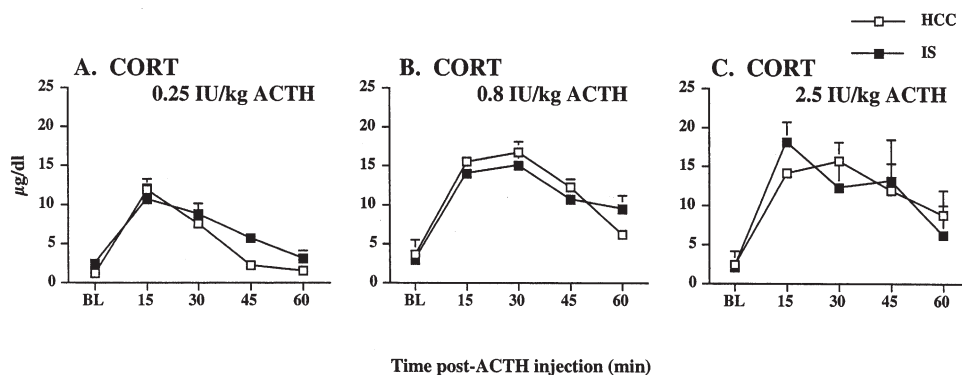


Fig. 6. Plasma CORT levels (means \pm SEM) following injection of 0.25 (A), 0.8 (B), and 2.5 (C) IU/kg ACTH in home cage control (HCC) or inescapably shocked (IS) rats 1 d following stressor treatment.

that the 0.8 IU/kg and 2.5 IU/kg doses induced a larger CORT response than the 0.25 IU/kg dose. IS treatment did not alter the CORT response to any dose of ACTH.

3.12. Experiment 5—methods

Effects of IS on DEX-induced suppression of the CORT response to elevated pedestal exposure. 49 rats ($n=5-7$ /group) were injected with 0, 1, 10, or 100 $\mu\text{g}/\text{kg}$ DEX 24 h after HCC or IS treatment. 90 min following injection, rats were placed on an elevated, uncovered square pedestal (33 cm^2 ; raised 61 cm above the ground). This procedure has previously been shown to elevate CORT levels (Pace et al., 2001). After 30 min, rats were removed from the pedestal and quickly decapitated. Trunk blood was collected for analysis of serum CORT levels.

3.13. Experiment 5—results

Effects of IS on DEX-induced suppression of the CORT response to elevated pedestal exposure. DEX dose-dependently suppressed the CORT response to elevated pedestal exposure, resulting in a significant effect of Drug [$F(3,41) = 46.651$, $p < 0.0001$]. ANOVA also revealed a significant Stress \times Drug interaction [$F(3,41) = 3.593$, $p < 0.05$]. Post hoc tests indicate that 1 $\mu\text{g}/\text{kg}$ DEX significantly suppressed the CORT response to elevated pedestal exposure in HCC, but not IS animals (Fig. 7). 10 and 100 $\mu\text{g}/\text{kg}$ DEX significantly suppressed the CORT response to elevated pedestal exposure in both HCC and IS treated animals. However, CORT levels from IS-100 $\mu\text{g}/\text{kg}$ DEX animals were significantly higher than those from HCC-100 $\mu\text{g}/\text{kg}$ DEX animals.

3.14. Experiment 6—methods

Effects of stressor controllability on DEX-induced suppression of HPA activity in response to LPS. Many consequences of IS are not present in animals exposed to

