Corticotropin Releasing Hormone (CRH) Antagonist Attenuates Adjuvant Induced Arthritis: Role of CRH in Peripheral Inflammation

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ABSTRACT. ***Objective.*** To determine whether a corticotropin releasing hormone (CRH) type 1-specific receptor antagonist, antalarmin, would alter the progression of inflammation in adjuvant induced arthritis (AIA) susceptible LEW/N rats by blocking local CRH mediated inflammatory responses or render AIA resistant F344/N rats more susceptible to AIA by blocking central CRH, thus reducing secretion of endogenous glucocorticoids.

Methods. F344/N and LEW/N rats were assigned to either drug or vehicle groups and treated with 20 mg/kg antalarmin or vehicle alone BID for 25 days by intraperitoneal injection. Arthritis was induced in both antalarmin and vehicle treated LEW/N and F344/N rats by subcutaneous injections at the base of the tail of incomplete Freund's adjuvant containing 10 mg/ml heat killed *Mycobacterium tuberculosis*. Control F344/N and LEW/N rats were maintained on either antalarmin or vehicle.

Results. Chronic blockade of CRH-R1 with systemic antalarmin significantly ameliorated AIA in LEW/N rats, reducing the severity of inflammation in peripheral joints, evidenced by clinical and histopathology scores, and weight loss associated with disease onset. Antalarmin neither induced nor exacerbated arthritis expression in F344/N or LEW/N rats, despite suppression of levels of adjuvant induced corticosterone, the major antiinflammatory glucocorticoid in rats.

Conclusion. Systemic blockade of CRH-R1 appeared to predominantly block peripheral proinflammatory effects of immune CRH, rather than the systemic glucocorticoid mediated antiinflammatory effects of hypothalamic CRH. Results indicate that chronic treatment with a CRH antagonist attenuates progressive inflammation induced degeneration of synovia, cartilage, and bone in arthritic joints, suggesting that antalarmin may have therapeutic potential in treatment of human autoimmune and inflammatory disorders. (J Rheumatol 2002;29:1252–61)

Key Indexing Terms:
CORTICOTROPIN RELEASING HORMONE RECEPTOR ANTAGONIST RATS
INFLAMMATION ADJUVANT INDUCED ARTHRITIS GLUCOCORTICOIDS

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation in joints and other tissues. The prevalence of RA varies worldwide between 0.5 and 2%[1]. Disease modifying agents to treat RA include the new generation of anti-tumor necrosis factor (TNF) and agents targeted to interleukin 1 (IL-1); however, the therapeutic response and tolerability to these compounds is varied. Elucidation of the role of corticotropin releasing hormone (CRH), as both an endocrine regulator of inflammation through the glucocorticoids and a direct autocrine regulator

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of inflammation in the periphery, suggests that drug design aimed at this hormone might provide new avenues for treatment of inflammatory diseases such as RA.

CRH is a major regulator of the hypothalamic-pituitary-adrenal (HPA) axis and principal coordinator of the stress response that also profoundly influences the immune system and plays a role in susceptibility and resistance to autoimmune diseases such as RA[2]. This occurs both indirectly through activation of the systemic hormonal stress response and directly through local modulatory actions on inflammatory responses[3]. Neuroendocrine CRH release plays an indirect immunosuppressive and antiinflammatory role through glucocorticoid release during activation of the HPA axis[4,5], while immune CRH, released at peripheral sites of inflammation, plays a direct immunostimulatory role as an autocrine or paracrine mediator of inflammation[6]. CRH is hypersecreted at inflammatory sites in experimental animal models of inflammation, and in autoimmune inflammatory diseases in humans[7]. Notably high concentrations of CRH and CRH mRNA have been observed in synovial tissue from acute and chronic streptococcal cell wall and adjuvant induced arthritis in Lewis rats[8], and in synovial tissues from joints of patients with RA[9,10], psoriatic arthritis, and sarcoid arthritis[10]. Further, inflammatory mediators including IL-1ß, TNF-α, and prostaglandin E_2 increase CRH mRNA in primary cultures of synoviocytes[10]. CRH binding sites and immunoreactive CRH-R1 receptors have been identified on a number of immune and immune-accessory cells, including human peripheral blood leukocytes[7], and in inflamed synovial tissues[9,10]. Proinflammatory actions of CRH include mast cell degranulation, leukocyte proliferation and cytokine secretion, monocyte chemotaxis, and production of oxygen radicals by macrophages[7,11].

