

## Prior Stressor Exposure Sensitizes LPS-Induced Cytokine Production

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Exposure to stressors often alters the subsequent responsiveness of many systems. The present study tested whether prior exposure to inescapable tailshock (IS) alters the interleukin (IL)-1 $\beta$ , tumor necrosis factor (TNF)- $\alpha$ , or IL-6 response to an injection of bacterial endotoxin (lipopolysaccharide; LPS). Rats were exposed to IS or remained as home cage controls (HCC); 24 h later animals were injected i.p. with either 10  $\mu$ g/kg LPS or equivalent volume sterile saline. IS significantly increased plasma TNF- $\alpha$ , IL-1 $\beta$ , and pituitary, hypothalamus, hippocampus, cerebellum IL-1 $\beta$  1 h, but not 2 h, after LPS, compared to controls. Additional animals were injected with LPS or saline 4, 10, or 21 days after exposure to IS and tail vein blood was collected and assayed for IL-1 $\beta$ . An enhanced plasma IL-1 $\beta$  response occurred 4 days after IS, but was gone by 10 days. These results suggest that exposure to IS sensitizes the innate immune response to LPS by resulting in either a larger or a more rapid induction of proinflammatory cytokines. © 2001 Elsevier Science (USA)

*Key Words:* stress; cytokine; interleukin-1; immune system; brain; sensitization

### INTRODUCTION

Exposure to stressful events often results in long-lasting changes in the responsiveness of a variety of systems. For example, repeated exposure to the same stressor (homotypic stress) often results in habituation of the hypothalamic–pituitary–adrenal (HPA) axis and brain stem catecholaminergic activity (Sakellaris & Vernikos-Danellis, 1975; Borrell, Torrellas, Guaza, & Borrell, 1980; Vernikos, Dallman, Bonner, Katzen, & Shinsake, 1982; Armario, Casterranos, & Balasch, 1984; Dobrakovova & Jurcovicova, 1984; Natelson et al., 1988; Pitman, Ottenweller, & Natelson, 1988; De Boer, Koopmans, Slangen, & Van der Gugten, 1990; Hauger, Lorang, Irwin, & Aguilera, 1990; Lachuer, Detton, Buda, & Tappaz, 1994). Conversely, exposure to a test stressor that is different (heterotypic stress) from that used during the initial repeated exposure results in sensitization of the HPA axis and brain stem catecholaminergic activity (Sakellaris & Vernikos-Danellis, 1975; Vernikos et al., 1982; Konarska, Stewart & McCarty, 1989; Lachuer et al., 1994). In addition, a single exposure to a stressor has been shown to sensitize central pathways involved in drug reward (Piazza & Le Moal, 1998), fear and anxiety (Agid, Kohn, & Lerer, 2000; Goenjian et al., 2000), and neuroendocrine responses (van Dijken et al., 1993; Schmidt, Binnekade, Janszen, & Tilders, 1996).

Stress-induced sensitization of neuronal pathways is of particular interest since it has been implicated in the pathogenesis of psychiatric disorders such as drug psycho-

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sis, panic, anxiety, posttraumatic stress, and depressive disorder (Shore, Tatum, & Vollmer, 1986; Engdahl, Dikel, Eberly, & Blank, 1997; Brown, Rush, & McEwen, 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, Eichler, Black, & Kocan, 1980; Leyton & Stewart, 1990), and has been argued to be the mechanism by which stressors enhance the rewarding properties of drugs (Piazza & Le Moal, 1998). Sensitization of the monoamine pathways is the most likely effect of stressors since they play a fundamental role in reward processes (Robinson & Berridge, 2000).

It has been suggested that stressors and activation of the immune system also lead to the stimulation of common neuronal pathways (Dunn & Welch, 1991; Dunn, Wang, & Ando, 1999). Activation of the innate or nonspecific immune system results in the production of proinflammatory cytokines such as interleukin-1 (IL-1 $\beta$ ) and IL-6 by phagocytic cells (Janeway, Travers, Walport, & Capra, 1999). During infection these proinflammatory cytokines stimulate inflammation of the infected site by inducing local dilation of blood vessels, upregulation of intercellular adhesion molecules (iCAMS), recruitment of immune cells to the infected area, and stimulation of infiltrating immune cells (Janeway et al., 1999). All of these responses are critical for localization and elimination of the invading pathogen. In addition, proinflammatory cytokines signal the brain, leading to activation of regions involved in the neurally mediated components of host defense (Dunn, 1993; Brady, Lynn, Herkenham, & Gottesfeld, 1994). This aspect of host defense has been called the "sickness response" (Kent, Bluthé, Kelley, & Dantzer, 1992a) and includes fever, increased NREM sleep, reductions in food and water intake, reduced exploration, reduced social behavior, hyperalgesia, HPA activation, and increased sympathetic nervous system activity (see Maier, Watkins, & Fleshner, 1994, for review). Together, these changes reduce the capacity of pathogens to replicate while simultaneously maximizing the host's ability to recover from the precipitating insult (Blalock, 1984; Roberts, 1991). Interestingly, brain-derived cytokines are induced in response to the peripheral cytokine signal (Laye, Parnet, Goujon, & Dantzer, 1994; Quan, Sundar, & Weiss, 1994) and these brain-derived cytokines are involved in mediating sickness responses (Krueger, Walter, Dinarello, Wolff, & Chedid, 1984; Sapolsky, Rivier, Yamamoto, Plotsky, & Valer, 1987; Hart, 1988; Dantzer & Kelley, 1989; Dascombe, Rothwell, Sagay, & Stock, 1989; Kluger, 1991; Kent et al., 1992b; Kent, Rodriguez, Kelley, & Dantzer, 1994; Maier, Watkins, & Nance, 2001). Thus, sickness responses can be blocked by intracerebral administration of the IL-1 receptor antagonist (IL-1ra), and induced by intracerebral injection of IL-1 $\beta$  (Rothwell & Luheshi, 1994; Schobitz, De Kloet, & Holsboer, 1994).