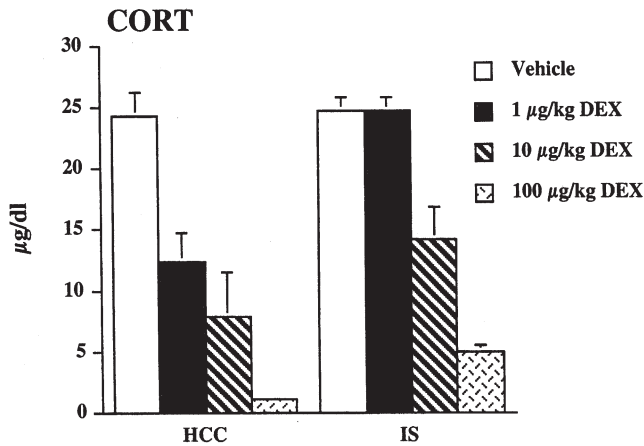


Fig. 7. CORT levels (means \pm SEM) in response to pedestal exposure in DEX pretreated home cage control (HCC) or inescapably shocked (IS) rats.

the same amount and duration of escapable shock (ES; Short and Maier, 1993; Amat et al., 1998a,b; Will et al., 1998). To determine whether the induction of DEX resistance depended upon stressor controllability, 43 rats ($n=6-8$ /group) were exposed to HCC, ES, or IS. The ES-IS procedure was identical to that previously described. Briefly, rats were restrained in Plexiglas wheel turn chambers and modified fuse-clip electrodes coated with electrode paste were securely fastened to the tail. Each rat received 100 tail shocks (1.0 mA) once per min on a variable intertrial interval ranging from 30 to 90 s. Each shock could be terminated by the rat turning a small wheel in the front wall of the chamber as previously described. Rats in the IS treatment group were paired (yoked) with a rat in the ES group and received the same intensity, duration, and pattern of shock. After termination of the stressor, rats were returned to their home cages.

24 h following the IS-ES procedure, rats were injected s.c. with 10 $\mu\text{g}/\text{kg}$ DEX or Veh. 90 min following the DEX or Veh injection, all rats were injected i.p. with 10 $\mu\text{g}/\text{kg}$ LPS. Rats were decapitated 2 h following LPS injection and trunk blood was collected for analysis of ACTH and CORT levels.

3.15. Experiment 6—results

Effects of stressor controllability on DEX-induced suppression of HPA activity in response to LPS. 10 $\mu\text{g}/\text{kg}$ DEX suppressed the ACTH and CORT responses to LPS in HCC treated animals. However, this dose of DEX was incapable of suppressing these same responses in IS and ES treated animals (Fig. 8). This resulted in significant Stress \times Drug interactions for ACTH [$F(2,37) = 5.635$, $p < 0.01$] and CORT [$F(2,37) = 8.317$, $p < 0.001$]. Post hoc tests indicate that DEX significantly blunted the ACTH and CORT responses to LPS in HCC, but not IS nor ES treated animals.

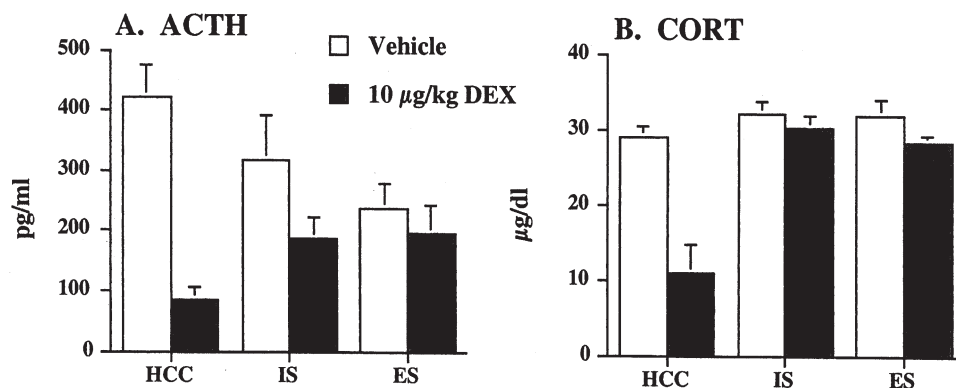


Fig. 8. ACTH (A) and CORT (B) levels (means \pm SEM) in response to LPS in DEX pretreated home cage control (HCC), escapably shocked (ES), or inescapably shocked (IS) rats.