In addition to the proinflammatory effect of CRH released locally at sites of inflammation, CRH released centrally from the hypothalamus has a largely immunosuppressive effect through activation of the HPA axis and release of glucocorticoids from the adrenals. Under physiological conditions, this negative feedback loop resulting from activation of the HPA axis by inflammatory mediators serves to regulate and restrain immune responses. In contrast, a hypoactive HPA axis is associated with an enhanced susceptibility to inflammatory disease[2,4,5]. In humans, fibromyalgia and chronic fatigue syndrome as well as autoimmune and allergic diseases including RA, Sjögren's syndrome, systemic lupus erythematosis, and allergic asthma and dermatitis[12-14] have all been associated with a hyporesponsive HPA axis. The immunosuppressive and antiinflammatory actions of even small doses of glucocorticoids on their target immune tissues and cells are well known and have made them invaluable as therapeutic agents in numerous autoimmune/inflammatory and allergic diseases.

Both association and intervention studies in animal models provide strong evidence for the role of blunted CRH and HPA axis responses in autoimmune/inflammatory disease. Inbred Lewis (LEW/N) rats with a hyporesponsive HPA axis are susceptible to autoimmune inflammatory disease, while Fischer (F344/N) rats that exhibit a hyperresponsive HPA axis are relatively resistant to inflammation[4,5]. The ability to manipulate the HPA axis and measure effects due to autoimmune disease expression in these inbred rat strains provides evidence for a causal relationship between blunted HPA axis responses and susceptibility to inflammatory disease. Interruption of the HPA axis at any point and by many means, including pharmacologic blockade of the glucocorticoid receptor with RU486[4], adrenalectomy[15], or hypophysectomy[16], renders inflammatory/autoimmune resistant rats susceptible to these diseases. Conversely, reconstitution of the HPA axis by intracerebroventricular fetal hypothalamic tissue transplantation reverses the susceptibility of LEW/N rats to autoimmune diseases[17].

In light of the multiple mechanisms by which CRH is involved in regulation of inflammation, we investigated whether administration of antalarmin, with its dual actions, would alter the progression of inflammation in adjuvant induced arthritis (AIA) in LEW/N rats by blocking local CRH mediated inflammatory responses, or render F344/N rats more susceptible to AIA by blocking central CRH, thus reducing secretion of endogenous glucocorticoids. Our previous results show that antalarmin penetrates into the central nervous system in rats and nonhuman primates, suppressing behavioral, autonomic, and endocrine actions of CRH[18], and also directly suppresses inflammation in an innate model of experimental inflammation induced by carrageenan[18-21].

Our results here show that chronic treatment with a CRH antagonist attenuated the progressive inflammatory induced degeneration of synovia, cartilage, and bone in arthritic joints and suggest that it has important therapeutic potential in the treatment of autoimmune and inflammatory disorders. These results also suggest that blockade of the peripheral proinflammatory effects of CRH by antalarmin play a relatively greater role in regulation of inflammatory arthritis in this model than central HPA axis blockade.

MATERIALS AND METHODS

Antalarmin. N-butyl-N-ethyl-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7Hpyrrolo[2,3-d]pyrimidine-4-yl]amine was as described[22]. For *in vivo* administration, it was dissolved in EtOH: Cremaphor EL (BASF, Mount Olive, NJ, USA): water (5:5:90) and injected intraperitoneally as warm (37ºC) freshly prepared solution.

