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Prior stressor exposure primes the HPA axis

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Abstract

Exposure to stressors often alters the subsequent responsiveness of many systems. The present study tested whether prior exposure to inescapable tailshock (IS) alters the corticosterone (CORT) or adrenocorticotropin hormone (ACTH) response to either an injection of bacterial endotoxin (lipopolysaccharide; LPS) or subsequent placement on a pedestal. Rats were exposed to IS or remained as home cage controls (HCC). 1, 4, 10, or 21 days later animals were injected i.p. with either 10 µg/kg LPS or equivolume sterile saline. Prior IS significantly increased plasma CORT 1 h, but not 2 or 5 h after LPS, compared to controls 1, 4, and 10 days, but not 21 days after IS. Exposure to IS 24 h earlier also significantly increased plasma ACTH 1 h after LPS. Additional animals were placed on a pedestal 24 h after IS, and plasma CORT was measured 15, 30, and 60 min later. IS significantly increased plasma CORT 15 min after pedestal exposure, but not after 30 or 60 min. These results suggest that exposure to IS sensitizes the CORT and ACTH response to subsequent HPA activation. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Exposure to stressful events often results in long-lasting changes in the responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis. For example, repeated exposure to the same stressor (homotypic stress) often results in a progressive decrease in the response of the HPA axis (Sakellaris and Vernikos-Danellis, 1975; Borrell et al., 1980; Vernikos et al., 1982; Armario et al., 1984; Dobrakovova and Jurcovicova, 1984; Natelson et al., 1988; Pitman et al., 1988; De Boer et al., 1990; Hauger et al., 1990; Cole et al., 2000). At least in some cases this habituation may occur because repeated stress leads to a progressive decrease in the response of brain stem catecholaminergic neurons in the medullary A1/C1 group (Lachuer et al., 1994) that provide input to the paraventricular nucleus of the hypothalamus. On the other hand, sensitization of neural pathways also is known to occur and often is observed when the organism is exposed to a test stressor that is different from that used during the initial repeated exposure (heterotypic stress). For example, prior exposure to chronic cold enhances HPA activity to ether (Vernikos et al., 1982) or peritoneal saline injection (Sakellaris and Vernikos-Danellis, 1975).

Stress-induced sensitization of neuronal pathways is of particular interest since it has been implicated in the pathogenesis of psychiatric disorders such as drug psychosis, panic, anxiety, post-traumatic stress, and depressive disorder (Shore et al., 1986; Engdahl et al., 1997; Brown et al., 1999; Agid et al., 2000; Goenjian et al., 2000). It is thought that cross-sensitization may occur between stressors and other stimuli if they activate a common neuronal pathway. For example, this process has been implicated in drug addiction because stressors and drugs of abuse activate overlapping neural circuitry (Antelman et al., 1980; Leyton and Stewart, 1990), and has been argued to be the mechanism by which stressors enhance the rewarding properties of drugs (Piazza and Le Moal, 1998).

It has also been suggested that stressors and activation of the immune system lead to the stimulation of common neuronal pathways (Dunn and Welch, 1991; Dunn et al., 1999). Activation of the innate or non-specific immune system results in the production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin-1 (IL-1) and IL-6 by phagocytic cells (Janeway et al., 1999). During infection these pro-inflammatory cytokines not only stimulate inflammation of the infected site but also signal the brain, leading to activation of regions involved in neurally mediated components of host defense (Dunn, 1993; Brady et al., 1994). Activation of the HPA axis is one such response. Elevated plasma glucocorticoids feed back to inhibit the synthesis and release of pro-inflammatory cytokines which protects an organism from septic shock during an inflammatory response (Bertini et al., 1988; Butler et al., 1989; Parant et al., 1991).

