Glucocorticoid sensitivity of interleukin-1 agonist and antagonist secretion: the effects of age and gender

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Daun, Jane M., Richard W. Ball, and Joseph G. Cannon. Glucocorticoid sensitivity of interleukin-1 agonist and antagonist secretion: the effects of age and gender. Am J Physiol Regulatory Integrative Comp Physiol 278: R855-R862, 2000.—Interleukin-1 (IL-1) is a primary mediator of inflammation that is regulated, in part, by the hypothalamic-pituitary-adrenal axis. The purpose of this study was to determine if gender- or age-related differences exist in the sensitivity of IL-1-producing cells to hydrocortisone. Peripheral blood mononuclear cells (PBMC) isolated from men and women (21–77 yr old) were incubated with hydrocortisone (0, 50, 100, 500, or 1,000 ng/ml) with or without lipopolysaccharide (LPS). Secretion of IL-1β and IL-1 receptor antagonist was inhibited in a dose-dependent manner (P = 0.001) without age- or gender-related differences. Hydrocortisone decreased soluble IL-1 receptor type II (sIL-1RII) secretion by unstimulated cells (P = 0.0001), but it increased secretion by LPS-stimulated cells (P = 0.0001) in all groups. Unstimulated cell supernatants from men contained greater concentrations of sIL-1RII than the supernatants from women (P = 0.011). Compared with men, PBMCs from women were less responsive to hydrocortisone inhibition of sIL-1RII secretion, regardless of age (P = 0.001), and compared with the follicular phase, sIL-1RII secretion was lower in the luteal phase of the menstrual cycle (P < 0.05). These data indicate that basal secretion and glucocorticoid modulation of sIL-1RII secretion by cultured PBMCs are gender dependent. Moreover, glucocorticoid influences on sIL-1RII secretion depend on the presence or absence of gram-negative bacterial toxins.

interleukin-1β; soluble IL-1 receptor type II; IL-1 receptor antagonist; human mononuclear cells

INTERLEUKIN-1 (IL-1) is a fundamental mediator of immune and inflammatory defense responses. However, IL-1 can also promote host-destructive processes. For example, it induces superoxide anion release by neutrophils, which can result in edematous lung injury and pancreatic β-cell death (16, 22). Additionally, IL-1 stimulates collagenase and proteoglycanase secretion from synovial cells, resulting in cartilage matrix degradation (33), and therefore has been associated with the pathogenesis of inflammatory diseases such as rheumatoid arthritis and lupus nephritis (12). IL-1 activity is mediated by two agonist isoforms, IL-1α, which is mainly cell associated and has juxtacrine actions, and IL-1β, which is the predominant secreted protein and has paracrine and endocrine actions. IL-1 is collectively defined as the two agonist isoforms. Because of the diverse, potentially destructive functions of IL-1, multiple control pathways have evolved to prevent undesirable tissue damage.

Several regulators acting at the site of inflammation modulate the biological action of IL-1. One regulatory pathway involves increased secretion of IL-1 receptor antagonist (IL-1ra) by activated monocytes. IL-1ra competitively inhibits the binding of IL-1 agonists to both type I and type II IL-1 receptors on target cells (2). Another regulatory pathway involves secretion of a soluble form of the IL-1 type II receptor (sIL-1RII), which binds secreted IL-1 before it reaches target cells thus preventing activation (8).

Glucocorticoids are potent systemic anti-inflammatory agents that are used clinically to modulate the inflammatory response. The endogenously produced glucocorticoid, cortisol (hydrocortisone), regulates IL-1 secretion through a feedback mechanism between the hypothalamic-pituitary-adrenal (HPA) axis and the monocyte (4, 32). This is most clearly demonstrated in animal models of endotoxemia, which are associated with increased ACTH and corticosterone secretion. IL-1 is a primary mediator of HPA axis activation, because pretreating animals with anti-IL-1 receptor antibodies diminished the endotoxin-induced increase in ACTH (30). Moreover, intravenous injection of IL-1β activates the hypothalamus, increasing corticotropin-releasing factor (CRF) and ACTH secretion (29). The net result is an IL-1-induced increase in glucocorticoid secretion, which, in turn, inhibits IL-1β secretion by decreasing IL-1 mRNA expression and stability (20, 21). This negative feedback loop limits inflammation and prevents a potentially detrimental overactive immune response.