Classically, it has been thought that the release of proinflammatory cytokines and the expression of "sickness behaviors" only occur when the immune system is activated by a pathogen. There is now evidence that exposure to a novel environment, restraint, foot shock, tail shock, or simple exposure to conditioned stimuli that were present during foot shock increase circulating levels of IL-6 (LeMay, Vander, & Kluger, 1990a; Zhou, Kusnecov, Shurin, DePaoli, & Rabin, 1993). Moreover, stressors such as inescapable tail shock (IS) increase brain levels of IL-1 $\beta$  (Nguyen et

al., 1998) and induce sickness responses such as fever and increases in acute phase proteins (Deak et al., 1997). Consistent with the idea that these sickness responses to IS are mediated by brain IL-1 $\beta$ , the responses can be blocked by intracerebroventricular (icv) administration of alpha-melanocyte-stimulating hormone, a functional IL-1 receptor antagonist (Milligan et al., 1999). Data such as these have led to the suggestion that activation of the acute phase response in reaction to stress may represent an anticipatory defensive immune response (Deak et al., 1997).

While it has been known that stress exacerbates inflammatory diseases such as psoriasis, asthma, and arthritis (Mei-Tal, Meyerowitz, & Engel, 1970; Thomason, Brantley, Jones, Dyer, & Morris, 1992), which are known to result from the activation of cells involved in innate immunity, little is known about the enhancing effects of stress on actual innate immune function. Deak, Nguyen, Fleshner, Watkins, and Maier, (1999) have shown that IS facilitates recovery from subcutaneous bacterial challenge and Dhabhar and McEwen (1996) have found restraint to enhance skin delayed-type hypersensitivity responses. In addition, Zhu et al. (1995) have shown that 5 days of cold water stress enhances proinflammatory cytokine production from *in vitro* stimulated peritoneal macrophages. However, no study has examined the effects of an acute session of stress on *in vivo* proinflammatory cytokine production following immune challenge. This is an important issue, as these cytokines are critical in mounting innate immune responses and in signaling the brain that infection has occurred.

In the present experiments we investigated whether exposure to an acute session of IS 1, 4, 10, or 21 days before administration of bacterial cell wall (lipopolysaccharide; LPS) would alter the cytokine response to LPS. Plasma IL-1 $\beta$ , IL-6, and tumor necrosis factor-alpha (TNF- $\alpha$ ), along with IL-1 $\beta$  levels in various brain regions were measured 1 and 2 h following ip administration of 10  $\mu$ g/kg LPS from rats exposed to IS 24 h prior, while only plasma IL-1 $\beta$  was measured from rats exposed to IS 4, 10, or 21 days prior to LPS challenge.

## MATERIALS AND METHODS

*Subjects.* Adult male Sprague Dawley rats (275–325 g; 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 2 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.

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

*LPS administration.* One, 4, 10, or 21 days after exposure to IS or serving as HCC, animals were injected ip with either 10  $\mu$ g/kg LPS (*Escherichia coli* endotoxin 0111: B4, Sigma Lot No. 17H4041) or equal volume sterile, endotoxin-free saline (Abbott Laboratories, North Chicago, IL). Preliminary studies showed that this dose results in a submaximal cytokine and corticosterone response (unpublished results).

*Plasma and tissue collection.* In some experiments animals were decapitated 60 or 120 min after administration of LPS or saline. Trunk blood was collected for later measurement of cytokines and endotoxin. Tubes were stored on ice and immediately spun in a refrigerated centrifuge. Plasma was aliquoted and stored at 20°C until time of assay. The pituitary and brain were quickly removed after decapitation. Brains were dissected on a frosted glass plate placed on top of crushed ice and brain structures, along with the pituitary, were placed in microfuge tubes and quickly frozen in liquid nitrogen. Brain tissue samples, which included hypothalamus, pituitary, hippocampus, cerebellum, and posterior cortex were stored at -70°C until the time of sonication.