Cross-sensitization of the HPA axis between stressors and immune stimuli has been observed. A single exposure to IL-1 results in enhanced HPA activity 11 days later upon subsequent exposure to footshocks (Schmidt et al., 1995). While exposure to chronic stress or an acute injection of IL-1 results in sensitization of the HPA axis after a period of several days, more rapid changes in HPA function have also been reported. For example, an intraperitoneal injection of TNF- α enhances HPA

activity upon a second challenge with TNF- α 1 day later (Hayley et al., 1999). These various observations have made it clear that there are multiple mechanisms of sensitization. Some of the sensitization effects develop slowly following presentation of the sensitizing agent, and are not present until several days to weeks later. In contrast, other sensitization phenomena develop quickly, and the very same event can induce both rapid and delayed sensitization, depending on the response to the event that is measured (Hayley et al., 1999). In addition, cross-sensitization can occur between stimuli that activate overlapping pathways (Antelman et al., 1980; Leyton and Stewart, 1990).

While it has been observed that cytokines can sensitize the HPA axis to subsequent stressors such as footshocks, it is not known whether the reverse is also true, namely whether a stressor would sensitize the HPA response to a subsequent immune challenge. In the present experiments we investigated whether exposure to an acute session of inescapable tailshock (IS) would sensitize the HPA response to an injection of bacterial cell wall (lipopolysaccharide; LPS) 1, 4, 10, or 21 days later. Plasma corticosterone (CORT) was measured 1, 2, and 5 h following i.p. administration of 10 $\mu\text{g}/\text{kg}$ LPS. Plasma adrenocorticotropin hormone (ACTH) was measured 1 h after injection of LPS in rats exposed to IS 24 h earlier. To determine if changes in HPA responsivity are specific to immune activation, additional animals were exposed to an elevated platform (pedestal) 24 h after exposure to IS. Plasma CORT was measured 15, 30, and 60 min after placement on the pedestal and plasma ACTH was measured 15 min after pedestal exposure.

2. Materials and methods

2.1. Subjects

Adult male Sprague Dawley rats (275–325 gms; Harlan Sprague Dawley, Inc., Indianapolis, IN) were individually housed in suspended wire cages (24.5 \times 19 \times 17.5 cm) with food and water available ad libidum. Colony conditions were maintained at 22°C on a 12-h light, 12-h dark cycle (lights on, 0700–1900 h). Rats were given at least two weeks to habituate to the colonies before experimentation. Care and use of animals were in accordance with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

2.2. Shock — stress protocol

Animals either remained in their home cages as controls (HCC) or were placed in Plexiglas tubes (23.4 cm length \times 7 cm width) and exposed to 100 5-s, 1.6-mA inescapable tailshocks (IS), with an average intertrial interval of 60 s. All stress procedures occurred between 0800–1000 h. After stressor termination, rats were returned to their home cages.

2.3. LPS administration

1, 4, 10, or 21 days after exposure to IS or serving as HCC, animals were injected i.p. with either 10 µg/kg LPS (*Escherichia coli* endotoxin 0111:B4, Sigma lot#17H4041) or equal volume sterile, endotoxin-free saline (Abbott Laboratories, North Chicago, IL.).

2.4. Pedestal — stress protocol

One day after exposure to IS or serving as HCC, animals were either placed on an elevated platform (32.4 cm square with a 2 cm lip, 64 cm above the ground) for 15, 30, or 60 min or remained in their home cage.

2.5. Plasma collection

In some experiments animals were decapitated either 60 min after administration of LPS or saline or immediately after 15, 30, or 60 min of pedestal stress. Trunk blood was collected in EDTA coated tubes for later measurement of ACTH and non-EDTA coated tubes for later measurement of corticosterone and endotoxin. Tubes were stored on ice and spun immediately in a refrigerated centrifuge. Plasma was aliquoted and stored at -80°C until time of assay.

2.6. Serial blood sampling procedure

In experiments in which serial blood samples were taken, baseline (BL) blood samples were obtained immediately prior to the administration of LPS or saline and blood samples were taken 60, 120, and 300 min later. To obtain blood samples, the rat was removed from its home cage, gently wrapped in a towel, and lightly restrained with a Velcro strap. The tail was exposed and a small nick was made in a lateral tail vein with a scalpel (no. 15 blade), and the tail gently stroked until a volume of approximately 200–300 µl of whole blood was obtained in microfuge tubes. The entire sampling procedure was accomplished within 2 min of approaching the cage. Samples were immediately spun in a refrigerated centrifuge, and plasma was aliquoted and stored at -20°C until the time of assay.

