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Inflammatory mediator-induced hypothalamic–pituitary–adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats

(corticosterone/interleukin 1α/corticotropin/serotonin/RU 486 glucocorticoids)

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ABSTRACT **Inbred Lewis (LEW/N) female rats develop an arthritis in response to group A streptococcal cell wall peptidoglycan polysaccharide (SCW), which mimics human rheumatoid arthritis. Histocompatible Fischer (F344/N) rats do not develop arthritis in response to the same SCW stimulus. To evaluate this difference in inflammatory reactivity, we examined the function of the hypothalamic–pituitary–adrenal (HPA) axis and its ability to modulate the development of the inflammatory response in LEW/N and F344/N rats. We have found that, in contrast to F344/N rats, LEW/N rats had markedly impaired plasma corticotropin and corticosterone responses to SCW, recombinant human interleukin 1α, the serotonin agonist quipazine, and synthetic rat/human corticotropin-releasing hormone. LEW/N rats also had smaller adrenal glands and larger thymuses. Replacement doses of dexamethasone decreased the severity of LEW/N rats' SCW-induced arthritis. Conversely, treatment of F344/N rats with the glucocorticoid receptor antagonist RU 486 or the serotonin antagonist LY53857 was associated with development of severe inflammatory disease, including arthritis, in response to SCW. These findings support the concept that susceptibility of LEW/N rats to SCW arthritis is related to defective HPA axis responsiveness to inflammatory and other stress mediators and that resistance of F344/N rats to SCW arthritis is regulated by an intact HPA axis–immune system feedback loop.**

A single intraperitoneal injection of group A streptococcal cell wall fragments (peptidoglycan group-specific polysaccharide; SCW) into euthymic LEW/N female rats induces severe, rapid onset, acute arthritis, followed by a chronic proliferative and erosive arthritis. Athymic LEW.rnu/rnu rats develop the rapid-onset acute-phase arthritis, but the chronic disease is significantly blunted, indicating that the late-, but not the early-, onset disease is thymus dependent. In contrast, histocompatible euthymic and athymic F344 rats develop only minimal, early-onset, swelling of the hind paws that rapidly subsides. These differences in disease pattern and severity are paralleled by the intensity of class II major histocompatibility antigen (Ia) expression in synovial tissues. The presence of a strain difference in the early-onset, thymic-independent phase of SCW arthritis in athymic LEW.rnu/rnu versus F344.rnu/rnu rats indicates that the thymic-independent phase of arthritis is genetically regulated and that the regulating factor or factors are operative very early in the disease (1). The mechanisms involved in this regulation are unknown.

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Corticosteroids are both potent endogenous anti-inflammatory and immunosuppressive agents and potent endogenous down-regulators of Ia expression (2–4). Corticosterone is released early in the course of inflammation, possibly through stimulation of the hypothalamic–pituitary–adrenal (HPA) axis by inflammatory mediators such as endotoxin and interleukin 1 (IL-1) and may be important in maintaining the normal feedback loop between the immune system and the central nervous system (5–14). We therefore compared the early corticotropin (ACTH) and corticosterone responses to SCW and IL-1α in inbred F344/N and LEW/N rats and outbred Harlan–Sprague–Dawley (HSD) rats. Since serotonin (5-HT) is also released during inflammation and down-regulates Ia expression (15), and since 5-HT pathways represent another route of hypothalamic–pituitary stimulation (16–23), we also compared the effect of the 5-HT agonist quipazine on acute ACTH and corticosterone responses in F344/N, LEW/N, and HSD rats. Furthermore, to evaluate the direct involvement of glucocorticoids in the observed SCW susceptibility of LEW/N rats and SCW resistance of F344/N rats, we examined the ability of replacement doses of glucocorticoids to suppress the SCW susceptibility of the former and the ability of a potent glucocorticoid antagonist RU 486 to reverse the SCW resistance of the latter. Data presented here indicate that HPA axis activation is defective in LEW/N rats and that the HPA axis plays a major role in regulating the development of SCW arthritis and probably other inflammatory conditions.