*Brain tissue processing.* Each tissue was added to 0.25–1.0 ml of cold Iscove's culture medium containing 5% fetal calf serum and a cocktail enzyme inhibitor (in mM: 100 amino-*n*-caproic acid, 10 EDTA, 5 benzamidine-HCl, and 0.2 phenylmethylsulfonyl fluoride). Total protein was mechanically dissociated from tissue using an ultrasonic cell disruptor (Heat Systems, Inc., Farmingdale, NY). Sonication consisted of 10 s of cell disruption at the setting 10. Sonicated samples were centrifuged at 14,000 rpm at 4°C for 10 min. Supernatants were removed and stored at 4°C until an ELISA was performed. Bradford protein assays were also performed to determine total protein concentrations in brain sonication samples.

*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 and 120 min later. To retrieve 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 was gently stroked until a volume of approximately 200–300  $\mu$ l of whole blood was obtained in microfuge tubes. The entire sampling procedure was accomplished within 2 min of approaching the cage to ensure nonstressed basal values. Samples were spun in a refrigerated centrifuge immediately, and plasma was aliquoted and stored at 20°C until the time of assay.

*Measurement of cytokines.* Cytokines were measured using commercially available ELISAs for rat IL-1 $\beta$ , TNF- $\alpha$  (R & D Systems, Minneapolis, MN), and IL-6 (BioSource, Camarillo, CA). The ELISAs were run according to the manufacturer's instructions. The rat IL-1 $\beta$  and TNF- $\alpha$  kits have a detection limit of <5 pg/ml and the IL-6 kit has a detection limit of <8pg/ml. The intra- and interassay precision is <10%.

*Measurement of plasma endotoxin.* Plasma levels of endotoxin were determined by an enzymatic assay, according to the procedure outlined by Bio-Whittaker (Cat. No. 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, brain, peritoneal, or spleen 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 15% of the animals were eliminated from the study due to no detectable endotoxin and they were evenly distributed between groups.

*Statistics.* Due to size and manageability, the experiments examining the cytokine response 4, 10, and 21 days after IS were run as separate experiments with their own controls and analyzed using a  $2 \times 2$  ANOVA between stress condition (IS vs HCC) and drug administration (saline vs LPS). The experiment examining the cytokine response 24 h after IS was analyzed using a  $2 \times 2 \times 2$  ANOVA between stress condition (IS vs HCC), drug administration (saline vs LPS), and time (1 h vs 2 h). Based on the a priori prediction that differences would be observed at the 1-h time point post hoc analysis was done using a Bonferonni corrected *t* test.