2.7. Measurement of plasma endotoxin

Plasma levels of endotoxin were determined by an enzymatic assay, according to the procedure outlined by Bio-Whittaker (cat# 50–648U; Walkersville, MD). The detection limit of the assay is 0.02 EU/ml. Plasma was diluted 1:10 for saline injected animals or 1:100 for LPS injected animals. Animals that were injected with LPS, but had no detectable levels of plasma endotoxin, also had no increase in plasma or brain cytokine levels compared to saline injected controls. Presumably, injections were made into an internal organ, which resulted in no detectable immune response. Therefore, these animals were eliminated from the study. Approximately 10% of the

animals were eliminated from the study due to no detectable endotoxin and were evenly distributed between groups.

2.8. *Measurement of plasma corticosterone*

Total plasma CORT levels were measured by RIA. Plasma samples (20 μ l) were diluted in 0.01 M PBS and heat inactivated for 1 h at 75°C. Samples and corticosterone standards (25–2000 pg/tube) were incubated overnight with antiserum (rabbit antibody B21–42; Endocrine Sciences, Inc., Tarzana, CA) and [3-H] corticosterone (20,000 cpm/tube). Antibody-bound steroid was separated from free steroid with dextran-coated activated charcoal. The assay sensitivity was approximately 0.5 μ g/ml for a 20 μ l plasma sample. Interassay and intraassay coefficients of variation were less than 9%.

2.9. *Measurement of plasma ACTH*

Plasma levels of ACTH were determined by RIA. Plasma samples (50 μ l) and ACTH standards (15.6–1000 pg/ml) were incubated overnight at 4°C with antiserum (rabbit antibody Rb7; courtesy of Dr. William Engeland, University of Minnesota) and 100 μ l of [¹²⁵I] ACTH. 100 μ l of goat anti-rabbit IgG (Calbiochem, La Jolla, CA, Cat # 539844) and 100 μ l of normal rabbit serum (Vector Laboratories, Burlingame, CA, Cat # S-5000) was added and allowed to incubate for 30 min before adding 2 ml of 5% polyethylene glycol (Sigma). Tubes were spun for 30 min at 4000 rpm at 4°C, decanted and pelleted radioactivity was measured using a gamma counter. The assay sensitivity was approximately 10 pg/ml for a 50 μ l plasma sample.

2.10. *Statistics*

Due to size and manageability, the experiments examining the corticosterone response 1, 4, 10, and 21 days after IS were run as separate experiments with their own controls, and analyzed using a 2 \times 2 \times 4 repeated measure ANOVA between stress condition (IS vs. HCC), drug administration (saline vs. LPS) and time (0, 60, 120, 300). The experiment examining the ACTH response 24 h after IS was analyzed using a 2 \times 2 ANOVA between stress condition (IS vs. HCC) and drug administration (saline vs. LPS). Post hoc analyses were done using a bonferonni corrected *t*-test.

3. Results

3.1. *Effects of prior stress on LPS induced plasma CORT*

In all experiments an injection of 10 μ g/kg LPS resulted in a significant increase in serum total CORT compared with time-matched saline injected controls. The maximum CORT response occurred 2 h after administration of LPS and started to return to basal levels by 5 h. Exposure to a single acute session of IS 24 h prior to

LPS administration resulted in a reliable increase in CORT compared with time-matched HCC values (Fig. 1A). A $2 \times 2 \times 4$ repeated measures ANOVA revealed a reliable interaction [$F(1,72)=3.73$; $P=0.015$] between time after LPS administration (0, 60, 120, 300 min), stress condition (IS vs. HCC), and drug administration (saline vs. LPS). As previously reported (Fleshner et al., 1995), exposure to IS results in a small increase in basal CORT values 24 h later. However, this small increase in CORT cannot explain the large differences observed between IS and HCC animals 60 min after injection of LPS as indicated by a second 2×2 ANOVA revealing a reliable interaction between stress condition (IS vs. HCC) and drug administration (saline vs. LPS) 60 min after injection of LPS [$F(1,24)=10.94$; $P=0.003$]. While the peak CORT response to 10 $\mu\text{g}/\text{kg}$ LPS did not differ between HCC and IS animals, CORT values may have reached maximum possible CORT values; therefore, lower concentrations of LPS and more timepoints would be needed to determine if IS would enhance peak CORT values.