4. Discussion

On a procedural note, DEX was selected as the ligand in these studies to allow for the measurement of endogenous corticosterone. The extrapolation of these results to other GCs (including CORT) is hindered by a few considerations, including differences in tissue access and receptor binding affinities between DEX and other GCs. Nonetheless, the present experiments show that exposure to a single session of IS induces a state of functional DEX resistance. Prior IS exposure impaired DEX-induced suppression of the proinflammatory cytokine response to LPS challenge. Furthermore, a single session of IS reduced the inhibitory effects of DEX on HPA axis activity (as assessed by circulating CORT levels) to either pedestal exposure or LPS challenge. Since pedestal exposure does not change brain or circulating cytokine levels (unpublished observations), the inability of DEX to suppress HPA activity most likely does not depend on the inability of DEX to suppress the proinflammatory cytokine response in IS animals. Suppression of the HPA axis by DEX remained altered up to 4 days after IS, while suppression of circulating proinflammatory cytokine levels returned to normal within 4 days of IS, further suggesting that resistance of the HPA axis and proinflammatory cytokine system to DEX may be independent. Failure of IS to potentiate CORT release to ACTH challenge does not conclusively rule out the possibility that increased adrenal sensitivity to low levels of ACTH contribute to elevated CORT levels in IS animals in the face of DEX administration. However, the inability of DEX to suppress the ACTH response to LPS in IS and ES animals suggests that more than the sensitivity of the adrenal gland to ACTH is altered. We also found that the resistance of the HPA axis to suppression by DEX was independent of the degree of behavioral control that the animal had over the shock session. Since escapably and inescapably shocked animals differ in their serotonergic responses to subsequent challenge (Amat et al., 1998a,b), the fact that ES and IS animals both showed DEX resistance suggests that these serotonergic alterations are not necessary for DEX resistance.

The induction of DEX resistance by IS is consistent with several behavioral and physiological consequences of IS exposure. IS produces many changes that are similar to those which occur during immune challenge or sickness, including acute phase responses, such as the emergence of sickness behaviors (e.g. decreased social interaction and food and water intake), alterations in acute phase proteins, and fever (Short and Maier, 1993; Deak et al., 1999). Additionally, IS primes the proinflammatory cytokine response to subsequent LPS challenge such that increases in proinflammatory cytokines are detectable earlier in animals previously exposed to IS (Johnson et al., 2002b). Furthermore, elevated basal CORT levels and a primed pituitary-adrenal axis can be observed for several days following a single exposure to IS (Fleshner et al., 1995; Johnson et al., 2002a). Since GCs negatively regulate several aspects of the acute phase response, sickness behaviors, transcription and translation of proinflammatory cytokines, and HPA axis activity, resistance to the effects of endogenous GCs could explain many changes observed after IS (Lee et al., 1988; Morrow et al., 1993; Johnson et al., 1996; Pezeshki et al., 1996).

As noted, GC resistance has been observed in many patients with affective disorders, AIDS, asthma and leukemia. Several mechanisms of GC resistance have been proposed, including decreased corticosteroid receptor expression, mutant receptors with low ligand binding affinity, impairments of heat shock proteins normally associated with unliganded corticosteroid receptors, and a hypothalamic-limbic system overdrive that overcomes suppression of the HPA axis by exogenous GCs (Amsterdam et al., 1989; Hala et al., 1996; Kojika et al., 1996; Leung et al., 1998). The resistance of proinflammatory cytokine secretion and HPA axis activity to DEX suppression in IS animals could have been produced by any of these mechanisms. In general, there may be increased drive on these systems or hyper(re)active counter-regulatory systems that interfere with DEX suppression. Conversely, there may be pharmacokinetic (e.g. increased DEX metabolism) or pharmacodynamic (e.g. alteration of GR number and/or function) related changes that alter tissue sensitivity to DEX. Since plasma concentrations of DEX did not differ between HCC and IS animals, altered metabolism of DEX most likely does not account for DEX resistance. Furthermore, although the effects of IS on cytokine secreting cell GR expression is unknown, previous studies have determined that IS does not alter expression of GR protein in the pituitary (Deak et al., 1999). Thus, if an IS-induced decrease in GC negative feedback leads to resistance of the HPA axis to DEX suppression, the decreased negative feedback is not the result of GR down regulation.