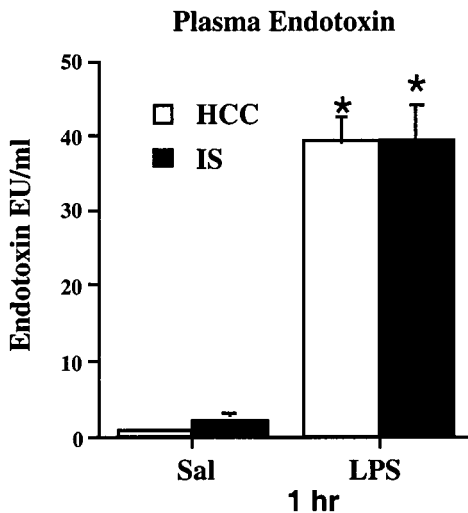
## RESULTS

### *Effects of Prior Stress on Plasma Endotoxin*

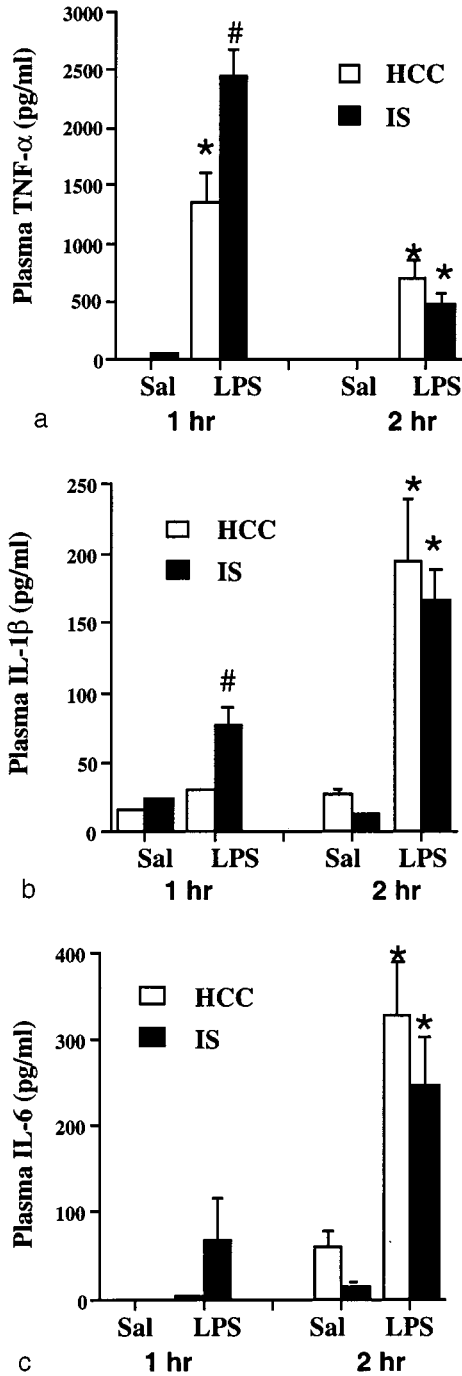
Plasma endotoxin levels for IS and HCC groups administered either LPS or saline are shown in Fig. 1. Low levels of plasma endotoxin were detectable in the saline control subjects. Administration of LPS produced large increases in plasma endotoxin after 1 h. Importantly, prior exposure to IS had no effect on basal or LPS administered plasma endotoxin levels.

### *Effects of Stress 24 h prior to LPS on Plasma Cytokines*

The plasma TNF- $\alpha$  values for the various groups are shown in Fig. 2a. Plasma TNF- $\alpha$  was not detectable in subjects who had not received LPS. However, LPS produced a large elevation in plasma TNF- $\alpha$  measured 1 h later. TNF- $\alpha$  levels at 2 h had substantially diminished, but were still above basal levels. Importantly, prior exposure to IS potentiated the plasma TNF- $\alpha$  increase to LPS at the 1-h time point, with the potentiation no longer evident 2 h after LPS. A  $2 \times 2 \times 2$  ANOVA revealed a reliable interaction between stress condition (IS vs HCC), drug administration (sa-



**FIG. 1.** Circulating plasma endotoxin 1 h after administration of lipopolysaccharide (LPS) or saline (Sal) in home cage control rats (HCCs) or rats exposed to inescapable tailshock (IS) 24 h prior. Bars represent means ( $n = 7-8$ ) plus standard errors. \*, significant difference ( $p < .05$ ) from saline-injected animals.



**FIG. 2.** Plasma levels of TNF- $\alpha$  (a), IL-1 $\beta$  (b), and IL-6 (c) 1 and 2 h after LPS or Sal administration in HCCs or rats exposed to IS 24 h prior. Bars represent means ( $n = 7-8$ ) plus standard errors. \*, significant difference ( $p < .05$ ) from saline-injected animals; #, significant difference ( $p < .05$ ) from saline- and HCC LPS-injected animals.

line vs LPS), and time (1 h vs 2 h) after LPS administration [ $F(1, 1,55) = 9.004$ ;  $p < .05$ ]. Post hoc analyses revealed a significant difference between HCC and IS animals administered LPS at 1 h ( $p < .05$ ), but not at 2 h ( $p = .43$ ). There were no statistical differences between saline groups at either time point.

Plasma IL-1 $\beta$  showed a different pattern (Fig. 2b). Plasma levels of IL-1 $\beta$  were detectable in the saline control subjects. Prior exposure to IS had no effect on basal levels of IL-1 $\beta$ . Although the plasma IL-1 $\beta$  response to LPS increased from the 1-h to the 2-h time point, IS again facilitated the cytokine response to LPS at the 1-h time point, but not at the 2-h time point. A  $2 \times 2 \times 2$  ANOVA did not reveal a reliable interaction between stress condition (IS vs HCC), drug administration (saline vs LPS), and time (1 h vs 2 h) after LPS administration [ $F(1, 1,55) = .607$ ;  $p = .44$ ]. However, post hoc analyses based on a priori predictions revealed a statistical difference between HCC and IS animals administered LPS at 1 h ( $p < .05$ ), but not at 2 h ( $p = .66$ ). There was no statistical reliable difference between saline groups at either timepoint. The pattern for plasma IL-6 was similar (Fig. 2c), but the post hoc analysis was not reliable at 1 h ( $p = .13$ ) or 2 h ( $p = .52$ ).