We repeated the above experiment 4, 10, and 21 days after the IS session (Fig.

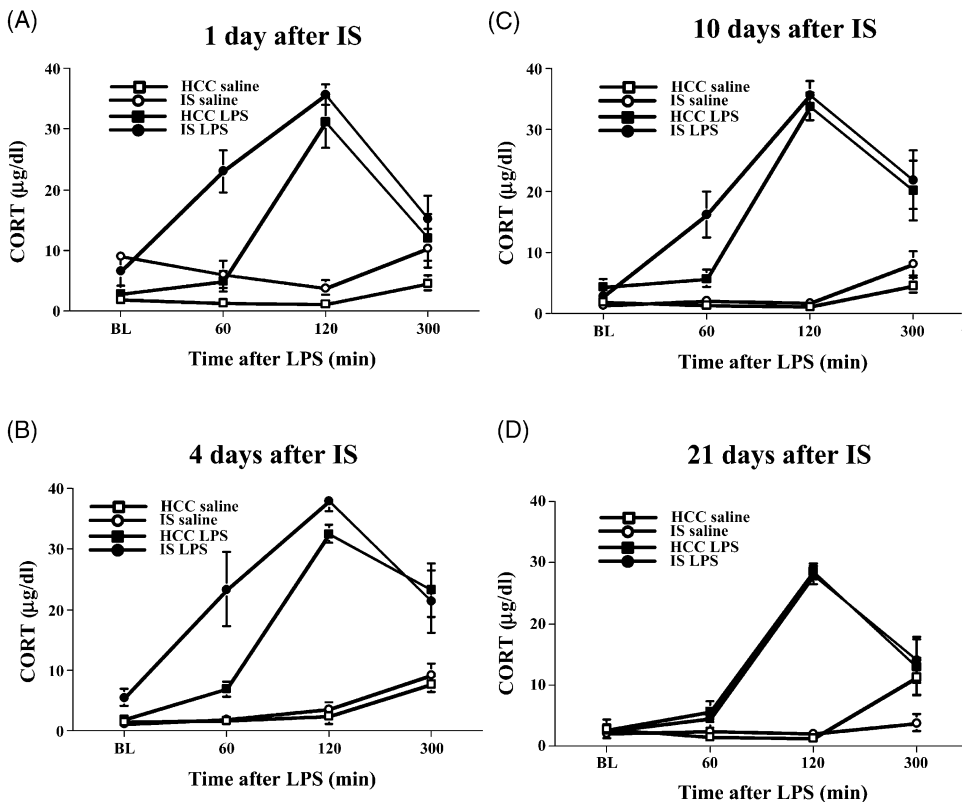


Fig. 1. Circulating plasma corticosterone 1, 2, and 5 h after administration of lipopolysaccharide (LPS) or saline in home cage control rats (HCC) or rats exposed to inescapable tailshock (IS) 1 day (A); 4 days (B); 10 days (C); or 21 days (D) prior. Data points represent means ($n=6-9$) plus standard errors.

1B–D). Both 4 and 10 days after the IS session there was a reliable interaction between stress condition (IS vs. HCC) and drug administration (saline vs. LPS) 60 min after the injection of LPS [$F(1,26)=9.66$; $P=0.005$ and $F(1,28)=5.57$; $P=0.026$, respectively]. The IS induced sensitization was no longer present 21 days after the shock session.