Systemically administered DEX penetrates CNS tissue poorly due to the presence of the multiple drug resistance gene encoded p-glycoprotein at the blood-brain barrier (Meijer et al., 1998). By acting as an extrusion pump, the p-glycoprotein limits access of DEX to the brain. Due to its low access to CNS tissue, small amounts of DEX (such as those used in the present studies) cannot effectively substitute for endogenous CORT at the level of the CNS. In response to reduced CORT at GC receptors in the CNS, there is an increase in secretion of central neuropeptides that are capable of activating ACTH secretion, mainly corticotropin releasing hormone (CRH) and arginine vasopressin (AVP). AVP synergizes with CRH to induce the release of ACTH from the anterior pituitary (Gillies et al., 1984) and several studies

have shown that both acute and chronic stressors can increase the percentage of CRH-containing neurons in the paraventricular nucleus of the hypothalamus that coexpress AVP (de Goeij et al., 1992; Tilders et al., 1993; van Dijken et al., 1993; Makino et al., 1995; Schmidt et al., 1995, 1996; Aubry et al., 1999). Of relevance to the present experiments, DEX resistance in aged animals has been attributed to the enhanced activity of hypothalamic AVP neurons (Hatzinger et al., 2000). Since GCs potently inhibit AVP production (Kovacs et al., 2000), this type of increased drive may ultimately reflect impaired central GC negative feedback. However, due to the peripheral site of action of DEX, the present experiments do not address whether IS does indeed induce central GC resistance.

IS-induced alterations in macrophage migration inhibitory factor (MIF) represent a particularly interesting possible mechanism to account for DEX resistance. MIF is a proinflammatory cytokine that antagonizes the anti-inflammatory effects of GCs. For example, MIF dose dependently reverses DEX induced suppression of the proinflammatory response to LPS *in vitro* (Calandra and Bucala, 1997; Bucala, 1998). Furthermore, low levels of GCs induce MIF production, while high to pharmacological levels of GCs inhibit MIF production (Calandra et al., 1995). Thus, MIF appears to be CORT inducible and act as a GC counter regulatory mechanism. Clearly any alteration that shifts the GC-MIF balance towards MIF would lead to GC resistance.

As noted previously, DEX resistance could arise from an impairment in the functioning of the peripheral GR. Recently a proinflammatory cytokine, IL-1 α , has been shown to impair DEX induced translocation of the GR to the nucleus (Pariante et al., 1999). Moreover, incubation of cells with IL-1 α led to a decrease in DEX induced GR mediated gene transcription (Pariante et al., 1999). Interestingly, IS exposure increases circulating and tissue levels of IL-1 β protein and administration of α MSH, a functional IL-1 antagonist, blocks the acute phase response and sickness behaviors induced by IS (Nguyen et al., 2000; Milligan et al., 1998). Whether this IL-1 β increase leads to impairments in GR function that underlie DEX resistance is unknown.

Although the present experiments do not implicate any particular mechanism, they do clearly demonstrate that a single session of a stressor can induce resistance to the effects of DEX as assessed by HPA and cytokine responses to LPS. Such GC resistance is capable of explaining a number of the enduring consequences that are produced by the same stressor, and may have implications for the understanding of a variety of stress-related disorders.

Acknowledgements

This work was supported, in part, by National Institute of Mental Health grants MH-45045, MH-00314, MH-55283 and MH-01558 and the Hughes Undergraduate Initiative.