MATERIALS AND METHODS

Animals. One-hundred-gram, virus antibody-free, female, inbred F344/N and LEW/N rats, and outbred HSD rats, purchased from Harlan–Sprague–Dawley, were acclimatized to 12-hr on/12-hr off light cycles, prior to i.p. injection of various inflammatory mediators.

Drugs and Inflammatory Mediators. SCW was prepared in sterile phosphate-buffered saline (PBS) as described (1) and was injected i.p. at a concentration of 0.02–2 mg of cell wall rhamnose per 5 ml of sterile PBS per 100 g. Recombinant human IL-1α (24) was a generous gift from P. Kilian and P. Lomedico (Hoffmann–La Roche). It was injected i.p. at doses ranging from 0.1 to 5 μg per 0.1 ml of sterile PBS per 100 g. Specific activity ranged from 3×10^8 to 2.5×10^9 units/μg. One unit of IL-1 activity was defined in the D10 cell

Abbreviations: SCW, streptococcal cell wall fragments; HPA, hypothalamic–pituitary–adrenal; IL-1, interleukin 1; ACTH, corticotropin; 5-HT, serotonin (5-hydroxytryptamine); CRH, corticotropin-releasing hormone.

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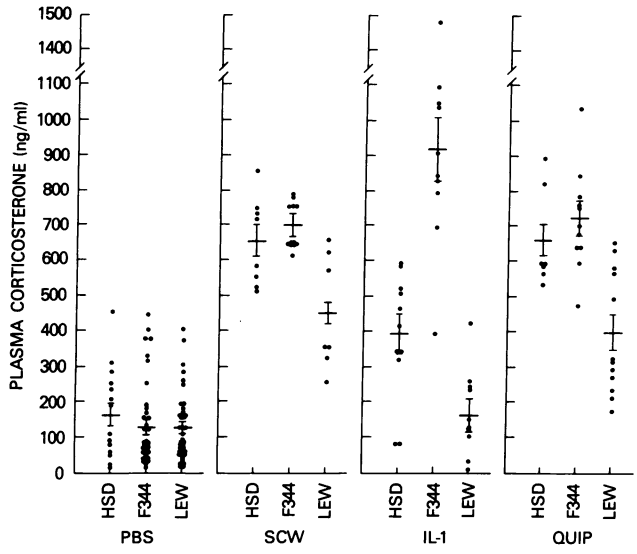

FIG. 1. Plasma corticosterone levels induced by SCW, IL-1α, or quipazine in inbred F344/N and LEW/N rats, and in outbred HSD rats. Rats of each strain were injected i.p. with one mediator, as shown: SCW (2 mg of cell wall rhamnose), 1 μg of recombinant IL-1α, 1 mg of quipazine or PBS control. Corticosterone was determined in plasma collected 60 min postinjection. The 1-hr time point of corticosterone measurement and the doses of mediators used were those found to be associated with maximal corticosterone responses in time course and dose–response experiments (see Figs. 2 and 3). Horizontal lines represent means ± SEM of each group.

bioassay as described (24). Endotoxin levels in final concentrations injected were <0.0013 EU/100 μl. Quipazine was purchased from Sigma. It was injected i.p. at doses ranging from 0.1 to 1 mg per 0.1 ml of sterile water per 100 g. Dexamethasone for cell culture was purchased from Sigma and was used in doses ranging from 0.01 to 100 μg per ml of sterile normal saline per 100 g. The glucocorticoid receptor antagonist RU 486 (25, ‖) was a generous gift from Roussel-UCLAF (Paris). It was suspended in sterile normal saline for i.p. injection at doses ranging from 0.03 to 3 mg per ml per 100 g. The 5-HT antagonist LY53857 [6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, 2-hydroxy-1-methylpropyl ester (*Z*)-2-butenedioate] (26), was a generous gift from M. Cohen, Lilly Research Laboratories, Eli Lilly. It was injected twice daily i.p. at doses ranging from 0.03 to 0.3 mg per 0.1 ml of sterile water per 100 g. Rat/human corticotropin-releasing hormone (CRH) was purchased from Peninsula Laboratories and was used at doses ranging from 0.01 to 8 μg per ml of sterile normal saline per 100 g.

