Resistance of central nervous system interleukin-6 to glucocorticoid inhibition in monkeys

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Reyes, Teresa M., and Christopher L. Coe. Resistance of central nervous system interleukin-6 to glucocorticoid inhibition in monkeys. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R612–R618, 1998.—The ability of both exogenous and endogenous glucocorticoids (GCs) to inhibit proinflammatory cytokine production was investigated in vivo. Specifically, we investigated the effects of elevated GC levels on interleukin (IL)-1-induced release of IL-6 into both blood and cerebrospinal fluid (CSF). Three experiments were conducted in rhesus macaques to elevate corticoid levels for at least 4 h before administration of IL-1β. The first study used dexamethasone pretreatment, the second utilized ACTH to stimulate endogenous cortisol release, while the third relied on a psychological challenge to stimulate the hypothalamic-pituitary-adrenal axis. Contrary to our a priori predictions, none of these treatments attenuated the IL-1-induced release of IL-6 into CSF. Additionally, the pattern in the blood response was similar, such that the IL-6 response was not blocked, although there was a trend toward a reduction of this response. These data indicated that the IL-1-induced IL-6 response is for the most part resistant to corticosteroid influence, such that even when a partial inhibition was sometimes evident in blood, cytokine release in the central nervous system was not affected.

interleukin-1; cerebrospinal fluid; rhesus monkey; cortisol; dexamethasone

CYTOKINES ARE CRITICAL TO the initiation and propagation of an immune response. On antigen detection, cytokines are released from activated immune cells and act both locally and at distant sites to regulate the response. Cytokines often work in a cascading manner, such that one cytokine leads to the release of other cytokines. One group of proinflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α), are among the first cytokines released in response to bacterial infection or severe trauma. In the bloodstream, TNF-α is detected first, followed closely by an acuterise in IL-1 levels, which in turn leads to the more prolonged release of IL-6 (5). Although beneficial, if this response proceeds unchecked, there may be pathological consequences. Excessively high levels of these cytokines lead to tissue damage and are an important mediator of septic shock fatalities (20).

The proinflammatory cytokines are also potent activators of the hypothalamic-pituitary-adrenal (HPA) axis, leading some investigators to hypothesize that the release of glucocorticoids (GCs) may provide negative feedback critical for preventing an inflammatory overreaction (19). In keeping with this idea, many investigators have used GCs in experimental paradigms to block the cytokine cascade. For example, several studies have reported that GCs can block the lipopolysaccharide (LPS)-induced rise in plasma IL-6. One study in rats found corticosterone pretreatment resulted in a nearly complete inhibition of the IL-6 response to LPS, whereas adrenalectomy augmented the response (27). A similar study found that dexamethasone (Dex) treatment reduced and adrenalectomy enhanced the LPS-induced IL-6 response in both plasma and cerebrospinal fluid (CSF) (4).

But the GC-cytokine relationship is not always so straightforward. The dose of GC may be an important factor to consider, as other studies have found different results at lower, more physiological, levels of GCs. For example, a low dose (20 mg) of cortisol had no effect on human lymphocytes stimulated with LPS in vitro, even though a high dose (80 mg) significantly suppressed IL-6 release (7). Furthermore, using an isolated perfused rat liver system, Liao et al. (17) found that low concentrations of corticosteroids actually enhanced LPS-induced IL-6 release, whereas IL-6 levels were reduced only after high levels of corticosterone. In considering the relationship between corticosteroids and cytokine biology, the type of corticosteroid utilized must be considered because synthetic steroids, such as Dex, may act preferentially through a different receptor. For example, an exogenously administered corticosteroid can block the lipopolysaccharide (LPS)-induced rise in plasma IL-6 from a glioblastoma cell line (8). Therefore, in the following studies we used both pharmacological GC administration (Dex) as well as ACTH administration and a psychological stressor to elevate endogenous GC (cortisol) levels.