#### *Effects of Stress 24 h prior to LPS on Brain IL-1 $\beta$ Protein*

Brain and pituitary IL-1 $\beta$  levels are showed in Figs. 3a–3e. Tissue levels of IL-1 $\beta$  were detectable in the saline control subjects. Prior exposure to IS had no effect on basal IL-1 $\beta$  levels 24 h later. Tissue IL-1 $\beta$  response to LPS increased from the 1-h to the 2-h time point in HCC animals, and once again IS facilitated the cytokine response to LPS at the 1-h time point, but not at the 2-h time point. A  $2 \times 2 \times 2$  ANOVA did not reveal a reliable interaction between stress condition (IS vs HCC), drug administration (saline vs LPS), and time (1 h vs 2 h) after LPS administration in any brain region. However, post hoc analyses revealed a statistically significant difference between HCC and IS animals administered LPS at 1 h for the hypothalamus ( $p = .05$ ), cerebellum ( $p < .05$ ), and pituitary ( $p < .05$ ), but not at 2 h ( $p = .83$ ), ( $p = .56$ ), and ( $p = .55$ ), respectively. No statistically significant differences were observed between HCC and IS animals 1 h after LPS administered in the hippocampus ( $p = .09$ ) and cortex ( $p = .11$ ), or at the 2-h time point ( $p = .65$ ) and ( $p = .34$ ), respectively.

#### *Effects of Stress 4, 10, and 21 Days prior to LPS-Induced Plasma IL-1 $\beta$*

As previously shown, plasma levels of IL-1 $\beta$  were detectable in the saline control subjects and prior exposure to IS had no effect on basal levels of IL-1 $\beta$ . Plasma IL-1 $\beta$  responses to LPS increased from the 1- to the 2-h time point. Animals injected with LPS 4 days after exposure to IS had a very similar response to animals that had been exposed to IS 24 h before LPS. That is, IS enhanced plasma IL-1 $\beta$  1 h, but not 2 h, after LPS (Fig. 4a). A  $2 \times 2$  ANOVA revealed an interaction between stress condition (IS vs HCC) and drug administration (saline vs LPS) 1 h after LPS administration [ $F(1, 25) = 7.55$ ;  $p < .05$ ]. LPS administration 10 or 21 days after IS did not result in significant differences in plasma IL-1 $\beta$  compared to controls (Figs. 4b and 4c).

## DISCUSSION

Exposure to a single session of IS resulted in the enhancement of proinflammatory cytokine release in response to LPS administered 24 h later. Prior exposure to IS