Hormone Assays. Plasma corticosterone was quantitated with a radioimmunoassay (27) kit purchased from Radioassay Systems Laboratories (Carson, CA). ACTH levels were determined by radioimmunoassay as described (28). Inter- and intraassay control variabilities for corticosterone were 1.2% and 3.4%, respectively; inter- and intraassay control variabilities for ACTH were 8.0% and 2.8%, respectively.

RESULTS

Corticosterone Responses to SCW, IL-1α, and Quipazine in Outbred HSD Rats Versus Inbred F344/N and LEW/N Rats. Intraperitoneal SCW, IL-1α, and the 5-HT agonist quipazine all induced marked plasma corticosterone responses in F344/N rats 1 hr after i.p. injection (Fig. 1). In contrast, these agents induced only minimal (SCW, quipazine) or absent (IL-1α) plasma corticosterone responses in LEW/N rats ($P < 0.01$). Outbred HSD rats exhibited mean corticosterone responses intermediate between the low LEW/N and high F344/N responses. Corticosterone responses of HSD rats showed a wide spread and fell into two groups: one overlapping the low LEW/N responses, and the other overlapping the high F344/N responses.

Time Course Kinetics of Plasma ACTH and Corticosterone Responses to SCW, IL-1α, and Quipazine in LEW/N Versus F344/N Rats. Fig. 2 shows that while plasma ACTH peaked at 30–60 min postinjection in both F344/N and LEW/N rats, the LEW/N plasma ACTH response to SCW, IL-1α, and quipazine was consistently lower than the F344/N response at all time points. Similarly, the LEW/N plasma corticosterone response was lower than the F344/N response at all time points. Total time-integrated plasma ACTH and corticosterone responses to SCW, IL-1α, or quipazine were significantly less in LEW/N rats than in F344/N rats (Table 1). In F344/N rats, compared to LEW/N rats, plasma ACTH increased >3-fold as much in response to IL-1α, >2-fold as much in response to SCW, and >1.6-fold in response to quipazine. F344/N rats increased plasma corticosterone >2-fold in response to SCW and IL-1α, and 1.4-fold in response to quipazine when compared to LEW/N rats.

Dose–Responses of Plasma ACTH and Corticosterone to SCW, IL-1α, Quipazine, or Rat/Human CRH in LEW/N Versus F344/N Rats. Figs. 3 and 4 show that at all mediator doses tested, LEW/N rats had lower plasma ACTH and corticosterone levels than F344/N rats.

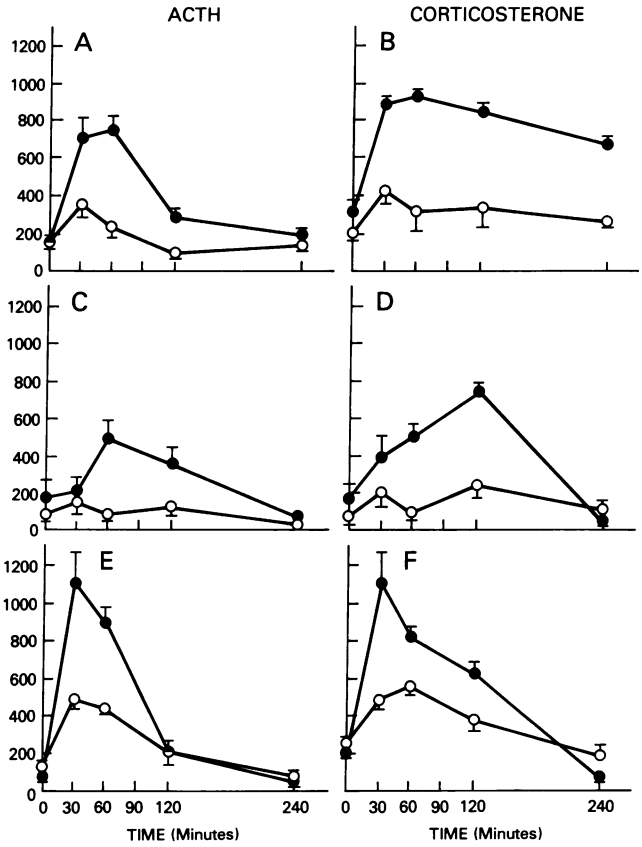

FIG. 2. Time course of plasma ACTH (pg/ml) and corticosterone (ng/ml) responses to SCW (*A* and *B*), human recombinant IL-1α (*C* and *D*), or quipazine (*E* and *F*) in F344/N (●) versus LEW/N (○) rats. Plasma ACTH and corticosterone were quantitated by radioimmunoassay at various time points up to 4 hr after i.p. injection of each agent shown. Data shown are means ± SEM of a minimum of five animals per experimental group.