Discrepancies may also be apparent between in vitro and in vivo systems, or with the use of different species, especially rodents that are “corticoid sensitive.” Therefore, it was important to extend these investigations to an in vivo model, as well as to explore these questions in a primate model. In addition, very little research has evaluated whether elevated corticoids in the blood compartment actually alter cytokine expression in the central nervous system (CNS). In previous work with rhesus monkeys, we have shown that IL-1 leads to the release of IL-6 into both blood and CSF (23, 24). The goal of the following studies was to investigate the impact of elevated GC levels on this aspect of the proinflammatory cytokine cascade. After establishing normative values in study 1, we tested whether Dex would inhibit the IL-1-induced IL-6 response in study 2. In studies 3 and 4, we investigated the effect of increased endogenous cortisol by pretreating with ACTH, maximally...
elevating cortisol levels, or psychologically challenging the monkeys, a moderate stressor that results in cortisol levels intermediate between baseline and ACTH-stimulated values.

MATERIALS AND METHODS

Subjects

A total of 47 juvenile rhesus monkeys (age 12–20 mo) was used in four separate studies at the Harlow Center for Biological Psychology. Study 1 involved 18 animals (13 females, 5 males), study 2 involved 17 animals (13 females, 4 males), study 3 involved 4 males, and study 4 involved 8 animals (7 males and 1 female). Because these animals were prepubertal, sex was not a significant factor influencing the IL-1-induced IL-6 response (24). Animals were either pair housed in standard cages (0.9 x 1.8 x 0.9 m) or group housed with peers (1.9 x 1.7 x 1.9 m), with a 14:10-h light-dark schedule, lights on at 0600. Animals were fed once daily, had water available ad libitum, and received fruit three times weekly. All procedures were approved by the Institutional Animal Use and Care Committee at the University of Wisconsin.

Materials

Dex (Steris Laboratories, Phoenix, AZ) was received in solution; lyophilized ACTH (Parke-Davis, Morris Plains, NJ) was reconstituted in sterile water. Recombinant human IL-1β (Biosource International, Camarillo, CA) was provided in sterile PBS with 0.1% BSA, pH 7.2. Final dilutions were prepared using a 0.05% rhesus monkey albumin (Sigma, St. Louis, MO) solution. All solutions were filter sterilized (0.2 µm). Stock solutions were stored at –70°C; dilute solutions were stored at 4°C for no more than 2 wk.

Assays

IL-6 in blood and CSF was quantified using Quantikine ELISA from R&D Systems (Minneapolis, MN) following procedures specified in the provided protocols. Both the high-sensitivity (range 0.094–10 pg/ml) and the regular (range 0.7–300 pg/ml) IL-6 kits were used to ensure that values below 10 pg/ml were accurate. Cortisol was determined in duplicate by antibody-coated tube RIA (GammaCoat kit; Incstar, Stillwater, MN). Inter- and intra-assay coefficients for this kit average below 5%.

Procedures

Study 1: Normative data. To determine the normal response to IL-1, 18 animals were utilized to verify our previously published control and stimulated values of IL-6. On the basis of our prior work, a 2-h time point was selected because the IL-1 response appears to peak between 1 and 3 h after IL-1 administration (24). IL-6 and cortisol values were determined 2 h after injections in 15 animals: 7 received IL-1β (0.75 µg/kg iv) and 8 received the vehicle control solution (0.05% rhesus monkey albumin, 0.2 ml iv). Three other animals served as baseline, un.injected controls. These data provided standard control values (baseline vs. IL-1 induced) to be used in comparisons in three subsequent studies. After administration of IL-1β or vehicle, animals were returned to the home cage and sampled 2 h later under light anesthesia (ketamine, 15 mg/kg im). In all four studies, IL-1 injections were given at 1300 and samples were collected at 1500. Blood (2 ml) was collected via femoral venipuncture and centrifuged at 2,000 rpm to separate plasma, which was aliquoted and frozen at –70°C. CSF (0.5 ml) was collected via insertion of a 25-gauge needle between vertebrae C2 and C3. CSF samples were placed immediately on ice, centrifuged at 2,000 rpm, transferred to a clean tube, and frozen at –70°C. Any CSF sample with visible signs of red blood cell contamination was discarded.