3.2. Effects of prior stress on LPS induced plasma ACTH

To examine the effects of prior exposure to IS on different levels of the HPA axis, animals were decapitated 60 min after an injection of LPS or saline and trunk blood collected and assayed for ACTH. Basal levels of ACTH were detectable in all animals and exposure to IS had no effect on basal ACTH levels 24 h later. However, exposure to a single acute session of IS 24 h prior to LPS administration resulted in a reliable increase in ACTH 60 min after LPS, compared with control values (Fig. 2). A 2×2 ANOVA revealed a reliable interaction [$F(1,28)=7.38$; $P=0.011$] between stress condition (IS vs HCC) and drug administration (saline vs. LPS). This indicates that sensitization of the CORT response does not occur solely at the level of the adrenal gland, but there is also a more rapid release of ACTH from the anterior pituitary.

3.3. Effects of prior stress on pedestal induced plasma CORT

Placing animals on a pedestal resulted in a significant increase in plasma CORT compared with home cage controls. Plasma CORT values reached peak levels between 15 and 30 min and started to return to basal levels by 60 min. Exposure to a single acute session of IS 24 h prior to exposure to the pedestal resulted in a

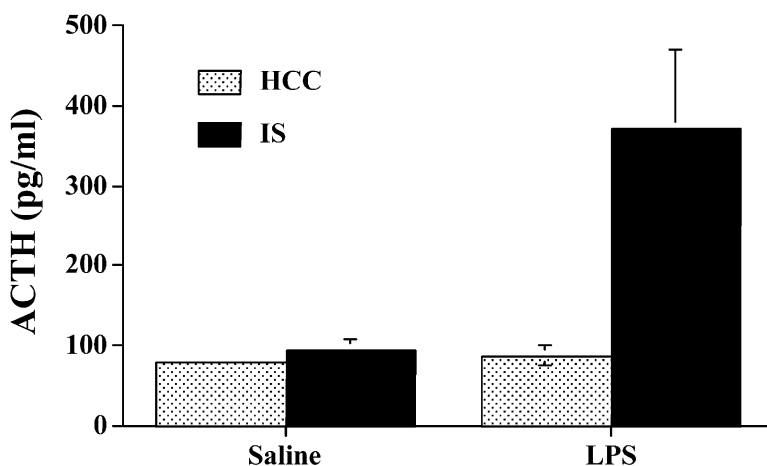


Fig. 2. Circulating plasma ACTH 1 h after administration of LPS or saline in HCC or rats exposed to IS 1 day prior. Data points represent means ($n=8$) plus standard errors.

reliable increase in CORT compared with time-matched HCC values (Fig. 3(A)). A 2×4 ANOVA revealed a reliable main effect of prior stress exposure (HCC vs IS) [$F(1,84)=15.96$; $P=0.0001$]. The interaction between prior stress exposure (HCC vs IS) and time on the pedestal (0, 15, 30, 60 min) was also reliable [$F(3,84)=4.768$; $P=0.004$]. Further analysis revealed statistical differences again in baseline CORT values ($P=0.003$) 24 h after exposure to IS and after placement on the pedestal for 15 min ($P=0.0003$), but not after 30 ($P=0.252$) or 60 ($P=0.727$) min of pedestal exposure.

3.4. Effects of prior stress on pedestal induced plasma ACTH

To examine the effects of prior exposure to IS on different levels of the HPA axis, additional animals were decapitated 15 min after placement on the pedestal and