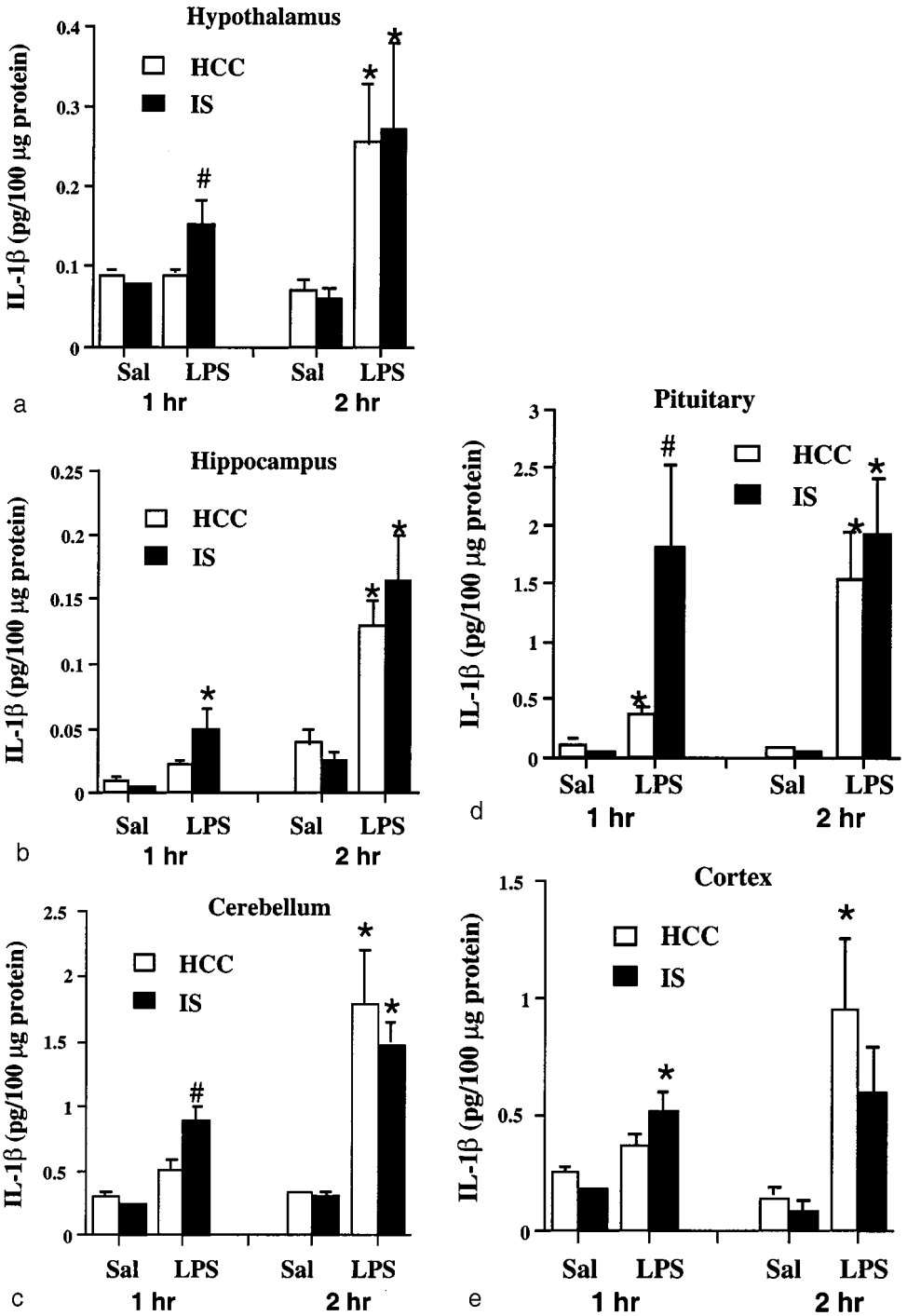
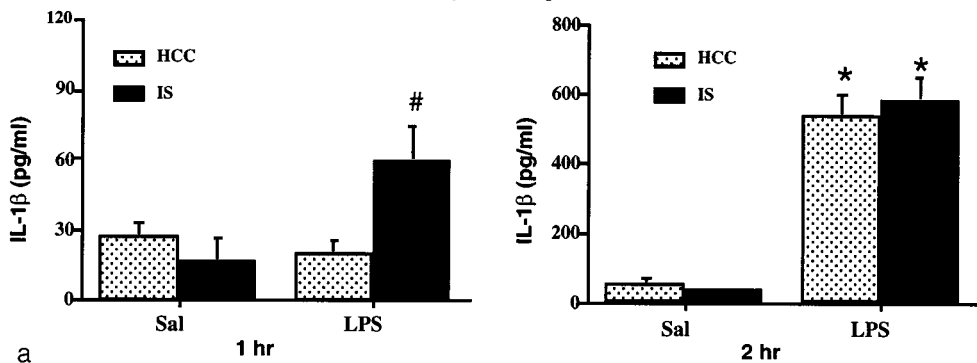
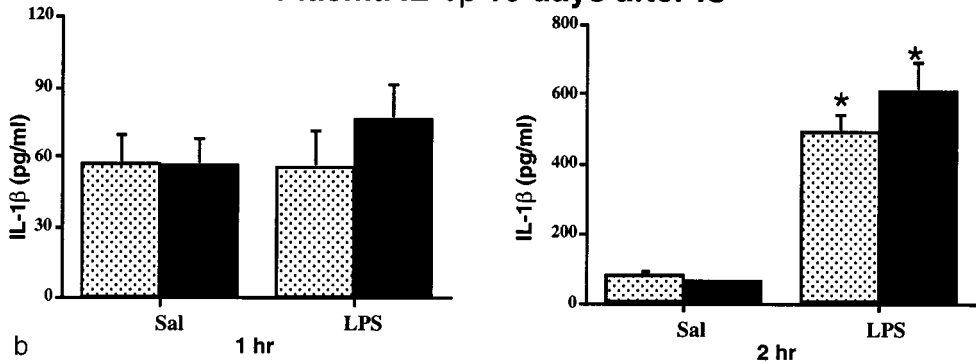


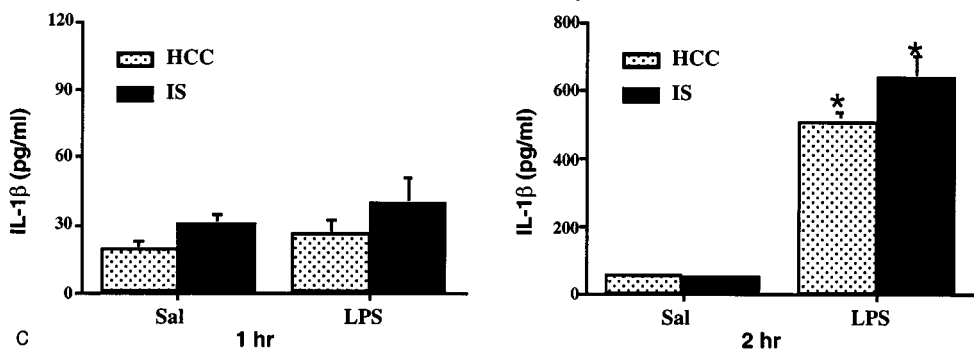
FIG. 3. IL-1 $\beta$  levels in the hypothalamus (a), hippocampus (b), cerebellum (c), pituitary (d), and cortex (e) 1 and 2 h after LPS or Sal administration in HCCs or rats exposed to IS 24 h prior. Bars represent means ( $n = 7-8$ ) plus standard errors. \*, significant difference ( $p < .05$ ) from saline-injected animals; #, significant difference ( $p < .05$ ) from saline- and HCC LPS-injected animals.

