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Acute And Persistent Effects Of Interleukin-1ß And Stress On The HPA Axis

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By

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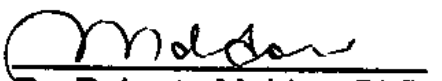
Approved By:



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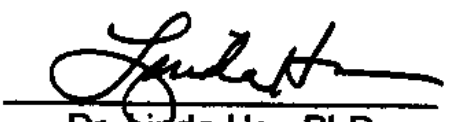

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Abstract

Stress has been shown to persistently elevate circulating levels of corticosterone in rats. One important mediator of stress is the cytokine IL-1β, which is capable of activating the HPA axis. The present study sets out to examine the persistent effects of IL-1β and the involvement of glucocorticoid receptors on corticosterone levels and to compare these effects to those produced by an inescapable tail shock stress. Persistent effects were also investigated in animals stressed during their circadian corticosterone trough in comparison to animals stressed during the circadian peak. The 1st experiment revealed that stressing rats during the PM hours, when circulating levels of corticosterone are high, produced similar results in persistent trough corticosterone elevations as observed with AM stress. In experiment 2, subjects were given a single i.p. injection of IL-1β and a glucocorticoid receptor (GR) antagonist. An acute effect (1 hour later) on corticosterone elevation was produced following the IL-1β injection; however, it did not remain persistently elevated. The GR antagonist did not affect acute or persistent corticosterone levels. In Experiment 3, IL-1β was administered in conjunction with a mild stressor in order to determine if the mild stress would potentiate the effects of IL-1β and lead to a persistent increase in plasma corticosterone levels. Persistent changes were not seen in this experiment either. Thus, the relationship between stress and peripheral IL-1β activation remains unclear at this time.

Introduction

Exposure to stress whether, physical or emotional, has important behavioral and physiological implications. A stressful stimulus can be considered as anything that disrupts the body's homeostatic state. Some behavioral changes seen after stress in laboratory animals are: suppression of exploratory behavior, induction of grooming behavior, increased conflict behavior, decreased food intake and decreased sexual behavior (Ottenweller et al, 1992; Servatius et al, 1994a; Servatius et al, 1995; Arborelius et al, 1999). A cascade of events occurs following a stressful experience, which is referred to as the stress response.

Short term elevations of hormonal mediators released during the stress response, like glucocorticoids and catecholamines, serve to maintain "allostasis", which literally means "maintaining stability through change." The body releases these hormones in order to adapt to the acute stress encountered, which is considered a protective mechanism. After the threat has been overcome, these hormonal mediators will gradually return to normal levels. However, if an organism encounters stress repeatedly or the stress response is not successfully turned off after a stressful experience, these hormonal mediators will remain elevated leading to "allostatic load". During this state of "allostatic load" the body will experience the damaging effects of chronically elevated glucocorticoids and catecholamines. This can lead to pathologies including anxiety disorders, hostile

and aggressive states, substance abuse, and cardiovascular disease. (McEwen, 2000a; McEwen, 2000b)

Countless studies have also uncovered a strong connection between tragic life experiences and Posttraumatic Stress Disorder (PTSD), thus revealing the importance of understanding the stress response and how it affects the overall health of the human population. PTSD usually develops within several weeks of an extreme trauma including: rape, severe physical assault, or a serious accident or injury. PTSD is characterized by re-experiencing the traumatic event in the form of nightmares or intrusive recollections, numbing of responsiveness to the outside world, including diminished interest in activities and detachment from others. Memory impairment, sleep disturbances, and exaggerated startle response are also symptoms of PTSD (Horowitz et al, 1980).

Studies have shown that PTSD is not a rare occurrence within the human population. For instance, it is estimated that approximately 1.07 million U.S. teenagers currently suffer from PTSD (van der Kolk et al, 1996). Also, 15.2% of Vietnam veterans are documented as having PTSD 20 years after the end of the war (van der Kolk et al, 1996). Animal models have been developed to understand the neurobiology of PTSD and other stress related disorders.

Physiologically, corticotropin releasing hormone (CRH) is released from parvocellular neurons in the paraventricular nuclei (PVN) of the hypothalamus after a stress is encountered. The CRH is then secreted in the median eminence and transported in the portal-hypophysial vessels to the anterior pituitary. The anterior pituitary then releases ACTH into circulation, which acts on the adrenal

cortex to release cortisol (corticosterone in rats). This is termed the hypothalamic-pituitary-adrenal axis (HPAA). (Ganong, 1999)

Cortisol, when secreted from the adrenal cortex, has many actions to help the body cope with the stress encountered and to ultimately return the body back to its homeostatic state. Cortisol exhibits effects on intermediary metabolism of fats, carbohydrates, and proteins in order to increase the amount of plasma glucose present. This rise in circulating glucose provides the energy needed for vital organs like the heart and the brain. The anti-inflammatory actions of cortisol also serve to suppress immune system cells in circulation. Cortisol is also important in maintaining vascular reactivity to norepinephrine and epinephrine, important neurotransmitters that are involved in the sympathetic response to stress. All of these actions, in addition to many others, are necessary for an organism to survive the threat that stress poses on the body.

It has been shown from many studies that the HPAA becomes activated with exposure to inescapable stressors, resulting in increased levels of circulating corticosterone in rats immediately following stress and for several days after (during the circadian trough) (Ottenweller et al, 1992; Ottenweller et al, 1994; Servatius et al, 1994a; Brennan et al, 2000). Inescapable stress has also been shown to affect other endocrine axes as well, i.e. thyroid and reproductive (Servatius et al, 1994b; Servatius et al, 2000; Servatius et al, 2001). Thus, this implies that "stress" affects many other axes in the body in addition to the HPAA, and perhaps other systems as well as the endocrine and nervous systems, further adding to the complexity of the stress response.

In most of the studies conducted to investigate the persistent effects of stress on corticosterone levels, rats are stressed during the light phase of their light: dark cycle and persistent effects are seen for days following stress, during the circadian trough (Ottenweller et al, 1992; Ottenweller et al, 1994; Brennan et al, 2000). There remains a question whether stress conducted during the dark phase of the light: dark cycle would produce similar effects on corticosterone levels seen the next morning, due to the possibility that persistent elevations in corticosterone could be the result of an anticipatory response. In other words, the persistent elevations of corticosterone seen after stress may be caused by the animal's ability to prepare itself for another stressful experience, which could be achieved through the use of learning cues (i.e. the light switching on at the beginning of the light phase) that the animal has associated with the previous stressful event.

The HPAA is regulated via a negative feedback mechanism. The feedback action of cortisol/corticosterone occurs in the anterior pituitary corticotrophs and the parvocellular neurons of the PVN in the hypothalamus and involves the binding of glucocorticoid receptors. Glucorticoid receptors (GR) are widespread throughout the brain; however, they are enriched in the PVN, the hippocampus, and the septum. They are also found at high density in the ascending monoaminergic neurons of the brain stem. (Joels and De Kleot, 1992; Arborelius et al, 1999). Glucorticoid receptors (GR) are only occupied by corticosterone during the stress response or at the circadian peak when circulating levels of corticosterone are high (Ratka et al, 1989; De Kleot et al,

1994). When circulating levels of corticosterone are low (i.e. circadian trough), the high affinity mineralocorticoid receptors (MR) are occupied. MRs are mainly found in the hippocampus and the septum (i.e. limbic structures) where they mediate the control of basal HPAA activity (Joels and DeKloet, 1992; Arborelius et al, 1999). MR and GR receptors are cytoplasmic and upon activation they are translocated into the nucleus where they act as transcription factors (Deak et al, 1999).