Study 2: Dexamethasone pretreatment. This study utilized a between-subjects design to assess the effect of Dex on the IL-1-induced IL-6 response. Animals were pretreated with Dex (0.5 mg/kg im) either acutely [for 4 h (n = 4)] or overnight [for 28 h (n = 8)] before injection with IL-1β. This dose of Dex is 30 times that used in the Dex suppression test (12), a dose that reliably suppresses cortisol production in rhesus monkeys (as was evident in all animals in the present experiment), yet just below the dose used clinically to treat acute inflammatory conditions in humans. The 4-h pretreatment involved a single injection, while the 28-h pretreatment was a series of three injections, at 28, 16, and 4 h before IL-1β injection (see Fig. 1). This dose of Dex is effective for at least 12 h, ensuring that at the point of IL-1β administration, Dex would be present. After IL-1β (0.75 µg/kg iv) was administered, animals were returned to the home cage and sampled 2 h later under light anesthesia (ketamine, 15 mg/kg im). As a further control condition, five other animals received the 4-h Dex treatment with no subsequent injection of cytokine. Sampling procedures were identical to study 1.

Study 3: ACTH pretreatment. This study used a within-subjects design (n = 4). Animals were administered ACTH (1 USP/kg im) 4 h before receiving an injection of either IL-1β (0.75 µg/kg iv) or the vehicle control solution (0.05% rhesus monkey albumin, 0.2 ml iv) (see Fig. 1). There was 1 wk

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Study 4

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Fig. 1. Experimental design. Study 1 established normative values. Study 2 tested 4- or 28-h dexamethasone (Dex) pretreatments, study 3 utilized a 4-h ACTH pretreatment, and study 4 evaluated interleukin (IL)-1β administration after a 4-h psychological challenge. Arrows indicate time points at which treatments were delivered, numbers indicate duration of treatment in hours, and syringes indicate time of sampling. Injections of IL-1β or vehicle were given at 1300, and samples were collected at 1500.
between experimental days, and the order of conditions was balanced across subjects (albumin/IL-1 or IL-1/albumin). This dose has been found to maximally stimulate adrenocortical responses for at least 4 h (elevating cortisol to 70–80 µg/dl) with a return to baseline values by 24 h (3), again ensuring that GC levels would be elevated at the point of IL-1 ß administration. Animals were then returned to the home cage and sampled 2 h later. Sampling procedures were identical to study 1.

Study 4: Psychological challenge (stress). All animals in this study were pair housed. The psychological challenge involved a social isolation paradigm followed by an acute arousal period with the experimenter present (13). First, the pair was separated and subjects were housed alone (0.75 × 0.75 × 0.71 m) in a quiet room for 3.75 h. An experimenter then entered the room without making eye contact with the animal and stood with her profile to the animal. After 10 min, the experimenter turned to face the animal directly and maintained eye contact for an additional 10 min. This challenge reliably elevates cortisol levels in juvenile monkeys (cortisol levels average 54.4 µg/dl immediately after the challenge, undisturbed baseline values range between 15 and 20 µg/dl; unpublished data from our lab). Similar to study 2 and study 3, IL-1 ß was given after 4 h of GC elevation. Monkeys received either an injection of IL-1 ß (0.75 µg/kg iv) or the vehicle solution. Both subjects in a pair received identical injections. The pair was then reunited in the home cage and left undisturbed for 2 h, at which time samples were collected (see Fig. 1). Sample procedures were identical to study 1.

Statistics

Study 1, study 2, and study 4 used one-way ANOVA with Scheffé’s post hoc comparisons, whereas study 3 used independent t-tests with Bonferroni corrections for multiple comparisons. Sex of subject was not evaluated as a variable because we have shown previously that its effect on the IL-6 response is minor in prepubertal animals (23).

RESULTS

Study 1: Normative Data

IL-1 ß resulted in a dramatic release of IL-6 into both CSF and blood [CSF: F(2,15) = 15.11, P < 0.0003; blood: F(2,15) = 21.20, P < 0.0001]. In the baseline and control conditions, IL-6 was nearly undetectable (below 2 pg/ml in both CSF and blood), whereas injections of IL-1 ß significantly increased IL-6 in both CSF and blood (187.5 ± 41.7 and 540.3 ± 102.5 pg/ml, mean ± SE, respectively). These normative data were used for comparative purposes in the next three studies.