Plasma IL-1 $\beta$  4 days after IS

a

Plasma IL-1 $\beta$  10 days after IS

b

Plasma IL-1 $\beta$  21 days after IS

c

**FIG. 4.** Plasma IL-1 $\beta$  levels 1 and 2 h after LPS or Sal administration in HCCs or rats exposed to IS 4 days (a), 10 days (b), or 21 days (c) prior. Bars represent means ( $n = 6-8$ ) plus standard errors. \*, significant difference ( $p < .05$ ) from saline-injected animals; #, significant difference ( $p < .05$ ) from saline- and HCC LPS-injected animals.

significantly increased plasma IL-1 $\beta$  and TNF- $\alpha$  1 h after immune stimulation, while showing a tendency in this direction with regard to IL-6. Since in response to LPS IL-1 $\beta$  stimulates the release of IL-6 (LeMay, Otterness, Vander, & Kluger, 1990b; Romero Schettini, Lechan, Dinarello, & Reichlin, 1993; Luheshi et al., 1996), it is the last in the cascade of proinflammatory cytokines to be induced. Thus, a slightly later time point may be needed to observe a significant enhancement in IL-6 levels. All of these cytokines were significantly elevated 2 h after LPS stimulation, but IS did not potentiate the cytokine responses at this time point. This pattern suggests that prior exposure to IS results in a more rapid release of proinflammatory cytokines. While there was no difference in cytokine levels 2 h after LPS administration, more time points would be needed to determine whether IS alters peak cytokine values.

A more rapid production of brain IL-1 $\beta$  was also observed in IS animals. This may reflect the more rapid peripheral immune response, since peripheral IL-1 $\beta$  has been shown to stimulate the production of central IL-1 $\beta$  (Laye et al., 1994; Quan et al., 1994). However, LPS has been shown to bind to brain endothelial cells and stimulate the release of IL-1 $\beta$  and IL-6 (Fabry et al., 1993; Corsini et al., 1996) and so it is possible that the enhanced central cytokine response to LPS is due to IS sensitization of central pathways. In this study IS resulted in a more rapid release of cytokines both peripherally and centrally, but more studies are needed to determine which cell types are sensitized after IS.

Exposure to IS resulted in facilitation of proinflammatory cytokine release as measured by the plasma IL-1 $\beta$  response to LPS for a period of days. LPS administration 4 days after IS resulted in a more rapid increase in plasma IL-1 $\beta$  compared to controls, similar to what was observed when LPS stimulation occurred 24 h after IS. However, the enhanced plasma IL-1 $\beta$  response was no longer present by 10 days after IS. It should also be noted that the 2-h IL-1 $\beta$  levels in the 4-, 10-, and 21-day post-IS studies were approximately three times larger than those in the previous 24-h experiment. Further studies have shown that this is due to the method of blood collection, serial tail vein nicks vs decapitation (unpublished observation).

One potential explanation for the more rapid increase in peripheral and central cytokines after an ip injection of LPS in animals that had received IS could be that the transport of the endotoxin from the peritoneal cavity to the blood stream was more rapid in IS animals. While a complete time course was not conducted to determine exactly when endotoxin levels began to increase in the blood after LPS, no difference was found in endotoxin levels 1 h after LPS between IS and control animals, which is the time point at which the enhancement in cytokine levels was observed. Therefore, it is unlikely that the more rapid induction of proinflammatory cytokines can be explained by a shift in the kinetics of endotoxin transport from the peritoneal cavity in animals exposed to IS.

Previous research has shown that stress enhances certain types of immune cell activity. Persoons, Schornagel, Breve, Berkenbosch, and Kraal (1995) have shown that IL-1 $\beta$  and TNF- $\alpha$  release from LPS-stimulated alveolar macrophages are enhanced immediately following 20 min of electric footshock. *In vivo* blockade of the autonomic nervous system or  $\beta$ -adrenoceptors completely blocked the stress-induced alterations in alveolar macrophage activity, suggesting that the sympathetic nervous system is involved in the stress-induced enhancement of IL- $\beta$  and TNF- $\alpha$ . Chronic cold water stress has also been shown to enhance TNF- $\alpha$  and IL-6 production from LPS-stimulated peritoneal macrophages collected soon after stress (Chancellor-

Freeland et al., 1995; Zhu et al., 1995). *In vivo* pretreatment with the substance P antagonist RP67,580 blocked the cold water stress-induced increase in IL-6 (Zhu et al., 1995) and pretreatment with capsaicin diminished the stress-induced enhancement of IL-6 and TNF- $\alpha$  (Chancellor-Freeland et al., 1995), suggesting that substance P is involved in the macrophage stress response. These studies suggest that immediately after acute and/or chronic stress the innate immune response is enhanced. The present data support the notion that stress enhances the innate immune response and adds the new findings that these effects occur *in vivo* and are long-lasting, since LPS was administered 24 h to 4 days after exposure to the stressor.