‖Philibert, D., Deraedt, R. & Teutsch, G., Eighth International Congress of Pharmacology, 1981, Tokyo, p. 668 (abstr. 1463).

Table 1. Total time-integrated plasma ACTH and corticosterone responses to SCW, IL-1α, or quipazine in LEW/N and F344/N rats

	F344/N	LEW/N	*n*	*P*	F/L
	ACTH, ng·ml^{-1}·min^{-1}				
SCW	94.5 ± 4.7	40.3 ± 1.8	20	<0.001	2.3
IL-1	64.9 ± 5.4	20.4 ± 3.7	23	<0.001	3.2
Quipazine	95.5 ± 12.0	57.3 ± 5.5	20	<0.05	1.6
	Corticosterone, μg·ml^{-1}·min^{-1}				
SCW	184.9 ± 3.9	70.3 ± 11.9	32	<0.001	2.6
IL-1	104.5 ± 3.7	38.9 ± 7.9	33	<0.001	2.7
Quipazine	119.6 ± 5.4	87.1 ± 4.4	20	<0.01	1.4

Data represent means ± SEM of total time-integrated plasma ACTH and corticosterone in F344/N and LEW/N rats in response to i.p. SCW (2 mg of cell wall rhamnose per rat), IL-1α (1 μg per rat), or quipazine (1 mg per rat). Data were derived by calculation of the area under time course curves shown in Fig. 2. F/L, ratio of total time-integrated plasma ACTH or corticosterone in F344/N (F) rats versus LEW/N (L) rats.

Pituitary, Adrenal, and Thymus Weights in F344/N Versus LEW/N Rats. Pituitary weights, although not significantly different, were greater in F344/N compared to LEW/N rats (Table 2). F344/N adrenal gland weights were slightly but significantly greater than adrenal gland weights from age-matched LEW/N rats ($P < 0.01$). LEW/N thymus weights were significantly higher than F344/N thymus weights ($P < 0.01$) in age-matched rats.

Effects of Dexamethasone Treatment on LEW/N Rats and of RU 486 or LY53857 on F344/N Rats Treated with SCW. Table 3 shows that not only did dexamethasone in the pharmacologic doses totally suppress the arthritis induced by SCW, but physiologic doses (1 μg daily or 0.5 μg twice daily) also significantly suppressed the severity of arthritis as determined by arthritis index compared to SCW plus saline-treated controls ($P < 0.05$).