Animal studies suggest that gender-related differences exist in the immune activation of the HPA axis. For example, central injection of IL-1β in female rats resulted in higher plasma ACTH and corticosterone concentrations than in males (29). In humans, age- and gender-related differences in the sensitivity of the HPA axis to feedback inhibition by glucocorticoids have been reported. In healthy individuals, cortisol regulates its own secretion by inhibiting CRF and ACTH secretion through a feedback mechanism. Heuser et al. (17) demonstrated that dexamethasone pretreatment in women did not inhibit CRF-stimulated secretion of...
ACTH and cortisol to the same magnitude as in men. In older subjects, stimulation of the HPA axis resulted in a more prolonged secretion of ACTH and cortisol compared with younger subjects (17). Furthermore, cortisol infusion was less effective at inhibiting the secretion of ACTH in older subjects (40). Gender differences in the sensitivity of the HPA axis to feedback inhibition by glucocorticoids are more pronounced with increasing age. Compared with older men, cortisol concentrations in older women remained elevated for a longer period of time after HPA axis activation (15). Thus there is an age- and gender-related decline in the ability to turn off the HPA axis after activation, resulting in a stronger and more prolonged HPA response.

Paradoxically, the incidence of inflammatory diseases such as Hashimoto’s thyroiditis, systemic lupus erythmatosus, and rheumatoid arthritis is much greater in women compared with men (1). Furthermore, the risk of inflammatory diseases increases as the individual ages (19). These outcomes may be reconciled if a reduced sensitivity to cortisol-mediated feedback inhibition on inflammatory cytokine secretion exists in women and in the elderly compared with young men. Although age and gender differences in the sensitivity of the HPA axis are well documented, the sensitivity of the mononuclear cell to inhibition by glucocorticoids has not been examined between genders or with aging. The purpose of this study was to test the hypothesis that secretion of the principal soluble IL-1 isoforms (IL-1β and IL-1ra) and soluble receptor (type II) by mononuclear cells of women and older individuals is less sensitive to inhibition by hydrocortisone than the cells of young men.

### MATERIALS AND METHODS

Subjects. All subjects were healthy, not taking any medication, and they gave an informed consent to participate in this study. Six healthy women (28.8 ± 1.7 yr of age, not taking oral contraceptives) and six healthy men (27.5 ± 2.3 yr of age) were included in this study. Baseline data from the young men (Table 1) have been included in a previously reported study (11). The women were tested twice, once during the midfollicular phase and once during the midluteal phase of the menstrual cycle. To examine the effects of age, seven healthy postmenopausal women not receiving hormone replacement therapy (62.1 ± 1.6 yr of age) and seven healthy older men (66.2 ± 3.4 yr of age) were recruited. Subjects representing all age and gender groups were recruited and tested concurrently. All procedures were approved by the Pennsylvania State University Human Investigation Review Committee.

Blood samples. A venous blood sample was drawn from each subject between 0800 and 0900 via the antecubital vein into a heparinized syringe. An aliquot of the blood was removed and centrifuged, and the plasma was separated and frozen at −70°C until analysis of plasma sIL-1RII and cortisol concentrations. The remaining blood sample was used for mononuclear cell isolation and determination of IL-1 isoform and soluble receptor secretion in cell culture.

Cell isolation and culture. Peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation with Ficoll-Hypaque (Histopaque, Sigma, St. Louis, MO). The mononuclear cell layer was aspirated and washed three times with 0.9% NaCl. The cells were resuspended in phenol red-free RPMI supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 100 mM HEPES, 0.2 mM L-glutamine (all from Sigma), and 1% heat-inactivated autologous plasma (56°C for 1 h). Sodium hydrocortisone (Solu-Cortef, Upjohn, Kalamazoo, MI) was added to the cultures in concentrations of 0, 50, 100, 500, or 1,000 ng/ml. In stimulated cultures, 1 ng/ml lipopolysaccharide (LPS) Escherichia coli (E. coli 055: B5, Sigma) was added 60 min after the hydrocortisone. Cells were incubated in 24-well polystyrene plates (Corning Glass Works, Corning, NY) at a density of 2.5 × 10⁶ cells/ml for 24 h at 37°C in a humidified 5% CO₂ chamber. After incubation, the supernatants were collected and centrifuged to remove unbound proteins and LPS. The supernatants were analyzed for IL-1β, IL-1ra, and sIL-1RII concentrations. All containers used for cell culture were disposable and endotoxin free; all solutions were injectable grade or endotoxin tested.