Animals. All procedures were in accord with the NIH guide for the care and use of laboratory animals under a protocol (CH 91-036) approved by the Animal Care and Use Committee of the NIMH. Virus antibody-free male Fischer (F344/N) and Lewis LEW/N rats (Harlan Sprague Dawley; Indianapolis, IN, USA) were maintained in restricted access housing under 12 h light/dark and standard lighting, temperature, and cage conditions. Male animals were used because of concerns regarding potential modula-

tion by estrogens on the expression of inflammatory diseases[2]. Animals were allowed 7–10 days after shipping to acclimate to the housing facility. At 6–7 weeks of age, the rats were lightly anesthetized with CO_2 and implanted with subcutaneous microchip identification capsules (Lab Trac Animal ID System, Avid, Norco, CA, USA) to allow observers to evaluate and rate individual animals for disease severity while blind to treatment group assignment. There were 8 groups of animals, 8 animals per group, consisting of adjuvant and naive F344/N rats treated with antalarmin or vehicle and adjuvant and naive LEW/N rats treated with antalarmin or vehicle.

Induction of arthritis and drug treatment. F344/N and LEW/N rats were assigned to either drug or vehicle study groups and treated with 20 mg/kg antalarmin or vehicle alone BID for 25 days beginning on Day –1 by IP injections. On Day 0, arthritis was induced in both antalarmin and vehicle treated LEW/N and F344/N rats by subcutaneous injections at the base of the tail of 0.1 ml of incomplete Freund's adjuvant (Difco Laboratory, Detroit, MI, USA) containing 10 mg/ml heat killed *Mycobacterium tuberculosis* (Difco). Control F344/N and LEW/N rats were maintained on either antalarmin or vehicle for comparison with the adjuvant injected animals. Body weight was recorded daily. F344/N animals were killed on Day 23 and LEW/N rats on Day 24 by decapitation between 10:00 AM and 1:00 PM. Each animal received either 20 mg/kg antalarmin or vehicle 90 min prior to decapitation and blood was collected for hormone analysis.

Clinical evaluation. The onset and severity of disease was monitored daily and evaluated independently by 3 observers. To quantitatively evaluate the severity of the arthritis, we used a scoring system that correlates the arthritis severity with joint size[23]. Briefly, the wrist, midforepaw, ankle, and midfoot joint were rated 0–4 based on the degree of swelling and the ability to bear weight. The presence of arthritis in each of the 3 joints of the 4 lateral digits of each paw was also recorded: presence = 1, absence = 0. Summing the numbers for each individual joint yielded the total score. Thus the maximum score per animal was 80, 20 per extremity.

Hormone and cytokine measurement. Trunk blood was collected in tubes containing EDTA. Plasma ACTH and corticosterone were measured by radioimmunoassay kits (ICN Biomedicals, Orangeburg, NY, USA).

Histopathology. The bilateral forepaws and hind paws were excised, denuded of skin, fixed in 10% buffered formalin, and decalcified in 50% formic acid and 50% citrate buffer. After paraffin embedding, sections were serially cut and then stained with routine H&E. The ankle and wrist joints were evaluated for the presence of inflammation in the intraarticular synovial membrane, tendon sheaths, joint capsule, and periarticular soft tissue. Each joint was evaluated independently and scored from zero to 4+ depending on the degree of inflammation. Zero was assigned to joints with no inflammatory cells (equivalent to controls) and 4+ to joints with severe acute and chronic inflammation involving the entire synovial membrane and secondarily involving periarticular soft tissues.