In addition to the HPAA, the immune system has also been shown to be activated by stressful stimuli. Nguyen et al (2000) found that there is an increase in Interleukin-1β (IL-1β) protein, a proinflammatory cytokine released by activated macrophages and monocytes, in the hypothalamus and the pituitary following a 2hr inescapable stress protocol (Nguyen et al, 2000). This suggests that IL-1β is an important mediator of stress. It has also been found that the HPAA becomes activated in response to immune stimuli, resulting in increases of circulating ACTH and corticosterone levels. Earlier studies have shown that administration of an immune stimulant (i.e. lipopolysaccharide) leads to an increase in corticosterone mediated by IL-1β (Rivier et al, 1989). Lipopolysaccharide (LPS) is a subcellular component of gram-negative bacteria, which activates the immune system when encountered by an organism. Subsequently, it was found that IL-1β administered alone either intracerebroventricularly (i.c.v.), intraperitoneal (i.p.), or intravenously (i.v.) could produce an acute rise in circulating corticosterone and ACTH (Matta et al, 1990; Besedovsky et al, 1991; Rivier, 1993; van der Meer et al, 1996; Schmidt et al, 1996). A study conducted

by Harbuz et al (1992) also found that a single i.p. injection of IL-1β can produce a persistent rise in corticosterone seen 24 hr later (at the circadian trough). The rise in corticosterone seen after immune system activation has suppressive effects on the immune system and helps prevent "overshoot" of an immune response, and thus aids in reestablishing the body's homeostatic state.

IL-1 exists in two forms IL-1α and IL-1β. These two forms only share a 26% homology, but share a single receptor. Many studies have revealed that the effects of IL-1 on the HPAA are primarily achieved through actions of IL-1β (Rivier et al, 1989; Suda et al, 1990; Harbuz et al, 1992). Although, it is now widely accepted that proinflammatory cytokines, especially IL-1, are released following immune system activation caused by the presence of infectious or inflammatory processes leading to activation of the HPAA, the mechanisms by which IL-1 stimulates the HPA axis remain unclear. Several studies have suggested a hypothalamic site of action (Sapolsky et al, 1987; Suda et al, 1990), while others have suggested that the pituitary may be the site of action (Brown et al, 1987; Bernton et al, 1987). Still other data indicate that IL-1 can stimulate corticosterone release directly from adrenal cell cultures (Winter et al, 1990; Tominaga et al, 1991).

One proposed model of the mechanism by which IL-1 acts suggests that an immunological stimuli leads to an increase of CRF release from activated lymphocytes. This lymphoid CRF then acts on macrophages to release IL-1. IL-1 can stimulate the release of hypothalamic CRF, pituitary ACTH, and release of ACTH from B-lymphocytes, which all lead to increased output of glucocorticoids

(i.e. corticosterone) from the adrenal cortex. IL-1 is also released from macrophages during psychological or physical stimuli. In this case, the macrophages are activated via hypothalamic CRF (Blalock, 1994). This model suggests that IL-1 acts at the hypothalamus, the pituitary, and the adrenal gland, perhaps explaining why so much controversy exists in the literature on IL-1's mechanism of action.

In addition to causing an increase in plasma corticosterone levels, the immune stimulant LPS, also induces fever. This resultant fever is mediated by many cytokines including IL-6, IL-1β, β-IFN, γ-IFN, and TNF-α (LeMay et al, 1990; Ganong, 1999). Related studies have shown that the LPS induced fever is inhibited by endogenously produced corticosterone released due to the stress response. It has been shown that intracerebroventricular delivery of a glucocorticoid receptor antagonist (RU-38486) potentiates the fevers (4 hrs following LPS) produced in response to LPS. Also, infusion of RU-38486 resulted in larger increases in core body temperature following exposure to an open field (a mild stressor). These results suggest that corticosterone exerts an inhibitory effect on fevers within the central nervous system. (McClellan et al, 1994; Morrow et al, 1996). Unpublished data from our laboratory suggests that peripheral administration of a glucocorticoid receptor antagonist (RU-38486) prior to inescapable stress will attenuate plasma corticosterone levels 24 hrs. following stress (at the circadian trough). It is not clear, however, whether the GR is acting peripherally or centrally in this case. Given all of the data compiled on stress and the immune system, the present study was originated to understand the

relationship between IL-1β and the persistent activation of the HPA axis following stress.

The 1st experiment (AM/PM Stress), was performed to investigate whether a stress administered near the circadian peak (PM stress) would still produce persistent changes in adrenal activity seen when stress is administered near the circadian trough (AM stress). Previous studies have suggested that animals stressed during the dark cycle do not show a significant rise in circulating corticosterone levels the next morning during the circadian trough. One study conducted by Tannenbaum et al. (1997) exposed animals to restraint stress either during the light cycle (AM) or the dark cycle (PM) and then measured both acute and persistent corticosterone levels. They found that a persistent rise in corticosterone was only evident in those subjects stressed during the AM hours (Tannenbaum et al, 1997). Experiment 1 was conducted in order to answer the question as to whether persistent elevations in corticosterone following stress are due to an anticipatory response or as a result of neuroendocrine changes.

Experiment 2 was designed to determine if a single administration of IL-1β would mimic the persistent rise in circulating corticosterone seen with inescapable stress. A study conducted by Harbuz et al (1992), suggests that administration of a single dose of IL-1β can produce persistent activations of the HPAA resulting in elevated plasma corticosterone and ACTH levels 24 hours later. We were also interested in whether a glucocorticoid receptor antagonist would attenuate the persistent effect produced by IL-1β in a similar fashion as shown by the unpublished data from our laboratory discussed previously.

Due to our failure to see a persistent effect in corticosterone levels with a single administration of IL-1β, we designed experiment 3 (IL/Stress) to determine if stress at a lower threshold could potentiate IL-1β's effects and produce a persistent elevation in plasma corticosterone seen 24 hrs later.

In this study the acute and persistent effects of stress and IL-1β on the hypothalamic-pituitary-adrenal axis were investigated. First, the effects of the time of stress (i.e. during the light period vs. the dark period) on persistent corticosterone elevations were tested. Then IL-1β and stress were administered and plasma corticosterone levels were measured at different time points. One question we hoped to answer was whether administration of IL-1β could mimic the persistent effects on corticosterone normally seen after an inescapable stress protocol. The role of GR receptors during the stress response elicited by IL-1β was also examined using a glucocorticoid receptor antagonist.

Methods

AM/PM Stress Experiment (Experiment 1):

Subjects

Adult male Sprague Dawley rats (225-250g) were purchased from Charles River (Kingston, NY). All were housed in single cages in isolation boxes; each box can house 16 animals. The boxes are sound attenuating with regulated temperature, air quality, humidity, and light: dark cycle (12hr: 12hr). Lights on occurred at 0700. Food and water were given *ad libitum.* Animals were allowed to habituate for one week before being subject to an experimental protocol.

Experimental Design and Procedure

Subjects were assigned to one of three groups: Control (n=6), AM-Stress (n=6), and PM-Stress (n=6). A baseline blood sample was taken from all of the animals at 0800. At 0900, the AM-Stress group was exposed to the stress protocol and at 1630 the PM-Stress group was exposed to the same protocol. The stress regimen consisted of placing animals in plexiglass restraint tubes (Fischer Scientific, USA) and delivering 40, 2.0 mA tail shocks (3s duration) over a 2 hr time period. Each tail shock was separated by an average of 180 seconds. Another blood sample was taken immediately following stress (1100 or 1830). The control group was sampled at 1830 also. Morning (0800) and

evening (1730) blood samples were collected for 3 days following the stress protocol.

Blood samples were taken from the tail vein and collected in heparinized capillary tubes. The tubes were immediately spun in a centrifuge to separate the plasma. The plasma was then transferred into clean capillary tubes and stored in the freezer (-20°C) until the assay was performed.