Cytokine measurements. The IL-1ra and sIL-1RII concentrations were measured by ELISAs developed in our laboratory. For the IL-1ra ELISA, 96-well high binding ELISA microtiter plates (Corning Glass Works) were coated with 50 µl of a 20 µg/ml protein A solution (Sigma) in 15 mM Na₂CO₃ and 34.8 mM NaHCO₃, pH 9.6 (coating buffer), and incubated overnight at 4°C (25). The plates were washed with PBS, pH 7.0, with 0.5% Tween 20 (PBST), followed by the addition of 50 µl of 4 µg/ml of monoclonal IL-1ra antibody (R&D Systems, Minneapolis, MN) in coating buffer and incubated overnight at 4°C. The plates were washed with PBST blocked with 200 µl of 1% BSA-PBS for 1 h followed by washing. Recombinant human IL-1α standards (R&D Systems) or culture supernatants (100 µl) were added to the wells, and the plates were incubated overnight at 4°C. The plates were washed followed by sequential incubations with 100 µl of 0.2 µg/ml biotinylated polyclonal IL-1α antibody (R&D Systems) and conjugated streptavidin-peroxidase (Pierce, Rockford, IL). After washing the plates, 100 µl of 2,2'-azinobis(3-ethylbenthiazoi-

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<th>Table 1. Age and gender characteristics of the control and LPS-stimulated secretion of IL-1 isoforms and soluble receptor</th>
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Values are means ± SE. All interleukin (IL)-1 proteins are expressed as pg/ml. Control secretion of IL-1β was below detection limit of ELISA (10 pg/ml) in all groups. LPS, lipopolysaccharide; sIL-1RII, soluble IL-1 receptor type II; IL-1ra, IL-1 receptor antagonist. Symbols represent significant difference from control *P = 0.0001, †P = 0.001.
RESULTS

IL-1β isoform and soluble receptor secretion in the absence of hydrocortisone. In unstimulated PBMC, IL-1β secretion was undetectable in all groups, whereas both IL-1α and sIL-1RII were detected. No significant age-related differences were observed in these cultures (Table I). However, when sIL-1RII secretion was analyzed by gender, irrespective of age, cells from the men secreted significantly greater amounts (540 ± 104 pg/ml) compared with the women (244 ± 57 pg/ml, P = 0.011, Fig. 1A). Secretion of sIL-1RII by cells from the young women was significantly higher in the follicular phase compared with the luteal phase (P = 0.048, Fig. 1B).

Stimulation of PBMC with LPS significantly increased both IL-1β and IL-1α secretion but significantly decreased sIL-1RII secretion in all groups. There were no significant age- or gender-related differences between the groups for the LPS-stimulated cultures.

Plasma sIL-1RII and cortisol. Plasma concentrations of sIL-1RII were also examined to determine if age or gender differences existed. Overall, plasma sIL-1RII concentrations were higher in the men (7.8 ± 0.5 ng/ml) than in the women (6.7 ± 0.2 ng/ml, P = 0.047). When stratified by age group, plasma sIL-1RII was significantly greater in the young men (8.32 ± 0.63 ng/ml) compared with the young women (6.48 ± 0.20 ng/ml, P = 0.033, Fig. 1C), but not the older men compared with the older women (P = 0.85). With the exception of one outlier in the older male group, a significant correlation was observed between in vitro sIL-1RII secretion by unstimulated cells and circulating plasma concentrations (r = 0.405, P = 0.03, data not shown).

Endogenous cortisol may influence the secretion of IL-1 isoforms by PBMC, therefore plasma cortisol concentrations were determined from the same blood used to isolate PBMC. No significant differences in plasma cortisol concentrations were detected between the groups. Plasma cortisol concentrations were highest in the older women (17.6 ± 2.4 µg/dl) and lowest in the older men (13.8 ± 2.4 µg/dl).

Hydrocortisone regulation of IL-1β secretion. IL-1β secretion in unstimulated cultures was not detectable by the methods employed. In LPS-stimulated cultures, hydrocortisone significantly decreased IL-1β secretion in a dose-dependent manner (P < 0.0001). As shown in Fig. 2, there were no significant age- or gender-specific differences in the sensitivity of PBMC to hydrocortisone. In fact, PBMC sensitivity to hydrocortisone for IL-1β secretion was almost identical for all the groups examined.