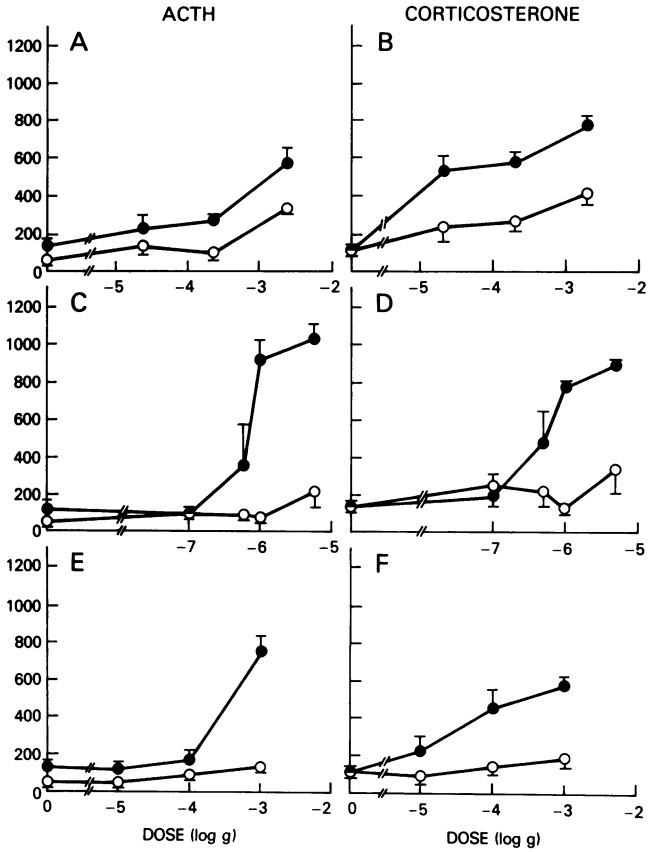

FIG. 3. Dose–responses of plasma ACTH (pg/ml) and corticosterone (ng/ml) responses to SCW (*A* and *B*), human recombinant IL-1α (*C* and *D*), or quipazine (*E* and *F*) in F344/N (●) versus LEW/N (○) rats. Various doses of mediators shown were injected i.p., and plasma ACTH and corticosterone were quantitated by radioimmunoassay 60 min postinjection. Data shown are means ± SEM of a minimum of five animals per group.

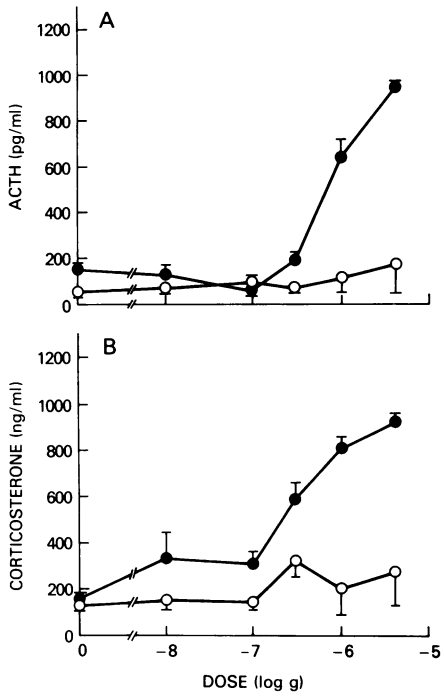

FIG. 4. Dose–response of plasma ACTH and corticosterone to various concentrations of human CRH in F344/N (●) versus LEW/N (○) rats. CRH was injected i.p., and plasma ACTH and corticosterone were measured by radioimmunoassay 60 min postinjection. Data are means ± SEM of a minimum of five animals per experimental group.

Table 4 shows the effect of treatment of F344/N rats with SCW plus the corticosterone receptor antagonist RU 486, or SCW plus the 5-HT antagonist LY53857, compared to either agent alone. Minimal mortality was observed in F344/N rats treated with SCW alone, and no mortality was observed in F344/N rats treated with RU 486 or LY53857 alone. RU 486, which had no effect alone (3 mg daily), was highly toxic when administered i.p. together with SCW, resulting in 100% mortality. Doses of RU 486 as low as 0.03 mg daily, when administered to SCW-treated rats, were still associated with significant inflammatory morbidity and mortality compared to controls. RU 486 has previously been shown to exacerbate carageenin-induced inflammation without significant mortality (25). Increased mortality in the SCW arthritis model was probably related to the severe peritonitis that developed in association with the combined i.p. administration of the two agents. At doses of RU 486 low enough to permit survival, surviving rats developed acute arthritis, in some cases of moderate severity (e.g., mean arthritis index = 4.5 at 0.3-mg RU 486 dose). Concurrent treatment of F344/N rats with SCW and the 5-HT antagonist LY53857 was not associated

Table 2. Pituitary, adrenal, and thymus weights in age-matched LEW/N and F344/N rats

Strain	Pituitary wt, mg (*n*)	Adrenal wt, mg (*n*)	Thymus wt, mg (*n*)
F344/N	9.6 ± 0.5 (10)	15.6 ± 0.5 (27)	260.0 ± 8.9 (10)
LEW/N	8.5 ± 0.4 (13)	13.3 ± 0.7 (26)	307.3 ± 8.2 (10)
P value	NS	<0.01	<0.01

NS, not significant.