Assay and Processing

Each sample was assayed for corticosterone using an ^{125}I kit purchased from ICN Biomedical Inc. (Carson, CA; RSL ^{125}I corticosterone kit #07-120102). All samples were diluted 1:80. Corticosterone amounts were analyzed by mixed ANOVA. The Bonferroni multiple comparison test was used for post-hoc analysis.

IL/GR Experiment (Experiment 2):

Subjects

Adult male Sprague Dawley rats (225-250g) were purchased from Charles River (Kingston, NY). All were housed in single cages in isolation boxes; each box can house 16 animals. The boxes are sound attenuating with regulated temperature, air quality, humidity, and light: dark cycle (12hr: 12hr). Lights on

occurred at 0700. Food and water were given *ad libitum*. Animals were allowed to habituate for one week before being subject to an experimental protocol.

Experimental Design and Procedures

Subjects were assigned to one of five groups according to body weight: Control (n=6), Vehicle-Vehicle (n=6), Vehicle-IL (n=6), GR-Vehicle (n=6), GR-IL (n=6). A baseline blood sample was taken from each animal at 0800 on the morning of the experiment. At 1000 all subjects except for the controls were injected once s.c. and once i.p. The IL-1β (PeproTech, NJ) was given i.p. at a dose of 6 μg/kg and was dissolved in sterile saline. The glucocorticoid receptor antagonist (GR), mifepristone (Sigma, MO) was given s.c. at a dose of 50 mg/kg and was dissolved in DMSO and propylene glycol (20 % DMSO). All animals were given the s.c injection first and then the i.p. injection immediately following. The Vehicle-Vehicle group was injected s.c with the GR vehicle and i.p. with sterile saline (IL-1β vehicle). The Vehicle-IL group received the GR vehicle s.c. and IL-1β i.p., the GR-Vehicle group received GR s.c. and sterile saline i.p., and the GR-IL group received GR s.c. and IL-1β i.p. Another blood sample was collected 1 hour following the injections. The next day a blood sample was taken at 0800. Blood samples were taken from the tail vein and collected in heparinized capillary tubes. The tubes were immediately spun in a centrifuge in order to separate the plasma. The plasma was then transferred into clean capillary tubes and stored in the freezer (-20°C) until the assay was performed.

Assay and Processing

Each sample was assayed for corticosterone using an ^{125}I kit purchased from ICN Biomedical Inc. (Carson, CA; RSL ^{125}I corticosterone kit #07-120102). All samples were diluted 1:200.

Corticosterone amounts were analyzed by mixed ANOVA. The control group was not included in the ANOVA, which enabled the data to be processed using a 2X2 design. The Bonferroni multiple comparison test was used for post-hoc analysis.

IL/Stress Experiment (Experiment 3):

Subjects

Adult male Sprague Dawley rats (225-250g) were purchased from Charles River (Kingston, NY). All were housed in single cages in isolation boxes; each box can house 16 animals. The boxes are sound attenuating with regulated temperature, air quality, humidity, and light: dark cycle (12hr: 12hr). Lights on occurred at 0700. Food and water were given *ad libitum*. Animals were allowed to habituate for one week before being subject to an experimental protocol.

Experimental Design and Procedures

Subjects were assigned to one of five groups according to body weight: Non-stress Vehicle (n=7), Non-stress IL (n=7), 5-Shock Vehicle (n=7), 5-Shock IL (n=7), and 40-Shock Vehicle (n=7). A baseline blood sample was taken from each animal at 0800 the morning of the experiment. All animals were injected at 0900 and immediately exposed to stress. The IL-1β (PeproTech, NJ) was given i.p. at a dose of 6 ug/kg and was dissolved in sterile saline.

The first group received a vehicle injection and was not exposed to the stress protocol (Non-stress Vehicle), the next group received an IL-1β injection and was not exposed to stress (Non-stress IL), the third group received a vehicle injection and was only exposed to 5 tail shocks, which lasts for approximately 15 minutes (5-Shock Vehicle), the fourth group received an IL-1β injection and was exposed to 5 tail shocks (5-Shock IL), and the last group received a vehicle injection and was exposed to the full stress protocol of 40 shocks over 2 hours (40-Shock Vehicle). The full stress regimen consisted of placing animals in plexiglass restraint tubes (Fischer Scientific, USA) and delivering 40, 2.0 mA tail shocks (3s duration) over a 2 hr time period. Each tail shock was separated by an average of 180 seconds. The animals exposed to 5 tail shocks entered the stress regimen 15 minutes prior to the end of the protocol and received the last 5 shocks only.

A blood sample was taken 1 hour following the stress protocol. The next morning at 0800 another blood sample was taken. Blood samples were taken

from the tail vein and collected in heparinized capillary tubes. The tubes were immediately spun in a centrifuge to separate the plasma. The plasma was then transferred into clean capillary tubes and stored in the freezer (-20°C) until the assay was performed.

Assay and Processing

Each sample was assayed for corticosterone using an ^{125}I kit purchased from ICN Biomedical Inc. (Carson, CA; RSL ^{125}I corticosterone kit #07-120102). All samples were diluted 1:200.

Corticosterone amounts were analyzed by mixed ANOVA. The 40SV group was not included in the ANOVA, which allowed the data to be processed as a 2X2 design. The 40SV group alone was analyzed using a mixed ANOVA to determine significant differences over sample time. The data was transformed (cubed root) in order to achieve normality. The Bonferroni multiple comparison test was used for post-hoc analysis.

Results

AM/PM Stress Experiment (Experiment 1):

The time when stress is administered does not affect persistent trough elevations in corticosterone. Significant main effects of Condition, $F(2,15)= 5.7$, $p< .02$ and Sample Time, $F(3,45)= 4.2$, $p< .01$ were seen in the evening plasma corticosterone levels. Post-hoc analyses showed the AM stress and PM stress groups differed from the control group only on the evening of stress (Figure 1). Morning samples showed significant differences due to the main effects of Sampling Time, $F(4,60)= 16.2$, $p< .0001$, and a Condition X Time interaction, $F(8,60)= 3.7$. $p< .005$. The AM and PM stress groups differed from the controls in their morning corticosterone levels on the first and second mornings following stress, which was confirmed by post-hoc analyses (Figure 2). Given these results, it seems that stressing rats close to either the peak or the trough in the circadian corticosterone cycle causes similar trough elevations for 2 days following stress.

Experiment 1: Evening Corticosterone Levels

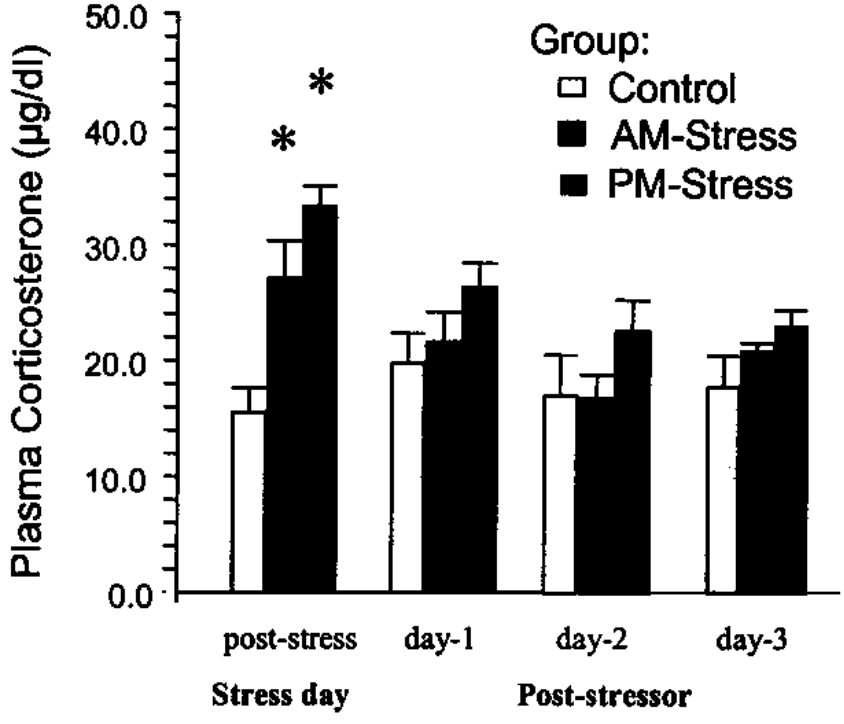

Figure 1: Evening or post-stress plasma corticosterone levels in Control, AM-Stress, and PM-Stress rats. An asterisk (*) represents a significant difference (Bonferroni multiple comparison, $p < .05$) between the post-stress samples for the AM and PM-Stress groups and the evening samples for the Control group.