References

- Amat, J., Matus-Amat, P., Watkins, L.R., Maier, S.F., 1998a. Escapable and inescapable stress differentially alter extracellular levels of 5-HT in the basolateral amygdala of the rat. *Brain Res.* 812, 113–120.
- Amat, J., Matus-Amat, P., Watkins, L.R., Maier, S.F., 1998b. Escapable and inescapable stress differentially and selectively alter extracellular levels of 5-HT in the ventral hippocampus and dorsal periaqueductal gray of the rat. *Brain Res.* 797, 12–22.
- Amsterdam, J.D., Maislin, G., Gold, P., Winokur, A., 1989. The assessment of abnormalities in hormonal responsiveness at multiple levels of the hypothalamic-pituitary-adrenocortical axis in depressive illness. *Psychoneuroendocrinology* 14, 43–62.
- Aubry, J.M., Bartanusz, V., Jezova, D., Belin, D., Kiss, J.Z., 1999. Single stress induces long-lasting elevations in vasopressin mRNA levels in CRF hypophysiotrophic neurones, but repeated stress is required to modify AVP immunoreactivity. *J. Neuroendocrinol.* 11, 377–384.
- Barnes, P.J., Greening, A.P., Crompton, G.K., 1995. Glucocorticoid resistance in asthma. *Am. J. Respir. Crit. Care Med.* 152, S125–140.
- Besedovsky, H.O., del Rey, A., Klusman, I., Furukawa, H., Monge Arditi, G., Kabiersch, A., 1991. Cytokines as modulators of the hypothalamus-pituitary-adrenal axis. *J. Steroid Biochem. Mol. Biol.* 40, 613–618.
- Bucala, R., 1998. Neuroimmunomodulation by macrophage migration inhibitory factor (MIF). *Ann. N.Y. Acad. Sci.* 840, 74–82.
- Buchman, A.L., 2001. Side effects of corticosteroid therapy. *J. Clin. Gastroenterol.* 33, 289–294.
- Buwald, B., de Boer, S.F., Schmidt, E.D., Felszeghy, K., Nyakas, C., Sgoifo, A., Van der Vegt, B.J., Tilders, T.J., Bohus, B., Koolhaas, J.M., 1999. Long-lasting deficiency dexamethasone suppression of hypothalamic-pituitary-adrenocortical activation following peripheral CRF challenge in socially defeated rats. *J. Neuroendocrinol.* 11, 513–520.
- Calandra, T., Bucala, R., 1997. Macrophage migration inhibitory factor (MIF): a glucocorticoid counter-regulator within the immune system. *Crit. Rev. Immunol.* 17, 77–88.