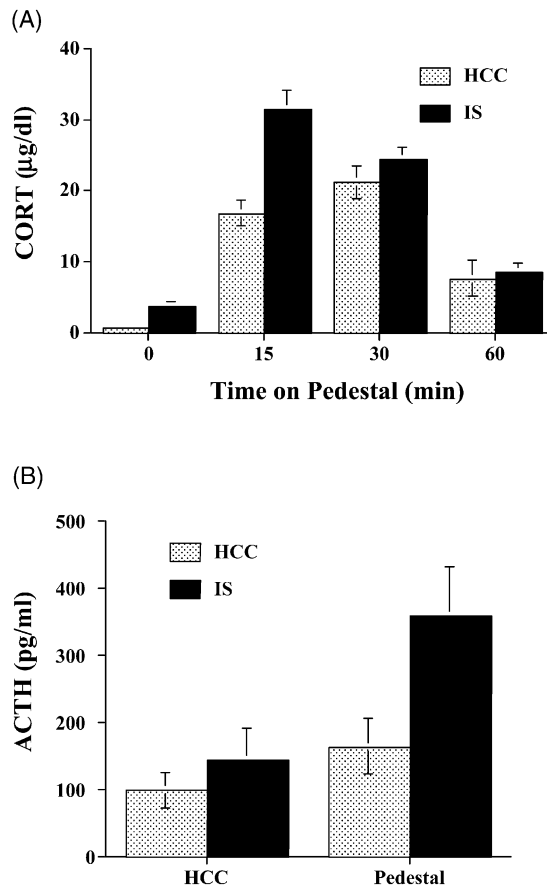


Fig. 3. Circulating plasma corticosterone 0, 15, 30, and 60 min (A); and plasma ACTH 15 min (B) after placement on a pedestal in HCC or rats exposed to IS 1 day prior. Data points represent means ($n=8-16$) plus standard errors.

trunk blood was collected and assayed for ACTH. 15 min of pedestal exposure increased ACTH. A 2×2 ANOVA revealed a reliable main effect [$F(1,27)=7.96$; $P=0.009$] of stress condition on day 2 (Ped vs HCC). Exposure to a single acute session of IS 24 h prior to pedestal exposure resulted in an increase in ACTH compared with time-matched HCC values (Fig. 3(B)). A 2×2 ANOVA revealed a reliable main effect [$F(1,27)=5.83$; $P=0.023$] of prior stress exposure on day 1 (IS vs HCC). Further analysis revealed that prior exposure to IS resulted in a significant increase in ACTH ($P=0.035$) when exposed to the pedestal compared to HCC. This indicates that sensitization of the CORT response does not occur solely at the level of the adrenal gland, but is also at the level of the anterior pituitary.

4. Discussion

In the present study we found that rats exposed to a single session of IS have a sensitized CORT response to subsequent challenge. Prior exposure to IS significantly increased the CORT response to either a subsequent immune challenge (LPS) or a psychological stressor (pedestal). In either case, the initial, rising phase of the CORT response was primed while the later phase of the CORT response remained unchanged. This suggests that IS does not just cause a shift in the time course of the CORT response upon subsequent challenge, but rather results in a longer period of time in which high levels of CORT are present and available to act at receptors. The sensitized CORT response appears 1 day after IS and, at least for immune stimulation, persists for 10 days, but not 21 days after IS.

A primed release of plasma ACTH was also observed in IS animals in response to LPS and pedestal challenge. This suggests that the primed CORT response to subsequent activation of the HPA axis is not mediated solely at the level of the adrenal gland, but also at least at the level of the anterior pituitary.

Activation of the HPA axis in response to a peripheral immune challenge depends on the production and release of both peripheral and central proinflammatory cytokines. Normally, an injection of LPS produces large increases in TNF- α , and virtually no increases in IL-1 β or IL-6 after 60 min (Bagby et al., 1994; Kakizaki et al., 1999; Hansen et al., 2000). Significant increases in IL-1 and IL-6 occur 90 min after an i.p. injection of 10 μ g/kg LPS (Hansen et al., 2000). Since these cytokines stimulate the HPA axis, CORT levels show a similar pattern; that is, little-to-no increase 60 min after LPS, small increases after 90 min, and maximum levels after 120 min (Hansen et al., 2000). We have recently shown that prior exposure to IS results in a more rapid release of proinflammatory cytokines upon subsequent LPS injection at the same dose as used in the present studies (Johnson et al., 2001). Animals previously exposed to IS had significantly increased TNF- α and IL-1 β 60 min after LPS injection compared to non-stressed controls. The primed cytokine response was present 1 and 4 days after IS but no longer occurred 10 days after IS. One possible explanation of why animals exposed to IS had a more rapid ACTH and CORT response to LPS in the present studies is that these animals have a more rapid cytokine response. However, while this might seem to be a plausible explanation for the

LPS-induced rapid ACTH and CORT response observed 1 and 4 days after IS, it cannot explain the sensitized CORT response 10 days after IS since the cytokine sensitization is then no longer present.