Hydrocortisone regulation of IL-1α secretion. In unstimulated cultures, hydrocortisone significantly decreased IL-1α secretion in a dose-related manner (P = 0.0001). Doses of hydrocortisone in the 50–100 ng/ml range decreased IL-1α secretion by 60–82%, and 1,000 ng/ml of hydrocortisone nearly abolished IL-1α secretion (Fig. 3A). There were no significant age- or gender-specific differences in IL-1α secretion. In LPS-stimulated cultures, hydrocortisone significantly decreased IL-1α secretion over the doses examined (P = 0.0001). In contrast to unstimulated conditions, the lower doses of hydrocortisone decreased IL-1α by only 30–45% (Fig. 3, A and B), whereas 1,000 ng/ml of hydrocortisone nearly abolished IL-1α secretion (Fig. 3A). There were no significant age- or gender-specific differences in IL-1α secretion. In LPS-stimulated cultures, hydrocortisone significantly decreased IL-1α secretion over the doses examined (P = 0.0001).
tions of hydrocortisone (500–1,000 ng/ml) did not cause further inhibition of sIL-1RII secretion in the cells from the men. These higher concentrations of hydrocortisone were less effective at inhibiting sIL-1RII secretion in the cells from the women. When the data were analyzed irrespective of age, cells from the women exhibited a significantly different dose response to hydrocortisone from the cells from the men (P = 0.0001).

As shown in Fig. 4B, hydrocortisone increased sIL-1RII secretion in LPS-stimulated cultures in a dose-dependent manner (P = 0.0001). High concentrations of hydrocortisone (500–1,000 ng/ml) increased sIL-1RII secretion considerably. For example, sIL-1RII secretion by the cells from the young men increased from 35 ± 20 to 591 ± 191 ng/ml, and the secretion by the cells from the young women increased from 143 ± 88 to 648 ± 188 ng/ml. The cells from the young women exhibited a significantly different dose response to hydrocortisone from the cells from the young men (P = 0.022).

DISCUSSION

The results of this study indicate that although hydrocortisone influences the secretion of all IL-1-related proteins studied, only sIL-1RII secretion was influenced in a gender-dependent manner. Mononuclear cells isolated from the men secreted more sIL-1RII under basal (non-LPS stimulated) conditions and were more responsive to high concentrations of
hydrocortisone than the cells from the women. Age did not affect glucocorticoid sensitivity to any of the proteins examined under any conditions. The cells were cultured in 1% autologous plasma, therefore it is possible that humoral factors were transferred that influenced cellular responsiveness. However, gender-specific differences in cytokine secretion have been observed for cells cultured with a synthetic serum replacement (10). The finding that the circulating concentration of sIL-1RII is greater in the men is consistent with a previous report that IL-1β binding capacity was higher in the plasma from the men compared with the women, and this binding capacity correlated with plasma sIL-1RII concentration (7).
Soluble IL-1RII appears to regulate IL-1 bioavailability by binding secreted IL-1 agonists (α or β). The addition of sIL-1RII to cultured fibroblasts or synovial cells decreased the IL-1-induced secretion of PGE2 and collagenase (6). Additionally, compared with wild-type controls, local phorbol 12-myristate 13-acetate-induced inflammation was reduced in transgenic mice (27). These studies implicate sIL-1RII as a natural inhibitor of IL-1-induced inflammation.

The mechanism(s) that determines gender differences in glucocorticoid-mediated inhibition of sIL-1RII secretion would not seem to be at the level of glucocorticoid receptor expression. Although decreased receptor numbers have been reported in the liver and thymus of female rats compared with male rats (13), no gender differences have been demonstrated in the glucocorticoid receptor number or affinity in human mononuclear cells (18, 36). Alternatively, progesterone can enhance the dissociation of glucocorticoids from their receptors (35), which may result in decreased glucocorticoid responsiveness. However, the present study included postmenopausal women whose progesterone concentrations would be low, thus reducing the possibility that progesterone is competing with pharmacological concentrations of hydrocortisone. Finally, if receptor expression were the key factor, then all three IL-1-associated proteins measured in this study would exhibit sex-related sensitivity to hydrocortisone.

Glucocorticoid receptor expression in mononuclear cells has been reported to decrease with age (3) which may compromise IL-1 regulation between the HPA axis and the monocyte. However, the present data indicate that the responsiveness of mononuclear cells from subjects in their seventh decade of life (on average) is similar to cells from subjects in their third decade.