While the mechanism by which IS sensitizes the cytokine response to LPS is unknown, a number of changes are known to occur in IS animals and some of these changes have the potential to alter the innate immune response. For example, exposure to IS has been shown to elevate positive acute phase proteins (Deak et al., 1997), increase core body temperature, and cause a small elevation in basal corticosterone levels (Fleshner et al., 1995). Each of these changes has been shown to enhance innate immune function (Liao, Keiser, Scales, Kunkel, & Kluger, 1995; Hasday, 1996; Wilckens & De Rijk, 1997; Jiang et al., 1999), but it is unclear whether any single or combination of these changes could result in a more rapid release of cytokines as observed in this study.

Stress has been thought to suppress cytokine responses to infection because high levels of glucocorticoids inhibit cytokine production and release (Berkenbosch, Wolvers, & Derijk, 1991; Fantuzzi, Di Santo, Sacco, Benigni, & Ghezzi, 1995). Indeed, the *in vivo* proinflammatory cytokine response to LPS is inhibited if the LPS is administered during or immediately after stress, when glucocorticoid levels are high (Goujon et al., 1995). However, in the present studies, LPS was administered after the large acute endocrine response to the stressor had subsided, and the cytokine response in the periphery and brain was enhanced. It has also been known that stress exacerbates inflammatory diseases (Mei-Tal et al., 1970; Thomason et al., 1992) such as psoriasis, asthma, and arthritis, which are known to result from immune activation. In addition, recent studies have suggested that stress can enhance some functional aspects of immune function for a period of days. Deak et al. (1999) have shown that stress facilitates recovery from subcutaneous bacterial challenge and Dhabhar and McEwen (1996) have shown that stress produces large and long-lasting enhancement of skin delayed-type hypersensitivity responses. These data along with the current finding that stress sensitizes the cytokine response to LPS suggests that at least part of the immune response is enhanced after an organism encounters a stressor, and an enhanced immune response after a stressor (e.g., predator-prey encounter) may be important in preventing or containing infection.

The present data add to the growing literature demonstrating cross-sensitization between stressors and stimuli that activate peripheral immune cells (Tilders & Schmidt, 1999). It has previously been shown that exposure to IL-1 $\beta$  and TNF- $\alpha$  sensitize subsequent endocrine, behavioral, and neurochemical responses to the same cytokine (Schmidt, Janszen, Wouterlood, & Tilders, 1995; Merali, Lacosta, & Anisman, 1997; Hayley, Brebner, Lacosta, Merali, & Anisman, 1999) and to footshock (Tilders & Schmidt, 1998). The present experiments indicate that cross-sensitization also occurs in the reverse direction, namely that exposure to a stressor can sensitize the response to a stimulus that activates cells of the immune system. Moreover, prior work has utilized IL-1 $\beta$  and TNF- $\alpha$  as sensitizing agents, and here it has been shown

that released IL-1 $\beta$  and TNF- $\alpha$  can themselves be sensitized by prior exposure to a stressor.

However, the existing data make it clear that there are multiple and different mechanisms of sensitization. Some of the sensitization effects that have been reported grow slowly following presentation of the sensitizing agent, and are not present until several weeks later (Schmidt et al., 1995). In contrast, other sensitization phenomena develop quickly (Hayley et al., 1999), 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). Rapid and delayed sensitizations have been argued to depend on different mechanisms (Tilders & Schmidt, 1999), and the cross-sensitization between IS and LPS seems to involve the more rapid sensitization.

Cross-sensitization between an initial immune-activating stimulus such as LPS or a cytokine and a subsequent stressor has been argued to be of potential importance for understanding depression (Tilders & Schmidt, 1999) and anxiety (Anisman & Merali, 1999). The experience of stressful life events has been implicated in the etiology of anxiety and affective disorders (Hammen, Davila, Brown, Ellicott, & Gitlin, 1992). Thus, it has been suggested that individuals who have received an immune stimulus might react to a stressor experienced during the cross-sensitization period in an exaggerated manner, thereby exacerbating the anxiogenic and depressogenic impact of the stressor. Interestingly, it has been suggested that the induction of proinflammatory cytokines in the periphery, and therefore in the brain, might also lead to depressed mood (Connor & Leonard, 1998) and anxiety (Connor, Song, Leonard, Merali, & Anisman, 1998). Thus, the cross-sensitization demonstrated here, in which an initial exposure to a stressor exaggerates the peripheral and central cytokine response to a bacterial stimulus, might also have implications for the etiology of anxiety or depression.

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