Data analysis. All data are presented as the mean ± SEM. Statistical significance was inferred at the $p < 0.05$ level unless otherwise noted. For clinical scores, the mean of the average scores of the 3 observers for each animal was determined and used to calculate the treatment mean for each day. Analysis of the clinical scores indicated that the scores assigned by the 3 observers were highly correlated; the Pearson product moment score was consistently > 0.9, $p < 0.0001$, for the 3 observers' scores on each day. Statistical significance was evaluated by MANOVA, repeated measures for body weight and clinical scores. Post-hoc nonparametric analysis was performed when appropriate. Because the hormone values were not distributed normally and exhibited unequal variances, these data were analyzed by Wilcoxon rank-sum test using PC-SAS Version 8. The Mann-Whitney nonparametric test was used to evaluate significance in histopathology scores in LEW/N-A-VEH vs LEW/N-A-ANT rats.

The results replicate the findings of a preliminary study (data not shown). Due to differences in the method used for clinical scoring (maximum score 20), the results presented here are from the second study only. The effect of antalarmin on the clinical and hormone profile for LEW/N and F344/N rats is essentially identical in the 2 studies.

RESULTS

The results are summarized in Table 1.

HPA hormones. Blockade of CRH-R1 receptors by BID injections of 20 mg/kg antalarmin significantly suppressed the pituitary-adrenal hormones in naive, non-adjuvant injected LEW/N rats (Figure 1, Table 1). LEW/N-N-VEH rats had significantly higher ($p < 0.05$) basal adrenocorticotropic hormone (ACTH) values (155 ± 34 pg/ml) than the chronic antalarmin treatment group, LEW/N-N-ANT (47 ± 9 pg/ml) (Figure 1a). Basal corticosterone values in naive antalarmin treated rats were barely detectable, 6 ± 1 ng/ml, and significantly lower compared to mean values of 33 ± 15 ng/ml in vehicle treated rats (Figure 1b). In adjuvant injected (AI) LEW/N rats, basal ACTH levels in the antalarmin treated rats were similar to the vehicle group, 187 ± 25 and 130 ± 21 pg/ml, respectively.

Antalarmin significantly suppressed basal plasma corticosterone values (17 ± 9 ng/ml) compared to vehicle treated (68 ± 21 ng/ml) adjuvant injected LEW/N rats. F344/N rats had higher and more variable basal ACTH values than did the naive LEW/N rats, despite frequent handling and housing in a quiet animal facility with restricted access. Neither adjuvant injection nor CRH-R1 antagonist treatment significantly altered basal plasma ACTH values in F344/N rats. However, in the adjuvant injected F344/N rats, antalarmin significantly suppressed basal plasma corticosterone compared to vehicle, 140 ± 31 vs 23 ± 6 ng/ml, respectively. In addition, adjuvant treatment increased plasma corticosterone values in F344/N rats as seen in the F344/N AI-VEH group (140 ± 31 ng/ml) compared to the F344/N N-VEH (17 ± 4 ng/ml) group of rats. The ratio of ACTH to corticosterone, a measure of adrenal responsivity, was not significantly different between any group for either F344/N or LEW/N, regardless of antalarmin treatment or adjuvant injection. Further, neither the adrenal weight nor the adrenal:body weight ratio was significantly altered by antalarmin treatment in either AI F344/N or LEW/N rats (data not shown).

Body weight. Non-adjuvant injected (naive) rats showed a 50–60% increase in body weight over the course of the experiment regardless of strain or treatment with antalarmin (Figure 2, Table 1). Both LEW/N and F344/N naive controls gained more weight than AI rats, with and without antalarmin treatment. Statistical analysis by MANOVA, using 3 day averages of body weight gain relative to Day –1, indicated that both naive LEW/N and naive F344/N gained significantly more weight than the adjuvant injected counterparts. Both antalarmin and vehicle treated AI LEW/N rats showed a steady increase in body weight until Day 12, the same day that the first clinical signs of joint inflammation appeared. Post-hoc comparison of the means indicated that AI LEW/N antalarmin treated rats achieved small but significantly greater increases in body weight than did AI LEW/N rats that were vehicle treated from Day 15 until the end of

Table 1. Summary of results.