Experiment 1: Morning Corticosterone Levels

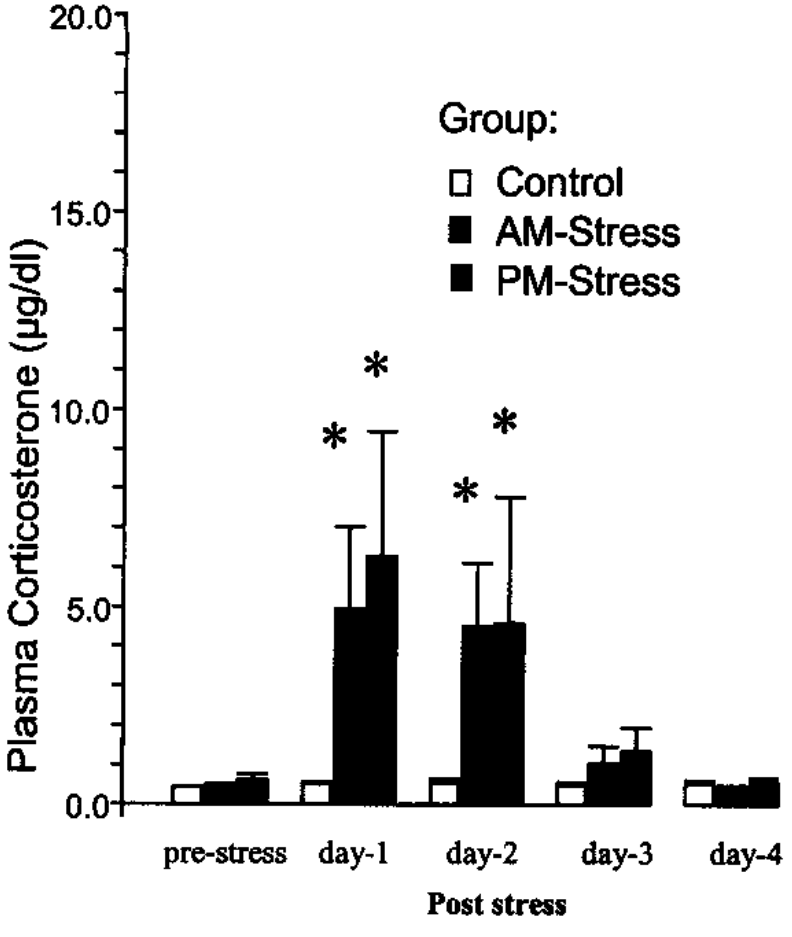

Figure 2: Morning plasma corticosterone levels in Control, AM-Stress, and PM-Stress rats. An asterisk (*) represents a significant difference (Bonferroni multiple comparison, $p < .05$) between the AM and PM-Stress group and the Control group on the first 2 mornings following stress and pre-stress

IL/GR Experiment (Experiment 2):

The baseline blood samples do not differ between groups (Figure 3). Figure 4 shows that IL-1β can influence plasma corticosterone levels 1 hour following injection. There was a significant main effect of IL-1β ($F(1,20)= 8.33$, $p< .01$) and of sample time ($F(3,60)= 13.69$, $p< .001$). Also, a significant difference in mean CORT levels due to an IL-1β X sample time interaction, $F(3,60)= 3.72$, $p< .02$ was seen. Post-hoc analysis revealed that the groups given IL-1β differed significantly from the groups that did not receive an IL-1β injection at the 1-hour post injection sample time point. Also, the subjects who received IL-1β were statistically different at the post injection time point than the same subjects at the baseline time point (Figures 3 and 4). However, IL-1β did not produce an effect 24 hrs later (Figure 5). Administration of the glucocorticoid receptor antagonist (GR) did not produce an effect at either time point (1 hour post injection or 24hr). See Figures 4 and 5. Although IL-1β produced an acute effect, no effect was seen 24 hrs later, which has been seen with other stressful stimuli at the circadian trough. The apparently high levels of corticosterone seen in the Veh-IL group at the 24 hr time point are accounted for by a single value.

Experiment 2: Baseline Corticosterone Levels

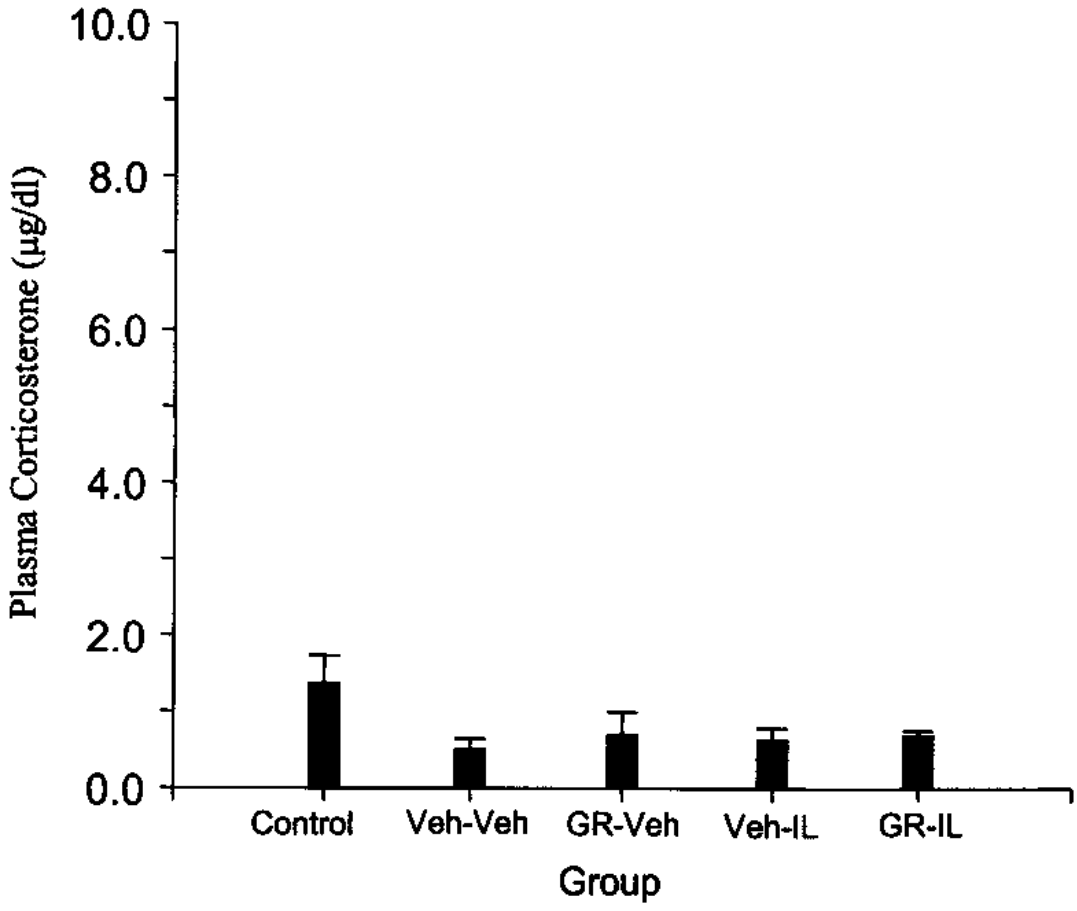

Figure 3: Baseline Plasma corticosterone levels in Control, GR-IL, GR-Veh, Veh-Veh, and Veh-IL groups. The baseline blood samples did not differ between groups.