An interesting finding of this study is that LPS differentially affected the secretion of IL-1 isoforms and sIL-1RII. Both IL-1β and IL-1ra secretion rates were significantly increased by LPS, but sIL-1RII secretion was decreased. These data are in accordance with others (26, 31) that demonstrated LPS decreased sIL-1RII mRNA expression in human mononuclear cells. Moreover, the activation state of the cell actually reversed the influence of hydrocortisone on sIL-1RII secretion in the present study. In unstimulated cells, hydrocortisone decreased sIL-1RII secretion, whereas in LPS-stimulated cells it markedly increased sIL-1RII secretion (18- to 33-fold). Brown et al. (5) have demonstrated that LPS and dexamethasone synergistically increase sIL-1RII secretion, although they did not report a dexamethasone-induced effect on sIL-1RII secretion in unstimulated cells. However, they did not detect basal levels of sIL-1RII as demonstrated in this study and by others (9, 31).

In contrast to the present data obtained with mixed mononuclear cell cultures, Colotta et al. (9) reported that dexamethasone significantly increased sIL-1RII secretion and mRNA expression in unstimulated, purified human monocytes. Conflicting data are reported regarding sIL-1RII secretion by unstimulated T cells (23, 24). However, phytohemagglutinin- or antigen-stimulated T lymphocytes do express IL-1RII mRNA and secrete the soluble receptor (23, 24). B lymphocytes also secrete sIL-1RII, however, the contribution of B lymphocytes in an isolated mononuclear cell preparation is ~5% (34). Hydrocortisone-induced decreases in sIL-1RII secretion may involve a negative influence of lymphocytes on the monocytes. The culture conditions used in the present study do correspond to in vivo conditions because unstimulated sIL-1RII secretion correlated with plasma sIL-1RII concentrations of the subjects.

Polyphagocytic neutrophils (PMN) also express IL-1RII and secrete a soluble form, however, regulation by LPS and glucocorticoids is different from that in mononuclear cells. LPS significantly increased sIL-1RII secretion by PMN (14), and dexamethasone increased both mRNA expression and secretion of sIL-1RII in unstimulated PMN (28). In contrast to the 60-kDa form secreted by PBMC, PMN secrete a 45-kDa form of sIL-1RII with the difference being attributed to glycosylation (9, 28). The differential regulation of sIL-1RII secretion by various cell types suggests that the function of secreted sIL-1RII may be dependent on the cellular source. It is possible that under basal conditions, circulating sIL-1RII originates from PBMC. During bacterial infection, however, the contribution of mononuclear cells declines, whereas the contribution of neutrophils increases. Van der Poll et al. (37) reported that administration of a low dose endotoxin did not change plasma concentrations of sIL-1RII, which may be due to a change in the relative contribution of PMN and PBMC to the total cellular secretion of sIL-1RII.

Counterbalancing the activity of IL-1β with specific IL-1 inhibitors provides important regulatory control of both systemic and local inflammation. The endogenously secreted anti-inflammatory cytokines IL-4 and IL-13 increase both sIL-1RII and IL-1ra secretion by mononuclear cells (9, 38, 39). In contrast, the present data demonstrated that hydrocortisone increased sIL-1RII secretion but decreased IL-1ra secretion in LPS-stimulated cells. Thus the mechanisms of glucocorticoid action are different from the mechanisms controlling regulation within the cytokine network.

Perspectives

Basal secretion of sIL-1RII by PBMC in vitro and plasma concentrations of sIL-1RII in vivo were significantly lower for the women compared with the men. Additionally, cells from the women were less responsive to regulation by hydrocortisone in terms of sIL-1RII secretion. It is well established that gender differences in the immune response exist. Women have better relative resistance to certain bacterial and viral infections than men and demonstrate increased responsiveness to a variety of antigens and mitogens. However, this increased immune responsiveness is associated with reduced tolerance of self-antigens, resulting in an increased incidence of autoimmune diseases (1). The reduced secretion and plasma concentrations of sIL-1RII observed in the women compared with the men in this study may result in an increase in the immuno-
stimulatory and proinflammatory potential of IL-1β. This may be one mechanism that contributes to gender-dependent differences in the immune response.

The results of this study also indicated that the activation state of the mononuclear cells determined how they responded to hydrocortisone in terms of sIL-1RII secretion. During gram-negative infection, cortisol is expected to increase sIL-1RII secretion, which, in turn, would downregulate LPS-induced IL-1 activity. However, several physiological stressors, including ultraviolet radiation and oxidative stress, induce IL-1β secretion; under these circumstances, cortisol may promote IL-1 activity by downregulating sIL-1RII.

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