Condition	ACTH, pg/ml	Corticosterone, ng/ml	Weight, % Gain	Arthritis, Clinical Score	Histopathology, Inflammatory Score
LEW/N N-VEH	155 ± 34	33 ± 15	62*	NA	NA
LEW/N N-ANT	47 ± 9**	6 ± 1**	59*	NA	NA
F344/N N-VEH	553 ± 130	17 ± 4†	60††	NA	NA
F344/N N-ANT	499 ± 221	47 ± 26	53††	NA	NA
LEW/N AI-VEH	187 ± 25	68 ± 21	7	23 ± 3	11 ± 1
LEW/N AI-ANT	130 ± 21	17 ± 9**	12**	12 ± 3**	7 ± 1**
F344/N AI-VEH	317 ± 92	140 ± 31†	28	~0	NA
F344/N AI-ANT	297 ± 59	23 ± 6**	29	~0	NA

* Significantly different from LEW/N AI-ANT, and -VEH, respectively, $p < 0.05$. ** Significant difference between vehicle/antalarmin pairs, $p < 0.05$. † Significant difference between F344/N N-VEH and F344/N AI-VEH, $p < 0.05$. †† Significantly different from F344/N AI-ANT, and -VEH, respectively, $p < 0.05$. NA: Not applicable; animals did not develop arthritis.

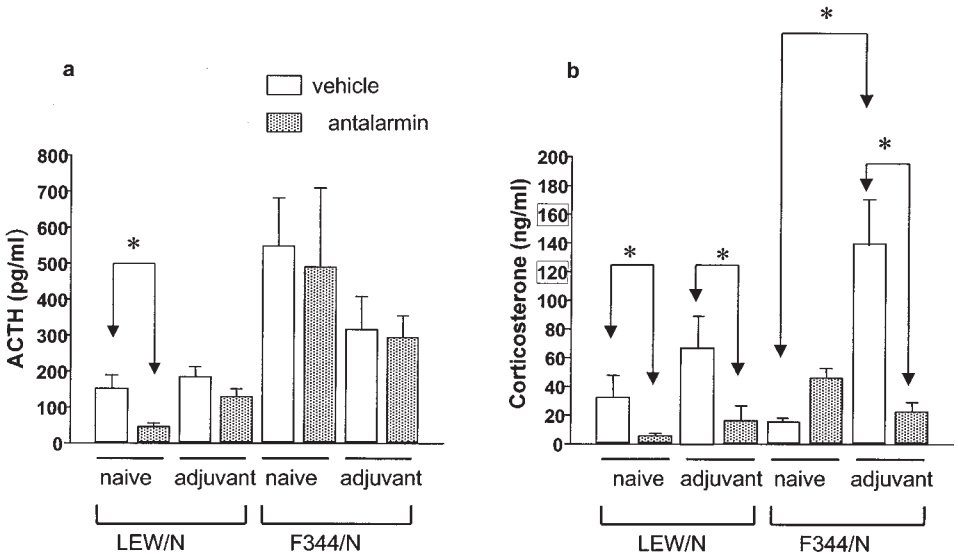


Figure 1. Plasma ACTH (a) and corticosterone (b) responses in naive and adjuvant injected F344/N and LEW/N rats, treated twice daily with either antalarmin or vehicle. Each point is the mean ± SEM of each animal's values. *$p < 0.05$, MANOVA followed by post-hoc Student t comparison of pairs.

the experiment. In contrast to the AI LEW/N rats, antalarmin treatment did not influence weight gain in F344/N rats.

Development of adjuvant induced arthritis. Consistent with our previous findings[4,5], F344/N rats were resistant to experimental arthritis (Figure 3a). Neither vehicle nor antalarmin treated AI F344/N rats developed significant clinical signs of arthritis. Both antalarmin treated and vehicle treated AI F344/N rats showed some transient swelling in digits, but never exhibited an average clinical score > 3 out of 80.