Experiment 2: 1 Hour Post-injection Corticosterone Levels

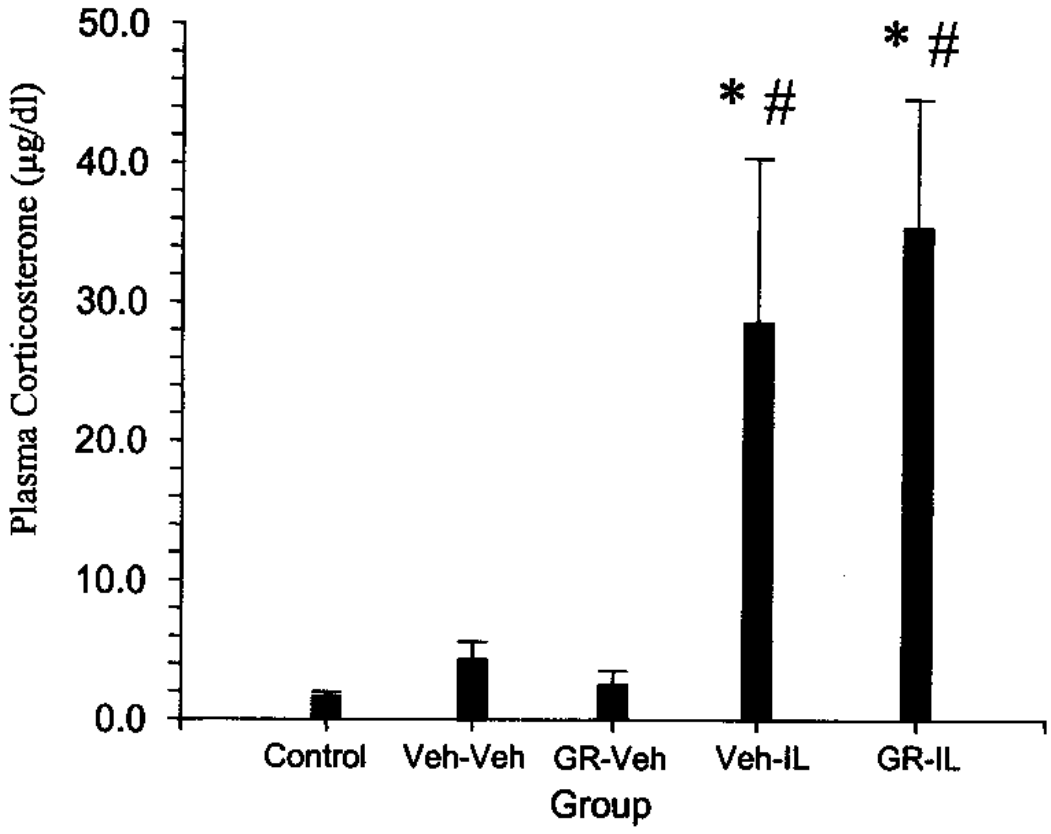

Figure 4: Post injection plasma corticosterone levels in Control, GR-IL, GR-Veh, Veh-IL, Veh-Veh groups. An asterisk (*) represents a significant difference (Bonferroni multiple comparison, $p<.05$) between the IL groups and the vehicle groups. There was also a significant difference between the IL group samples at the post injection time point as compared with the IL groups at the baseline time point (indicated by #).

Experiment 2: Next Morning Corticosterone Levels

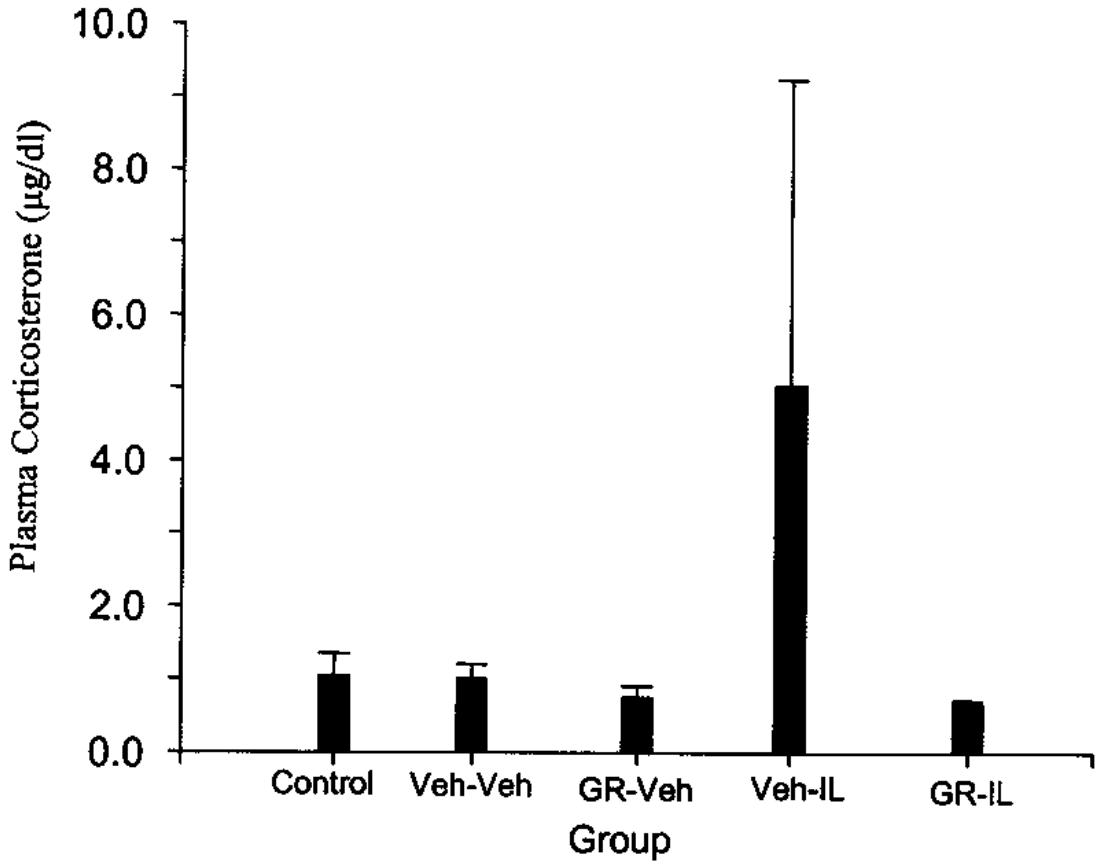

Figure 5: Next morning plasma corticosterone levels in Control, GR-IL, GR-Veh, Veh-IL, and Veh-Veh groups. No significant difference between groups was found. The apparently high level of corticosterone seen in the Veh-IL group was accounted for by a single value.