Table 3. Dexamethasone suppression of SCW arthritis in LEW/N rats (72 hr)

Dexamethasone dose, μg	Incidence of arthritis	Severity of arthritis, mean A.I. ± SEM
0	5/5	8.3 ± 2.1
0.1 (b.i.d.)	5/5	8.4 ± 1.8
0.5 (b.i.d.)	4/5	3.4 ± 1.5*
1.0 (QD)	5/5	2.0 ± 0.5*
10.0 (QD)	0/4	0*
100.0 (QD)	0/5	0*

LEW/N rats were injected with a single dose of SCW (2 mg of cell wall rhamnose per rat), followed by dexamethasone, at the doses indicated, or saline controls. Dexamethasone injections were given once (QD) or twice (b.i.d.) daily for a total of 72 hr, and severity of arthritis (A.I., articular index) was quantitated as described (2) at 72 hr post-SCW injection by a single-blinded observer. Briefly, A.I. is the sum of the severity of arthritis (scale, 0–4 where 4 is most severe arthritis) of each of the limbs. Maximum A.I. is 16.
*A.I. significantly less than A.I. of control animals (dexamethasone dose 0, saline only) ($P<0.05$).

with significant mortality, but it was associated with development of mild to moderate arthritis compared to control rats treated with either agent alone ($P < 0.05$). Although not all dose variables were explored, the data clearly show that blocking the effects of corticosterone or 5-HT in SCW-treated F344/N rats results in severe or even fatal systemic inflammatory disease.

DISCUSSION

In the experiments reported here, we have shown that acute corticosterone responses to SCW, IL-1α, and quipazine are severely depressed in arthritis-susceptible LEW/N rats compared to arthritis-resistant F344/N rats. Outbred HSD rats, which exhibit an intermediate mean susceptibility to SCW-induced arthritis with wide variability (29), also showed an intermediate mean and wide variability of corticosterone responses to these mediators. Replacement of corticosterone with physiologic doses of dexamethasone significantly suppressed the severity of SCW arthritis in LEW/N rats. Conversely, antagonism of corticosterone in F344/N rats, with the corticosterone receptor antagonist RU 486, was associated with increased mortality, exacerbation of inflammation, and development of mild to moderate acute arthritis in this otherwise resistant strain. It is clear from these studies that, whether present on a genetic basis, as in LEW/N rats, or on a pharmacological basis, as in RU 486-treated F344/N rats, a deficiency in the corticosterone response to SCW is associated with development of susceptibility to SCW-induced inflammatory disease.

Table 4. Effect of RU 486 or LY53857 on mortality and arthritis in F344/N rats treated with SCW

Agent(s) injected	Mortality	Incidence of arthritis in surviving rats	Severity of arthritis in surviving rats (A.I.)
SCW + saline	2/15	1/13	0.2 ± 0.05
Saline + RU 486 (3.0 mg)	0/6	0/6	0
SCW + RU 486 (0.03 mg)	1/5*	2/4	0.5 ± 0.3
SCW + RU 486 (0.1 mg)	2/5*	1/3	0.3 ± 0.3
SCW + RU 486 (0.3 mg)	3/5*	1/2	4.5 ± 4.5†
SCW + RU 486 (1.0 mg)	4/5*	0/1	0
SCW + RU 486 (3.0 mg)	5/5	—	—
Saline + LY53857 (0.3 mg)	0/7	0/7	0
SCW + LY53857 (0.03 mg)	1/5	2/4	2 ± 1.2†
SCW + LY53857 (0.3 mg)	0/5	2/5	2 ± 1.5

F344/N rats (five animals per group) were treated with a single injection of SCW (2 mg of cell wall rhamnose per rat), followed by daily i.p. injections of RU 486 or twice daily i.p. injections of LY53857 at doses indicated. Control animals were treated with SCW plus saline, RU 486 plus saline, or LY53857 plus saline. Articular index (A.I.) was quantitated by a single-blinded observer at 72 hr post-SCW injection. Maximum A.I. is 16.
*Surviving rats receiving RU 486 plus SCW showed ruffled fur and peritoneal inflammation at necropsy.
†$P<0.05$ compared to A.I. of SCW plus saline-treated controls.