In contrast, AI LEW/N developed clinical symptoms associated with arthritis, marked swelling and erythema, in peripheral joints, predominately ankles. Compared to the vehicle treated rats, antalarmin treated rats had significantly lower articular severity indices (Figure 3a). On Day 12, 6 vehicle treated and 2 antalarmin treated AI LEW/N rats developed arthritis. By Day 13, clinical signs were evident in 100% of the vehicle treated and 75% of antalarmin treated LEW/N rats. By Day 14, 87.5% of the AI LEW/N antalarmin treated animals had developed arthritic symptoms. The incidence of clinical expression remained at 100% for vehicle treated and antalarmin treated rats from Day 15 until the end of the study. The articular severity scores plateaued around Day 21–22, as previously observed[24]. Statistical analysis by MANOVA revealed that the clinical scores were significantly higher over time and in vehicle treated than antalarmin treated AI LEW/N rats ($p < 0.05$). The maximum clinical score for vehicle treated

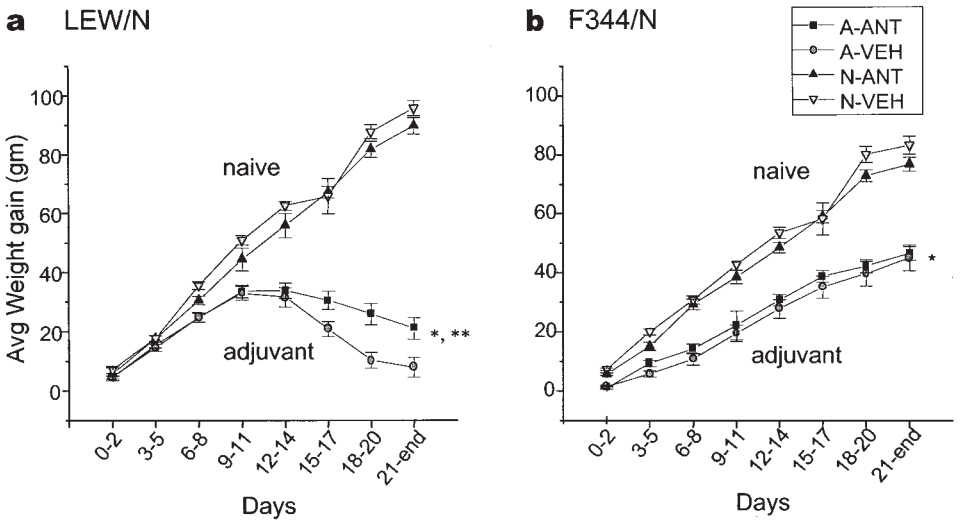


Figure 2. Average weight gain in naive and adjuvant, antalarmin treated and untreated LEW/N and F344/N rats before treatment with either adjuvant or antalarmin. Each point represents the group mean ± SEM (n = 8) of each animal's average 3 day body weight gain relative to Day –1. *p < 0.05 vs naive animals of the same strain, **p < 0.05 antalarmin vs vehicle treated adjuvant LEW/N rats from Day 15-17 to end (MANOVA followed by post-hoc t test).

animals was 23.8 ± 2.6 compared to 12.4 ± 2.8 (mean ± SEM) for antalarmin treated animals.

Histopathology scores, grading severity of inflammation in histologically stained fore and hindpaw sections from antalarmin treated and untreated AI LEW/N rats, also showed significantly more intense inflammatory damage in the joints of vehicle treated compared to antalarmin treated animals (11.03 ± 1.15 vs 6.75 ± 0.96; $p < 0.02$) (Figure 3b, Figure 4). Histopathology scores, the sum score of all 4 paws per animal, were highly correlated with the total clinical score (Spearman $r = 0.85$, $p < 0.0001$).