- Calandra, T., Bernhagen, J., Metz, C.N., Spiegel, L.A., Bacher, M., Donnelly, T., Cerami, A., Bucala, R., 1995. MIF as a glucocorticoid-induced modulator of cytokine production. *Nature* 377, 68–71.
- Cole, M.A., Kim, P.J., Kalman, B.A., Spencer, R.L., 2000. Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. *Psychoneuroendocrinology* 25, 151–167.
- de Goeij, D.C., Jezova, D., Tilders, F.J., 1992. Repeated stress enhances vasopressin synthesis in corticotropin releasing factor neurons in the paraventricular nucleus. *Brain Res.* 577, 165–168.
- Deak, T., Nguyen, K.T., Cotter, C.S., Fleshner, M., Watkins, L.R., Maier, S.F., Spencer, R.L., 1999. Long-term changes in mineralocorticoid and glucocorticoid receptor occupancy following exposure to an acute stressor. *Brain Res.* 847, 211–220.
- Deak, T., Meriwether, J.L., Fleshner, M., Spencer, R.L., Abouhamze, A., Moldawer, L.L., Grahm, R.E., Watkins, L.R., Maier, S.F., 1997. Evidence that brief stress may induce the acute phase response in rats. *Am. J. Physiol.* 273, R1998–2004.
- DeRijk, R., Sternberg, E.M., 1997. Corticosteroid resistance and disease. *Ann. Med.* 29, 79–82.
- Derijk, R., Van Rooijen, N., Tilders, F.J., Besedovsky, H.O., Del Rey, A., Berkenbosch, F., 1991. Selective depletion of macrophages prevents pituitary-adrenal activation in response to subpyrogenic, but not to pyrogenic, doses of bacterial endotoxin in rats. *Endocrinology* 129, 330–338.
- Fleshner, M., Deak, T., Spencer, R.L., Laudenslager, M.L., Watkins, L.R., Maier, S.F., 1995. A long-term increase in basal levels of corticosterone and a decrease in corticosteroid-binding globulin after acute stressor exposure. *Endocrinology* 136, 5336–5342.
- Gillies, G.E., Puri, A., Linton, E.A., Lowry, P.J., 1984. Comparative chromatography of hypothalamic corticotrophin-releasing factors. *Neuroendocrinology* 38, 17–24.
- Greenberg, L., Edwards, E., Henn, F.A., 1989. Dexamethasone suppression test in helpless rats. *Biol. Psychiatry* 26, 530–532.
- Hala, M., Hartmann, B.L., Bock, G., Geley, S., Kofler, R., 1996. Glucocorticoid-receptor-gene defects and resistance to glucocorticoid-induced apoptosis in human leukemic cell lines. *Int. J. Cancer* 68, 663–668.