IL/Stress Experiment (Experiment 3):

Stress, whether for 2 hours or fifteen minutes, lead to a significant increase in plasma corticosterone levels 1 hour following the stress session as depicted in Figure 7. The ANOVA for the IL groups and the 5S groups, revealed a significant main effect of stress ($F(1,24)= 12.09$, $p< .002$) and of sample time ($F(3,72)= 63.41$, $p< .001$). The interaction of stress X sample time also produced a significant effect, $F(3,72)= 10.24$, $p<.001$. The baseline blood samples did not differ between groups (Figure 6). The Bonferroni multiple comparison test showed that the subjects receiving 5 shocks over 15 minutes had significantly higher plasma corticosterone levels than the non-stress groups one hour following stress cessation. The ANOVA for the 40SV group vs. sample time produced a main effect of sample time, $F(2,12)= 13.88$, $p<.001$. Post-hoc analysis also revealed that the 40-shock group had significantly elevated corticosterone levels the next morning (sample time= 3) and at sample time 2 (1 hour after stress). See Figure 8. This 24 hr effect was not seen in the 5-shock group (Figures 7 & 8). IL-1β failed to produce an effect at sample time 2 (3 hrs. following injection) or at sample time 3 (24 hrs. following injection). Therefore, in this experiment IL-1β failed to produce both acute and persistent effects on plasma corticosterone levels.

Experiment 3: Baseline Corticosterone Levels

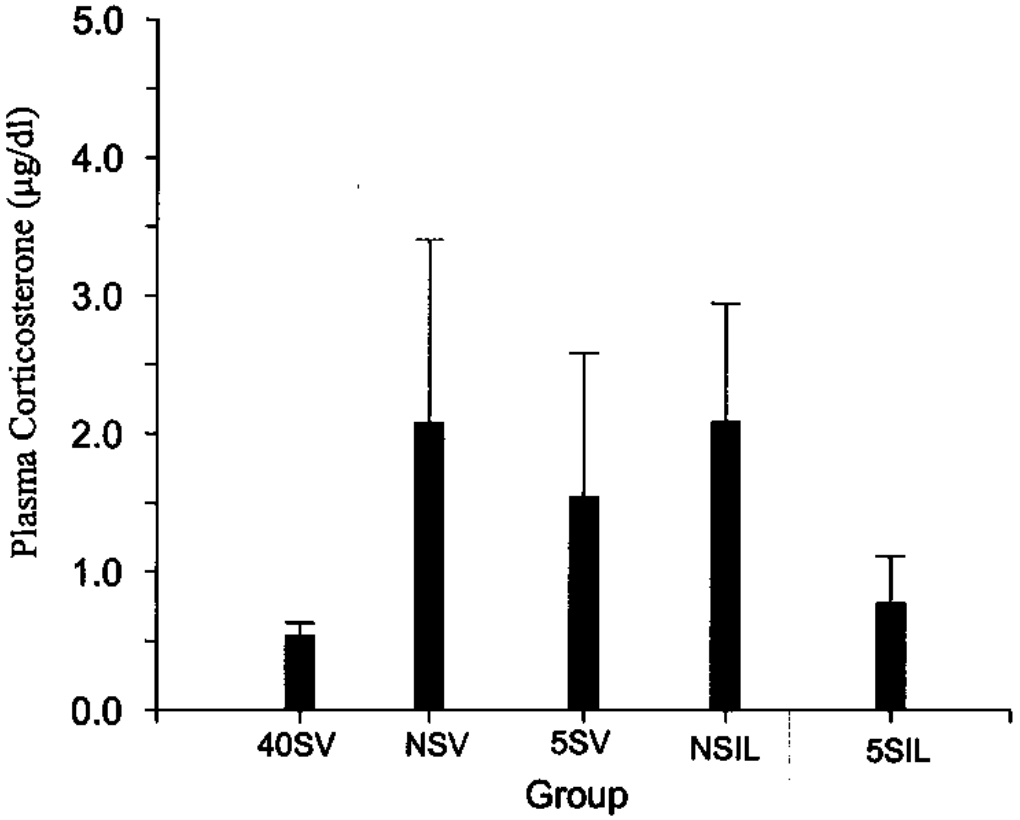

Figure 6: Baseline plasma corticosterone levels in 40SV, 5SIL, 5SV, NSIL, and NSV groups. The baseline blood samples did not differ between groups.

Experiment 3: 1 Hour Post-stress Corticosterone Levels

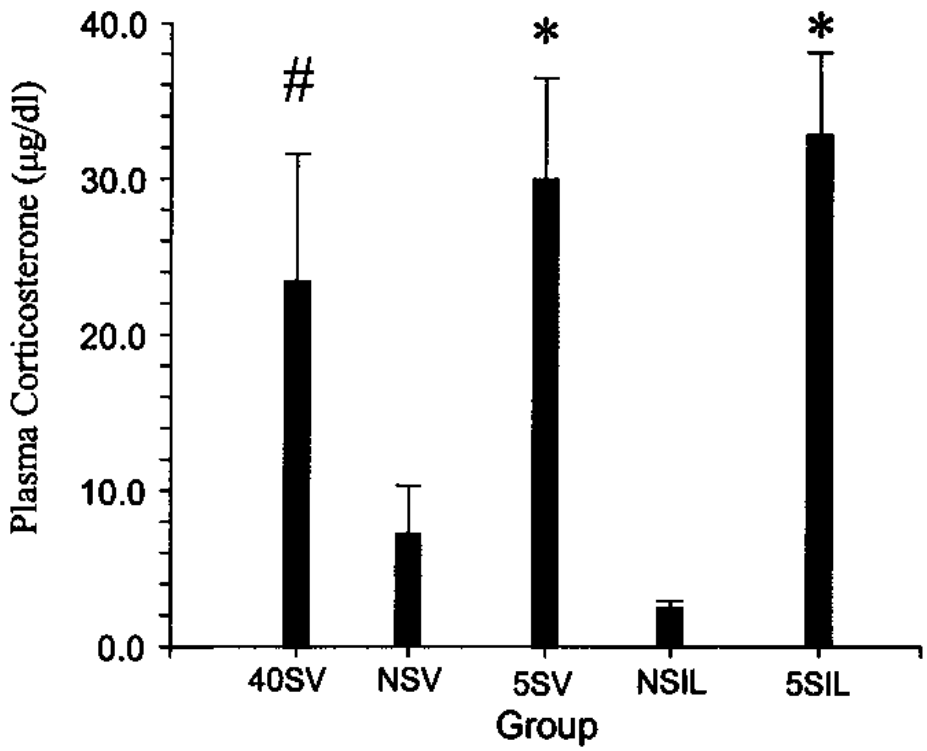

Figure 7: Post-stress plasma corticosterone levels in 40SV, 5SIL, 5SV, NSIL, and NSV groups. An asterisk (*) represents a significant difference (Bonferroni multiple comparison, $p<.05$) between the 5S groups and the NS groups. # indicates a significant difference between 40SV group at the I hour post-stress time point and the baseline time point.