Recent evidence has suggested that elevation of corticosterone during inflammation may result from stimulation of the HPA axis by inflammatory and immune mediators, such as endotoxin (bacterial lipopolysaccharide) and IL-1 (6–12). Since SCW are chemically related to endotoxin and stimulate the release of IL-1 from activated macrophages (30), SCW could increase corticosterone via IL-1 stimulation of the HPA axis. The studies presented here show that LEW/N rats have depressed corticosterone and ACTH responses to SCW and absent corticosterone and ACTH responses to IL-1α. These findings suggest that the defective HPA axis response to inflammatory mediators in LEW/N rats is at the hypothalamic and/or the pituitary level. The greater LEW/N corticosterone and ACTH response to SCW compared to IL-1α may be related to the more sustained nature of the stimulus, as well as to possible stimulation of the HPA axis at multiple levels by the many inflammatory mediators released by SCW, in addition to IL-1, such as interleukin 2 and tumor necrosis factor.

The smaller adrenal glands and the larger thymuses of LEW/N compared to F344/N rats are also consistent with deficient HPA axis responses and chronic mild hyposecretion of corticosterone. LEW/N rats also have depressed ACTH and corticosterone responses to exogenous CRH. This could be secondary to inadequate priming of the anterior pituitary corticotroph by endogenous CRH or other ACTH secretagogues, or to some inherent defect of this cell. It is impossible from the data presented here to precisely define the site of the defect, whether hypothalamic or pituitary, particularly since chronic over- or understimulation of the HPA axis, from whatever cause, could result in secondary changes in baseline and stimulated levels of CRH, ACTH, and corticosterone production, as well as secondary changes in adrenal and thymus sizes (4, 31).

The observation that LEW/N rats are deficient in ACTH and corticosterone responses to the 5-HT agonist quipazine, as well as to IL-1α and SCW, suggests that the defect in these rats is not solely related to impaired IL-1 stimulation of the HPA axis. The greater LEW/N corticosterone response to quipazine compared to IL-1α may result from the multiple pathways through which 5-HT and 5-HT agonists may stimulate the HPA axis since 5-HT is both a major CRH and a potent ACTH secretagogue (16–23). The physiologic relevance of such a potential route of 5-HT stimulation of the HPA axis is, however, not clear, since it would be dependent on adequate systemic concentrations of 5-HT released from platelets during inflammation reaching central sites. The importance of 5-HT pathways in intactness of the inflammatory mediator–HPA axis loop and arthritis resistance is also suggested by the development of arthritis in SCW-injected rats treated with the 5-HT_2 antagonist LY53857. However, the interpretation of the central effects of LY53857 in induction of arthritis is complicated by the drug's potential local anti-inflammatory activity, which is reflected in the lower mortality of the LY53857 plus SCW-treated animals compared to the RU 486 plus SCW-treated animals.

The data presented here, coupled with the markedly enhanced inflammatory disease in SCW-injected F344/N rats following pharmacologic interruption of the HPA axis, and suppression of arthritis severity in SCW-injected LEW/N rats following replacement doses of dexamethasone, provide strong evidence that arthritis susceptibility in the LEW/N rat,

and resistance in the F344/N rat, is regulated, at least partially, through corticosterone production and HPA axis responsiveness to inflammatory and possibly other stress mediators. LEW/N rats, therefore, represent a unique animal model for a genetically determined defect in the central nervous system–inflammatory/immune system feedback loop (i.e., the stress response). Whether of hypothalamic or pituitary origin, this defect is associated with increased susceptibility to arthritis in response to SCW, and could also contribute to the increased susceptibility to other experimentally induced inflammatory diseases observed in LEW/N rats (32–39).

The data may also have implications for susceptibility to rheumatoid arthritis in humans. Our data raise the possibility that susceptibility to rheumatoid arthritis may be related to defective HPA axis production of stress hormones in response to inflammatory and possibly other stress mediators. Therefore, evaluation of these responses in patients with rheumatoid arthritis may provide new insights into the pathogenesis of rheumatoid arthritis and other inflammatory/autoimmune diseases.

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