- Hatzinger, M., Wotjak, C.T., Naruo, T., Simchen, R., Keck, M.E., Landgraf, R., Holsboer, F., Neumann, I.D., 2000. Endogenous vasopressin contributes to hypothalamic-pituitary-adrenocortical alterations in aged rats. *J. Endocrinol.* 164, 197–205.
- Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23, 477–501.
- Janeway, C.A., Travers, P., 1994. Host defense against infection. In: Janeway, C.A., Travers, P. (Eds.), *Immunobiology*. Garland Publishing, Inc, New York.
- Johnson, J.D., O'Connor, K.A., Deak, T., Spencer, R.L., Watkins, L.R., Maier, S.F., 2002a. Prior stressor exposure primes the HPA axis. *Psychoneuroendocrinology* 27, 353–365.
- Johnson, J.D., O'Connor, K.A., Deak, T., Stark, M., Watkins, L.R., Maier, S.F., 2002b. Prior stressor exposure sensitizes LPS-induced cytokine production. *Brain Behavior and Immunity* (in press).
- Johnson, R.W., Propes, M.J., Shavit, Y., 1996. Corticosterone modulates behavioral and metabolic effects of lipopolysaccharide. *Am. J. Physiol.* 270, R192–198.
- Kaspers, G.J., Pieters, R., Klumper, E., De Waal, F.C., Veerman, A.J., 1994. Glucocorticoid resistance in childhood leukemia. *Leuk. Lymphoma* 13, 187–201.
- Kojika, S., Sugita, K., Inukai, T., Saito, M., Iijima, K., Tezuka, T., Goi, K., Shiraiishi, K., Mori, T., Okazaki, T., Kagami, K., Ohshima, K., Nakazawa, S., 1996. Mechanisms of glucocorticoid resistance in human leukemic cells: implication of abnormal 90 and 70 kDa heat shock proteins. *Leukemia* 10, 994–999.
- Kovacs, K.J., Foldes, A., Sawchenko, P.E., 2000. Glucocorticoid negative feedback selectively targets vasopressin transcription in parvocellular neurosecretory neurons. *J. Neurosci.* 20, 3843–3852.
- Lee, S.W., Tsou, A.P., Chan, H., Thomas, J., Petrie, K., Eugui, E.M., Allison, A.C., 1988. Glucocorticoids selectively inhibit the transcription of the interleukin 1 beta gene and decrease the stability of interleukin 1 beta mRNA. *Proc. Natl. Acad. Sci. USA* 85, 1204–1208.
- Leung, D.Y., de Castro, M., Szefer, S.J., Chrousos, G.P., 1998. Mechanisms of glucocorticoid-resistant asthma. *Ann. NY Acad. Sci.* 840, 735–746.
- Makino, S., Smith, M.A., Gold, P.W., 1995. Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor mRNA levels. *Endocrinology* 136, 3299–3309.
- Martignoni, E., Petraglia, F., Costa, A., Bono, G., Genazzani, A.R., Nappi, G., 1990. Dementia of the Alzheimer type and hypothalamus-pituitary-adrenocortical axis: changes in cerebrospinal fluid corticotropin releasing factor and plasma cortisol levels. *Acta Neurol. Scand.* 81, 452–456.
- Meijer, O.C., de Lange, E.C., Breimer, D.D., de Boer, A.G., Workel, J.O., de Kloet, E.R., 1998. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in *mdr1A* P-glycoprotein knockout mice. *Endocrinology* 139, 1789–1793.
- Miller, A.H., Pariante, C.M., Pearce, B.D., 1999. Effects of cytokines on glucocorticoid receptor expression and function. Glucocorticoid resistance and relevance to depression. *Adv. Exp. Med. Biol.* 461, 107–116.
- Miller, A.H., Spencer, R.L., Pulera, M., Kang, S., McEwen, B.S., Stein, M., 1992. Adrenal steroid receptor activation in rat brain and pituitary following dexamethasone: implications for the dexamethasone suppression test. *Biol. Psychiatry* 32, 850–869.
- Milligan, E.D., Nguyen, K.T., Deak, T., Hinde, J.L., Fleshner, M., Watkins, L.R., Maier, S.F., 1998. The long term acute phase-like responses that follow acute stressor exposure are blocked by alpha-melanocyte stimulating hormone. *Brain Res.* 810, 48–58.
- Modell, S., Yassouridis, A., Huber, J., Holsboer, F., 1997. Corticosteroid receptor function is decreased in depressed patients. *Neuroendocrinology* 65, 216–222.
- Morrow, L.E., McClellan, J.L., Conn, C.A., Kluger, M.J., 1993. Glucocorticoids alter fever and IL-6 responses to psychological stress and to lipopolysaccharide. *Am. J. Physiol.* 264, R1010–1016.
- Murphy, B.E., Dhar, V., Ghadirian, A.M., Chouinard, G., Keller, R., 1991. Response to steroid suppression in major depression resistant to antidepressant therapy. *J. Clin. Psychopharmacol.* 11, 121–126.
- Nasman, B., Olsson, T., Viitanen, M., Carlstrom, K., 1995. A subtle disturbance in the feedback regulation of the hypothalamic-pituitary-adrenal axis in the early phase of Alzheimer's disease. *Psychoneuroendocrinology* 20, 211–220.