Experiment 3: Next Day Corticosterone Levels

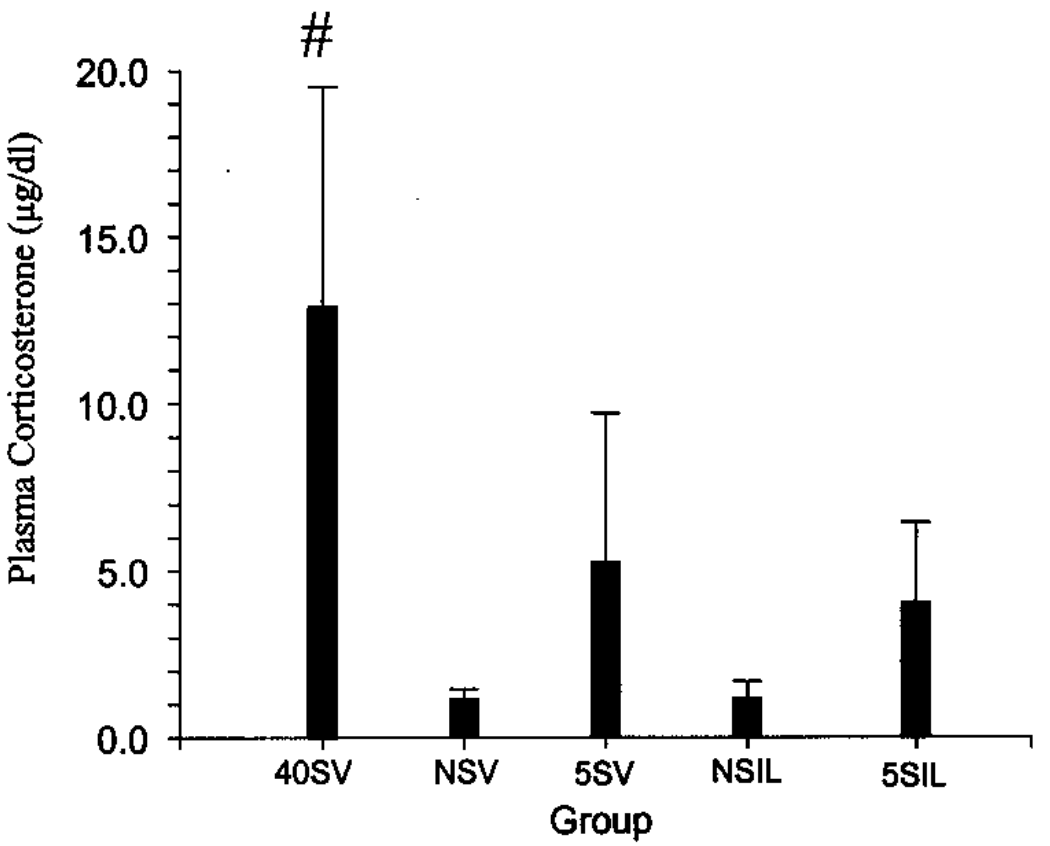

Figure 8: Next day plasma corticosterone levels in 40SV, 5SIL, 5SV, NSIL, and NSV groups. # indicates a significant difference between the 40SV group at the next day time point and the 40SV group at the baseline time point.

Discussion

The current study was performed in order to understand the neuroendocrine influence on persistent stress-induced increases in plasma corticosterone. There remains much speculation into the mechanism causing the persistent changes in HPAA activity seen following an acute stress like inescapable tail shock. It is known that a stress like inescapable tail shock not only produces a rise in corticosterone 24 hours following stress, but it also leads to a decrease in levels of corticosteroid binding globulin (CBG) (Fleshner et al 1995; Deak et al, 1999). CBG is partly responsible for regulating the bioactivity of corticosterone; when bound to CBG, corticosterone is not able to bind to its intracellular receptors and thus initiate its effects. Therefore, a reduction in CBG allows for more "free" corticosterone to be present in circulation, which can then go on to bind to its receptors.

It has been thought that a down regulation of glucocorticoid and mineralocorticoid receptors, resulting in reduced negative feedback, could be responsible for the persistent changes in HPAA activity seen after stress. However, this is not likely given the present research. A study by Deak et al (1999), found no change in the presence of whole cell glucocorticoid receptors following stress, suggesting that an acute stress will not lead to a decrease in the amount of receptors. Also, the finding that the large corticosterone increase caused by stress is not necessary to elicit a persistent elevation in corticosterone 24 hours later suggests that down regulation of receptors is probably not responsible for the persistent changes (Moldow et al, 2001). In that study, a

persistent rise in corticosterone was seen even after synthesis of corticosterone was inhibited during stress. If those large amounts of corticosterone are not present following stress then there is nothing there to cause down regulation of the receptors. The study by Moldow et al (2001) suggests that stress levels of corticosterone are not necessary to cause the persistent rise in corticosterone seen following stress, therefore, leading us to believe that other molecules involved in the stress response are responsible for the persistent elevations (i.e. CRH, norepinephrine, or IL-1β).

Persistent elevations in corticosterone seen following stress could be due to a decrease in negative feedback caused by increased drive to the HPAA that could override the negative feedback mechanism. Perhaps, the HPA axis becomes sensitized in response to a severe stressor in order to prepare the organism in case of a subsequent threat. The persistent elevations seen after a severe stressor could simply be the body's way of protecting itself in case a similar threat is encountered. However, the results of experiment 1 do not support this hypothesis. There still remain many questions as to why an acute stressor leads to persistent changes in HPA activity and how this occurs.

In the first experiment (AM/PM Stress), it was determined that stressing animals close to either the peak or the trough of the circadian corticosterone cycle causes similar elevations in trough corticosterone levels for 2 days following stress (Figure 2). A previous study by Tannenbaum et al (1997), suggested that animals subjected to a restraint stress during their circadian peak (during PM hours) did not show persistent elevations in corticosterone.

Persistent elevations in corticosterone were only seen in the animals that were stressed close to their circadian trough (during AM hours). In our study, an inescapable tail shock stress was used which most likely explains the difference in results, suggesting that 20 minutes of restraint stress is not severe enough to cause similar trough elevations.

A recent study from our lab lead us to believe that there should not be a difference in persistent corticosterone elevations in animals stressed during the circadian peak or the circadian trough. This study found that even if synthesis of corticosterone is blocked during stress, persistent elevations are still seen the next morning (Moldow et al, 2001). This suggests that the large corticosterone increase caused by stress is not necessary to elicit persistent corticosterone elevations. The results found in experiment 1 correspond to this idea, in that it should not matter if stress is encountered when circulating levels of corticosterone are low (during circadian trough) or when they are high (during circadian peak).

More importantly the results of experiment 1 also suggest that time cues or other learning cues (i.e. the lights turning on at the beginning of the light phase) are not responsible for persistent elevations of corticosterone. If the persistent rise in corticosterone seen following stress is simply an anticipatory response we would not have seen a rise in plasma corticosterone during the AM hours in those animals that received PM stress. However, those animals that experienced a PM stress showed similar elevations in corticosterone during the morning when compared to the AM-Stress group, suggesting that

neuroendocrine changes are responsible for the persistent corticosterone elevations seen following stress.

In the 2nd experiment (IL/GR), an acute elevation in plasma corticosterone levels was seen 1 hour following injection of IL-1β (Figure 4). This is in agreement with numerous studies (Matta et al, 1990; Besedovsky et al, 1991; Rivier, 1993; van der Meer et al, 1996; Schmidt et al, 1996;). However, it is not clear what IL-1β's mechanism of action is. It should be noted that IL-1β is not able to cross the blood brain barrier (BBB), but it does exhibit central nervous system effects. Most IL-1β is released by activated macrophages in the periphery; however, it can also be released in smaller amounts by CNS cells including, glial cells and neurons.

Given this information there has been much speculation into how IL-1β is initiating its effects on the HPA axis. Since IL-1β cannot cross the BBB in significant amounts, brain regions that are not protected by the BBB have been proposed as possible sites of action. These sites include hypothalamic regions like the median eminence, subfornical organ, and the organum vasculosum of the laminae terminals. Studies have found that direct administration of IL-1β into the median eminence (ME) leads to an increase in circulating ACTH levels (Matta et al, 1990; Rivier, 1993). This is most likely due to the hypothalamic CRF-containing neurons that terminate in the median eminence. These studies bring up the question of what is mediating IL-1β's actions on the ME that lead to the increases in ACTH and, subsequently, corticosterone that are seen following peripheral IL-1β administration.

The involvement of central catecholamines in this process remains controversial. Some studies suggest that the catecholamines are important in mediating IL-1β's effects (Dunn, 1988; Matta et al, 1990), while others have shown that they are not important in this process (Barbanel et al, 1990; Rivier, 1993). Although the ME has been found as a possible site of IL-1β's action on the HPA axis there remain many unexplored possibilities. Perhaps, peptides like vasopressin or oxytocin could be involved in IL-1β's mechanism of action. The involvement of prostaglandins has even been suggested. It is possible that IL-1β could signal a second messenger, like a prostaglandin, at the BBB border, leading to its HPA actions (Rivier, 1993).