Study 2: Dex Pretreatment

IL-6 was significantly elevated after injections of IL-1 ß, as reflected by a significant difference in CSF levels of IL-6 among the five conditions [F(4,27) = 13.45, P < 0.0001] (Fig. 2A). However, neither the 4-nor 28-h Dex pretreatment altered the IL-6 response to IL-1 ß in CSF. Administration of Dex alone without IL-1 ß also did not have any effect on IL-6, which remained in the low range found in the albumin controls. Similar to the CNS response, plasma IL-6 was elevated after all IL-1 ß conditions [F(4,27) = 3.53, P < 0.02] (Fig. 2B). Again, the 28-h Dex pretreatment did not significantly alter this IL-6 response in blood, and the low levels of IL-6 in the Dex control condition did not differ from untreated controls. However, the 4-h Dex pretreatment appeared to attenuate the humoral response somewhat, with plasma IL-6 levels reaching only 296.4 pg/ml in comparison to 540.3 pg/ml for the IL-1 ß alone condition. This difference was not statistically significant.

Effectiveness of the Dex dose was confirmed by measuring cortisol levels. Because Dex is a synthetic GC, endogenous cortisol production is inhibited in the presence of Dex. Normative cortisol values generated in study 1 were 25.6 ± 2.5 (mean ± SE, µg/dl) for albumin-injected animals and 71.9 ± 4.1 for IL-1 ß-injected animals, replicating the finding that IL-1 ß is a potent stimulus for the HPA axis. Cortisol levels in all Dex-treated animals (Dex only, Dex + albumin, and...
Dex + IL-1β) were under 5 µg/dl, ensuring that an effective dose of Dex had been administered.

Study 3: ACTH Pretreatment

IL-1β resulted in the typical increase in IL-6 in both CSF and blood, even after ACTH pretreatment. Compared with the ACTH + albumin control condition, there were significantly higher levels of IL-6 in both CSF (t₀ = 4.83, P < 0.0015) and blood (t₀ = 6.56, P < 0.0003) in animals that received an injection of IL-1β (Fig. 3, A and B). Compared with the IL-6 levels observed after IL-1 in study 1, the ACTH pretreatment did not significantly alter the CSF response to IL-1 (t₀ = 0.785, P = 0.22, NS), whereas it did reduce the IL-6 release into blood (t₀ = 1.875, P < 0.05). However, because of the Bonferroni correction required for the multiple statistical comparisons, this inhibitory effect of IL-6 release in the blood compartment was only marginally significant (adjusted significance: P < 0.025).

This dose of ACTH has been shown to stimulate cortisol release for at least 4 h with a decline to baseline by 24 h (3). Cortisol levels in animals that received ACTH followed by albumin (measured at 6 h post-ACTH administration) confirmed this finding. Cortisol values were starting to decline but were still above baseline: 33.2 ± 1.5 (mean ± SE, µg/dl) compared with normative values from study 1 of 25.6 ± 2.5. These levels were marginally higher than the normative values (t₁₀ = 2.001, P < 0.04) The cortisol response to IL-1 in ACTH + IL-1 animals (71.1 ± 6.3) did not differ from the normative cortisol response seen to IL-1 administration alone (71.9 ± 4.1).

Study 4: Psychological Challenge (Stress)

As observed in study 2 and study 3, the stress pretreatment (and resulting GC elevation) did not alter the IL-6 response in the intrathecal compartment. Both the IL-1 alone and the stress + IL-1 conditions comparably elevated IL-6 levels, significantly above levels in the stress + albumin vehicle condition [F(3,21) = 16.04, P < 0.0001] (Fig. 4A). As found in the prior experiments, the humoral release of IL-6 was more sensitive to the pretreatment. The stress pretreatment significantly attenuated the IL-6 response to IL-1 in blood (P < 0.002), although there still was a large release compared with the virtually undetectable IL-6 in control conditions [F(3,21) = 18.44, P < 0.0001] (Fig. 4B).