- Nguyen, K.T., Deak, T., Owens, S.M., Kohno, T., Fleshner, M., Watkins, L.R., Maier, S.F., 1998. Exposure to acute stress induces brain interleukin-1 β protein in the rat. *J. Neurosci.* 18, 2239–2246.
- Nguyen, K.T., Deak, T., Will, M.J., Hansen, M.K., Hunsaker, B.N., Fleshner, M., Watkins, L.R., Maier, S.F., 2000. Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1 β protein response to acute stress. *Brain Res.* 859, 193–201.
- Norbiato, G., Bevilacqua, M., Vago, T., Clerici, M., 1998. Glucocorticoid resistance and the immune function in the immunodeficiency syndrome. *Ann. NY Acad. Sci.* 840, 835–847.
- Norbiato, G., Bevilacqua, M., Vago, T., Baldi, G., Chebat, E., Bertora, P., Moroni, M., Galli, M., Oldenburg, N., 1992. Cortisol resistance in acquired immunodeficiency syndrome. *J. Clin. Endocrinol. Metab.* 74, 608–613.
- Oxenkrug, G.F., Pomara, N., McIntyre, I.M., Branconnier, R.J., Stanley, M., Gershon, S., 1983. Aging and cortisol resistance to suppression by dexamethasone: a positive correlation. *Psychiatry Res.* 10, 125–130.
- Pace, T.W.W., Cole, M.A., Kalman, B.A., Ward, G., Spencer, R.L., 2001. Acute exposure to a novel stressor further reduces the habituated corticosterone response to restraint in rats. *Stress* 4, 319–331.
- Pariante, C.M., Pearce, B.D., Pisell, T.L., Sanchez, C.I., Po, C., Su, C., Miller, A.H., 1999. The proinflammatory cytokine, interleukin-1 α , reduces glucocorticoid receptor translocation and function. *Endocrinology* 140, 4359–4366.
- Pezeshki, G., Pohl, T., Schobitz, B., 1996. Corticosterone controls interleukin-1 β expression and sickness behavior in the rat. *J. Neuroendocrinol.* 8, 129–135.
- Ramachandra, R.N., Sehon, A.H., Berczi, I., 1992. Neuro-hormonal host defence in endotoxin shock. *Brain Behav. Immun.* 6, 157–169.
- Ribeiro, S.C., Tandon, R., Grunhaus, L., Greden, J.F., 1993. The DST as a predictor of outcome in depression: a meta-analysis. *Am. J. Psychiatry* 150, 1618–1629.
- Schmidt, E.D., Janszen, A.W., Wouterlood, F.G., Tilders, F.J., 1995. Interleukin-1-induced long-lasting changes in hypothalamic corticotropin-releasing hormone (CRH)—neurons and hyperresponsiveness of the hypothalamus-pituitary-adrenal axis. *J. Neurosci.* 15, 7417–7426.
- Schmidt, E.D., Binnekade, R., Janszen, A.W., Tilders, F.J., 1996. Short stressor induced long-lasting increases of vasopressin stores in hypothalamic corticotropin-releasing hormone (CRH) neurons in adult rats. *J. Neuroendocrinol.* 8, 703–712.
- Sheridan, J.F., Stark, J.L., Avitsur, R., Padgett, D.A., 2000. Social disruption, immunity, and susceptibility to viral infection. Role of glucocorticoid insensitivity and NGF. *Ann. NY Acad. Sci.* 917, 894–905.
- Short, K.R., Maier, S.F., 1993. Stressor controllability, social interaction, and benzodiazepine systems. *Pharmacol. Biochem. Behav.* 45, 827–835.
- Stark, J.L., Avitsur, R., Padgett, D.A., Campbell, K.A., Beck, F.M., Sheridan, J.F., 2001. Social stress induces glucocorticoid resistance in macrophages. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280, R1799–1805.
- Tilders, F.J., Schmidt, E.D., de Goeij, D.C., 1993. Phenotypic plasticity of CRF neurons during stress. *Ann. NY Acad. Sci.* 697, 39–52.
- van Dijken, H.H., de Goeij, D.C., Sutanto, W., Mos, J., de Kloet, E.R., Tilders, F.J., 1993. Short inescapable stress produces long-lasting changes in the brain-pituitary-adrenal axis of adult male rats. *Neuroendocrinology* 58, 57–64.
- Weidenfeld, J., Yirmiya, R., 1996. Effects of bacterial endotoxin on the glucocorticoid feedback regulation of adrenocortical response to stress. *Neuroimmunomodulation* 3, 352–357.
- Will, M.J., Watkins, L.R., Maier, S.F., 1998. Uncontrollable stress potentiates morphine's rewarding properties. *Pharmacol. Biochem. Behav.* 60 (3), 655–664.