Many studies have also found that IL-1β can directly stimulate the release of CRF from the PVN in the hypothalamus (Sapolsky et al, 1987; Suda et al, 1990), while others have suggested a pituitary site of action (Brown et al, 1987; Bernton et al, 1987). Still others propose an adrenal site of action (Winter et al, 1990; Tominaga et al, 1991). Since IL-1β's effects on the HPA axis seem to occur on many levels, it is very difficult to determine what is actually occurring. Perhaps, IL-1β is capable of initiating many different effects within the CNS and the type of stress encountered by the body will determine which pathway becomes activated. All in all, the present study has confirmed that IL-1β is important in regulating the HPA axis, however, the mechanism underlying its actions needs to be explored further.

Another aim of this study was to investigate the persistent effects of IL-1β on the HPA axis and to test the involvement of glucocorticoid receptors in this

process. The involvement of glucocorticoid receptors was examined using a GR antagonist (RU-486). A persistent rise in corticosterone was not seen after administration of IL-1β in this study (See Figure 5). This is contrary to one study conducted by Harbuz et al (1992), where they saw an increase in plasma corticosterone levels 24 hours following a single injection of IL-1β. In this study they used a slightly higher dose than our dose of 6μg/kg on the same animal model. Also, they collected a trunk blood sample rather than a tail vein sample in our studies. Based on the similarities of the experiments it is difficult to explain why different results were obtained. Perhaps the rise in corticosterone seen was caused by the animals being exposed to the decapitation process and was not caused by the IL-1β administration at all. The higher dose of 2 μg/rat could also explain why they detected a persistent rise in corticosterone.

Effects of the GR antagonist were not seen either acutely or persistently (See Figures 4 and 5). One might expect to see an acute rise in corticosterone following administration of a GR antagonist and a stressor like IL-1β, since this would affect the negative feedback of corticosterone, which is mediated through GR receptors present in the brain. The reason we did not see an acute rise could be due to the fact that the GR antagonist was administered subcutaneously immediately before the i.p. injection of IL-1β. Perhaps there was not enough time allowed for the antagonist to block the GR receptors before they encountered the increased levels of corticosterone caused by the administration of IL-1β. The GR antagonist and the IL-1β were administered at the same time so that both drugs remained active for several hours in the body.

Data from our lab suggests that administration of a GR antagonist will attenuate the persistent rise in corticosterone seen the next morning following a severe stressor like inescapable tail shock (Unpublished, 2001). This effect was not seen in the present experiment, most likely because IL-1β was not able to elicit a persistent effect on corticosterone. Persistent effects on corticosterone are normally seen after severe stressors like inescapable tail shock (Ottenweller et al, 1992; Ottenweller et al, 1994; Deak et al, 1999; Brennan et al, 2000). The present study suggests that IL-1β administration is not a severe enough stressor to cause persistent effects on the HPA axis.

Experiment 3 of this study suggests that subjecting animals to 15 minutes of inescapable tail shock will elicit an acute rise in corticosterone, but will not lead to a persistent activation of the HPA axis seen the next morning. This leads us to believe that the severity and duration of a stressor is important in determining whether or not the HPA axis will become persistently activated. An increase in IL-1β has been shown to occur following inescapable tail shock (Nguyen et al, 2000). Taken together our data suggest that IL-1β is most likely not responsible for the persistent effects on the HPA axis that are seen following severe stressors like inescapable tail shock.

The third experiment (IL/Stress) was performed to determine if a mild stressor could potentiate the effect of IL-1β and thus lead to a persistent rise in corticosterone seen at the circadian trough the next morning. In order to accomplish this we paired administration of IL-1β with a 15-minute inescapable stress protocol. A persistent elevation of corticosterone was not seen when IL-1β

was paired with the mild stress or when it was administered alone (See Figure 8). This is consistent with our previous finding that IL-1β is not strong enough to lead to persistent activation of the HPA axis.

Corticosterone was elevated acutely (1 hour following stress) in those subjects exposed to the 15-minute, 5-shock stress protocol and those that received the full 2 hr, 40-shock stress protocol (Figure 7). However, only those subjects that received the full 2 hr stress protocol showed persistent elevations in corticosterone the next morning. This data clearly shows that the severity and duration of a stressor will affect the persistence of HPAA activation. The animals that received the 15-minute stress had similar levels of corticosterone 1 hour following stress to those animals that received the full 2 hr stress. In this case, it appears that the duration of the stress is responsible for the persistent rise in corticosterone seen in those subjects exposed to the 2 hr inescapable stress session.

Another finding of this experiment was that IL-1β alone did not produce an effect on corticosterone seen 3 hours following injection (Figure 7). There remains a discrepancy about the time course of IL-1β effects on ACTH and corticosterone in the literature. For instance some studies suggest that corticosterone should be elevated 3 hours following a single i.p. injection (Dunn, 1988; Berkenbosch et al, 1989; Suda et al, 1990; Harbuz et al, 1992; Schmidt et al, 1996;). Other studies, however, suggest that when IL-1β is administered i.v, corticosterone and ACTH levels are back down near basal levels as early as 1.5 hours following the injection (Matta et al, 1990; Besedovsky et al, 1991; Rivier,

1993). The literature suggests that when administered i.p. IL-1β should elicit effects on the HPA axis seen as long as 4-5 hours following the injection. However, our data does not go along with this trend in the literature. Perhaps administration of the IL-1β never elicited a rise in corticosterone due to improper preparation of the drug or other technical problems. Since a blood sample was not taken closer to the time of injection, it is very difficult to assess why we did not see a rise in corticosterone levels 3 hours later.

In conclusion, this study confirms the idea that a severe acute stress will lead to persistent changes in the physiology of the HPA axis. Many mediators are involved in the stress response and all of these factors need to be explored in order to understand what happens physiologically following stress. Although IL-1β has been shown to be an important mediator of stress, the results of this study suggest that most likely there are other mediators (i.e. other cytokines or hormones) contributing to the deleterious, permanent effects of stress on the body. Also the present study suggests that various types of stress have different effects on the HPA axis. Most likely each type of stress elicits a different reaction in the body. For example, an immune challenge may rely heavily on cytokines like IL-1β or TNFα to initiate the stress response, whereas a psychological stress may utilize different molecules that ultimately lead to the same outcome. Therefore, it is not possible to consider "stress" as one broad area, thus making research in this area even more challenging. A full understanding of the stress response is necessary to gain insight into the cause of stress related disorders

like anxiety, depression, and even PTSD, thus leading to the development of better treatments for these disorders.

Summary

The prevalence of diseases like depression, anxiety, and PTSD in today's society prove that exposure to stress can be extremely harmful to the human body. In the present study, both the acute and persistent physiological effects of stress were investigated. This was achieved by administering IL-1β, a cytokine known to stimulate the HPA axis, and also by administering a severe stress, inescapable tail shock.

The preceding studies confirm that severe, acute stress can elicit a long-term response in circulating corticosterone. However, this response was not achieved when IL-1β was given or when a mild stress was encountered, suggesting that some stressors are strong enough to lead to persistent changes in HPAA activity, while others are not. IL-1β is most likely not solely responsible for the persistent changes in HPAA activity seen following an acute stress, however, it can not be ruled out as an important mediator. These results also suggest that if stress is encountered at different times during the circadian cycle, it will not lead to differential effects on persistent HPAA activity.

This research has provided another step toward a full understanding of the body's response to a stressful experience. This brings us closer to the development of therapeutic interventions for stress related disorders.

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