As stated in MATERIALS AND METHODS, this challenge reliably elevates cortisol levels to an average 54.4 µg/dl immediately after the social isolation (unpublished data from our lab). Nevertheless, this manipulation produced a surprising effect on the HPA response to IL-1β. The stress pretreatment actually diminished the adrenal response to IL-1β (40.6 ± 4.2 vs. 71.9 ± 4.1 µg/dl, stress + IL-1 vs. normative IL-1, respectively), although this cortisol response was still significantly higher than the stress + albumin animals [23.7 ± 1.5 µg/dl; F(3,21) = 42.92, P < 0.0001]. The stressor-control animals were not significantly different from either the albumin controls or baseline controls, signifying that the reunion of the social pair elicited a rapid return to baseline cortisol levels.

**DISCUSSION**

As highlighted in this research, the influence of GCs on the cytokine cascade varies across different experimental paradigms. Studies investigating the effect of GCs on cellular functions, primarily done in vitro, have led to the commonly held belief that GCs are universally immunosuppressive. However, many authors have cautioned against a simplistic application of this principle to in vivo physiology (31). By exploring the physiological actions of GCs in vivo, the work presented here qualifies the prevailing view that these steroids inhibit all aspects of cytokine biology.

The most striking finding was the complete inability of Dex or endogenous GCs to alter IL-6 release into CSF. We have demonstrated previously that this CSF
IL-6 is brain derived (24), and the current work indicates that GCs do not readily block this central release. Similarly, GC elevations did not block IL-6 release into the blood, although there was a trend toward a reduction in this response. However, this reduction was only statistically significant following the psychological stressor, a condition involving not only GC elevation but additional stress hormones (i.e., the catecholamines) as well. In vitro studies typically find an inhibition of cytokine production and release by Dex. Two reports described a nearly complete blockade of the IL-1-induced IL-6 response in cultured fibroblasts treated with Dex (18, 32), and another study demonstrated a fivefold decrement in the IL-1-induced IL-6 response in cultured chondrocytes treated with cortisol (10). In our studies, both exogenous and endogenous corticosteroids slightly diminished but did not completely block IL-1-induced IL-6 release into the blood. Interestingly, a sustained 28-h Dex treatment did not have any inhibitory effect, even though one might have anticipated an even greater suppression of peripheral IL-6 sources after the more prolonged Dex treatment. Some compensatory process may have occurred [such as downregulation of the GC receptor (GCR) after prolonged exposure to this high-affinity ligand], allowing the IL-6 response to be elicited unhindered.

The cortisol data provided a means by which we could confirm that GC levels were successfully elevated at the point of IL-1 administration. They also provided some additional information on HPA responses to IL-1. IL-1 is known to stimulate hypothalamic corticotropin-releasing factor (26) and pituitary ACTH release (1), whereas direct stimulation of the adrenals by IL-1 is questionable. On the basis of our findings in study 2, it does not appear that IL-1 acts directly at the adrenal level; otherwise, cortisol release should still have occurred even after the Dex treatment. Inhibition of the hypothalamus and pituitary with Dex would not block a direct stimulation of the adrenal by IL-1. Furthermore, it was also of interest that the social stress and reunion in study 4, which is known to modify neuroendocrine responses centrally (6), also altered the cortisol response to IL-1. In this case, the cortisol response was almost 50% smaller than normal, presumably because a psychological process activated on reunion of the pair (possibly associated with increased hippocampal activity) modulated the hypothalamic response to IL-1. This finding concurs with many reports demonstrating an ability of social companionship in monkeys to modulate the HPA response to a stressor (in this case IL-1) (30).

In addition to IL-1, psychological stress has also been reported to stimulate the release of IL-6 into plasma. In rats, psychological stress (an open-field exposure) resulted in a significant elevation of plasma IL-6 (15). We did not confirm this type of stress-induced IL-6 response in monkeys. After the psychological stress (with no injection of IL-1), there was no detectable IL-6 in either the CSF or blood. We have also examined CSF IL-6 levels in animals after psychological stressors of longer duration (a 12-h social separation) and again did not find elevated levels of IL-6 in the CSF or plasma (unpublished data). Whatever mechanism leads to the stress-induced release of IL-6 in the rodents does not appear to occur readily in monkeys, at least under this type of psychological challenge.