To further examine whether the primed ACTH and CORT response 1 day after IS is dependent on a more rapid cytokine response to the LPS an alternative, non-immune stimulus was used to activate the HPA axis. Pedestal stress was chosen because it does not resemble the previous stressor (confinement in a tube receiving shocks) and it does not produce a maximal CORT response, which would prevent possible increases from being observed. It is known that exposure to some stressors elevates plasma IL-6 (LeMay et al., 1990; Zhou et al., 1993) and plasma and brain IL-1 (Nguyen et al., 1998), and that these cytokines may play a role in activation of the HPA axis (Shintani et al., 1995). Since exposure to IS sensitizes the cytokine response to LPS (Johnson et al., 2001), it is possible that IS may also result in sensitized cytokine responses to exposure to subsequent stressors, thereby enhancing HPA activation. However, no increase in plasma IL-6 or IL-1 or brain IL-1 was detected at any timepoint after exposure to the pedestal (data not shown). The fact that the pedestal induces a primed HPA response without a cytokine response, and the fact that the sensitized cytokine response to LPS does not have the same time course as the sensitized CORT response, suggest that the HPA and cytokine sensitization are independent.

Another possible set of mechanisms involved in IS sensitization of the HPA axis center on hypothalamic peptides. Exposure to chronic stress can increase arginine vasopressin (AVP) receptors in the anterior pituitary (Aguilera et al., 1994) and enhance the stimulatory effects of AVP on the release of ACTH and CORT (Hashimoto et al., 1988; Aguilera et al., 1994). AVP is a well known secretagogue that potentiates the ACTH releasing effect of CRH (Gillies et al., 1982). In addition, challenge with LPS, IL-1, footshock, repeated restraint, or brain surgery increases AVP stores in the median eminence seven days later (Schmidt et al., 1996). After IL-1 administration, AVP upregulation has been shown to occur in CRH containing neurons within the paraventricular nucleus of the hypothalamus (Schmidt et al., 1995). Moreover, subsequent activation of the HPA axis during the time of increased AVP stores results in enhanced ACTH and CORT release (Schmidt et al., 1995). It is not known whether IS results in the upregulation of AVP and/or a change in AVP receptors, and if such a change would match the more rapid onset of the HPA sensitization observed in the data presented.

The present data add to the growing literature demonstrating sensitization of the HPA axis after exposure to a stressor. It has previously been shown that exposure to chronic stress, whether continuous or intermittent, results in primed HPA responses to novel, acute stressors. Moreover, prior work has utilized IL-1 β and TNF- α as sensitizing agents to subsequent HPA responses to the same cytokine and to footshock. The present study indicates that a single session of inescapable tailshock is sufficient to sensitize the HPA response for 10 days and that this sensitization is present one day after IS. Sensitization occurs between IS and a second stressor (pedestal), and cross-sensitization between IS and an immune stimulus (LPS). The present study suggests that after exposure to IS there is a more generalized sensitization

ation of the HPA axis such that any subsequent activation of this system results in primed ACTH and CORT release. These various observations have made it clear that there are multiple mechanisms of sensitization. Some of the sensitization effects develop slowly following presentation of the sensitizing agent, and are not present until several weeks later (Schmidt et al., 1995) while other sensitization phenomena develop quickly. Rapid and delayed sensitization have been argued to depend on different mechanisms (Tilders and Schmidt, 1999), and the sensitization between IS and pedestal and the cross-sensitization between IS and LPS seem to involve the more rapid sensitization mechanism.

Sensitization of the HPA axis has been argued to be of potential importance for understanding psychopathologies such as depression (Pariante et al., 1995), and the experience of stressful life events has been implicated in the etiology of anxiety and affective disorders (Hammen et al., 1992). Thus, it has been suggested that individuals exposed to a traumatic stressor might react to a stressor experienced during the period of sensitization in an exaggerated manner, thereby exacerbating the anxiogenic and depressogenic impact of the stressor. Thus, the sensitization and cross-sensitization demonstrated here, in which an initial exposure to a stressor exaggerates the HPA response to a subsequent stressors or immune challenge might also have implications for the etiology of anxiety or depression.

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