Although we found some attenuation of the IL-6 response in blood, the fact that the CSF response was completely unaffected by elevated GCs requires some discussion. One explanation focuses on the cellular source for IL-6 released in CSF versus IL-6 released into blood. We have previously shown that IL-6 in CSF is brain derived and not a result of passive diffusion from the blood into CSF (24). Numerous CNS cell types are capable of producing IL-6, including astroglia, microglia, and neurons (11, 25), as well as endothelial cells of the blood-brain barrier (BBB) (29).

Fig. 4. CSF (A) and blood (B) levels of IL-6 after psychological stressor. Normative data from study 1 are presented at left. Stress alone (no IL-1b) did not increase IL-6 in either the CSF or blood (n = 4). Furthermore, the stressor did not affect the IL-1-induced IL-6 response in the CSF. However, there was a significant attenuation of the IL-6 response in blood (n = 6). **P < 0.002.
peripheral administration of Dex, a small amount does cross the BBB, but levels in CSF are lower than in plasma (28). Similarly, we have found that cortisol levels in CSF following an injection of IL-1 are only ~13% of the levels seen in blood (presumably only the free fraction; unpublished data). Furthermore, it has been suggested that Dex can “tighten” the BBB (9, 21) and could thereby further restrict GC access to the IL-6-producing CNS cells. Differences in GCR expression may also partially explain the lack of effect on the IL-6 responses in the CNS. In the rat, GCR staining in the CNS is found primarily in neural, nonglial cells (2), whereas nonneural, glial cells are a more likely source of CNS cytokines than neurons.

It is also necessary to reconcile our findings with studies that demonstrated a GC suppression of LPS-induced IL-6 release. During endotoxic challenge, there are several sequential waves of cytokines, with TNF-α appearing first, followed by IL-1, and finally IL-6 (5). It is possible that GCs affect events early in this cytokine cascade, primarily TNF-α and/or IL-1. In contrast, we directly initiated the cytokine cascade by administering IL-1, and GCs may not be able to stop the cytokine cascade after this point. It is known that Dex can stop the production and release of IL-1; it selectively inhibits the transcription of the IL-1β gene and can destabilize previously transcribed IL-1β mRNA (24). This may explain why GCs block LPS-induced IL-6 release (4, 27) but did not affect IL-1-induced IL-6 release. Furthermore, IL-6 may be more resistant to GC regulation than IL-1β or TNF-α (7). For example, exercise-induced elevations in cortisol were associated with lower LPS-induced TNF-α and IL-1 responses, whereas the IL-6 response was not affected (7). Furthermore, it was reported that IL-6 was 10–20 times less sensitive to Dex inhibition than IL-1β.

In addition to the proinflammatory cytokine cascade, similar discrepancies have been found with regard to GC actions on other immune responses. Rather than being simply inhibited by the presence of GCs, both lymphocyte proliferation (22) and cytolytic activity (16) exhibit a more complex, bidirectional relationship with GCs, with in vitro responses sometimes not affected or even enhanced by GCs. Thus, despite literally thousands of articles suggesting a universally immunosuppressive role for GCs, reviews of the literature continue to caution against this oversimplification (31). When considering complex cytokine cascades crossing from the periphery into the CNS, it is critical to delineate where in the pathway hormones can exert the greatest influence.

Perspectives

Cytokines are potent stimulators of the HPA axis. This finding has led some to hypothesize that the HPA response functions to prevent an inflammatory overreaction. In vitro studies support this conjecture, but the data presented in the preceding paper question whether it occurs as robustly in vivo. Certainly, the CNS cytokine response was unaffected by elevated GC levels, whereas the blood response was only slightly attenuated. Although it is clear that GCs can affect other aspects of inflammation, such as the acute phase response, it is unlikely that the physiological levels of GCs associated with increased cytokine production would be sufficient to block or eliminate the course of proinflammatory cytokine response to sepsis or endotoxic shock.

This research was supported by National Institute of Mental Health Grant MH-41659. T. Reyes is supported by a National Science Foundation Predoctoral Fellowship.

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Received 5 February 1998; accepted in final form 8 May 1998.

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