DISCUSSION

Our findings show a significant effect of the CRH-R1 antagonist antalarmin in altering the progression of inflammatory arthritis and also suggest a major role for CRH-R1 in inflammatory arthritis. First, chronic blockade of CRH-R1 with systemic antalarmin significantly ameliorated adjuvant induced arthritis in LEW/N rats, reducing both the severity of inflammation in peripheral joints and weight loss associated with disease onset. Second, treatment with antalarmin neither induced nor exacerbated arthritis expression in F344/N and LEW/N rats, despite suppression of adjuvant induced corticosterone levels, the major antiinflammatory glucocorticoid in rats. While these studies do not directly address the mechanism by which antalarmin suppresses adjuvant arthritis disease activity, they do indicate that the primary actions of antalarmin are through blockade of the peripheral proinflammatory effects of CRH. Thus, blockade of peripheral proinflammatory CRH has a relatively greater effect on disease outcome than blockade of central activation of pituitary-adrenal axis in this preclinical model of arthritis. This is consistent with findings[7,11] that peripheral CRH acts on local immune cells and blood vessels to elicit proinflammatory responses. In the carrageenan induced subcutaneous inflammation model, systemic administration of rabbit anti-CRH sera decreased inflammatory exudate volume and cellularity in rats[11], and antalarmin administration decreased exudate cellularity in rats[18] and mice[11]. Administration of CRH antiserum in the early stages of the disease attenuated the severity of experimentally induced uveitis in mice[25] and CRH and stress induced increases in vascular permeability and mast cell degranulation[26,27]. Further, CRH-R1 receptor deficient mice showed attenuated hind limb swelling in the turpentine induced inflammatory model[28].

Although pharmacological and surgical interruption of the HPA axis predisposes to inflammation[5,29], exogenous and endogenous glucocorticoids (i.e., dexamethasone, cortisol) in physiological as well as pharmacological doses ameliorate, but do not alter, the progression of arthritis in LEW/N rats[5]. Thus, while it seems counterintuitive that the diminished corticosterone levels associated with chronic antalarmin treatment did not induce inflammation in F344/N rats, the relative levels of corticosterone persisting in the 2 strains may help explain this observation. Despite the attenuation by antalarmin of basal corticosterone levels in the adjuvant treated rats, the level of corticosterone persisting may have been adequate to suppress arthritis in the inflammatory resistant F344/N rats in contrast to the susceptible LEW/N rats. Corticosterone levels in adjuvant treated rats were higher than naive animals in both F344/N and LEW/N rats; however, the mean corticosterone value in the adjuvant treated LEW/N was less than half that achieved by adjuvant

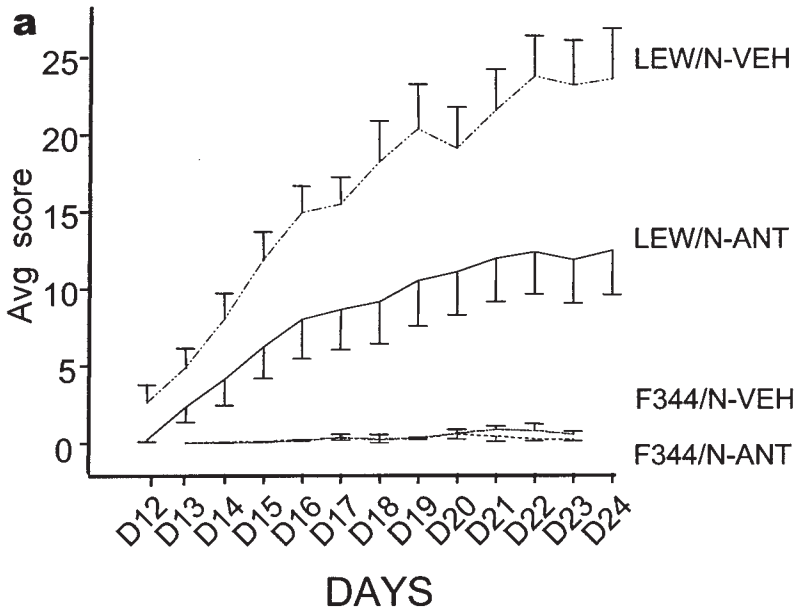


Figure 3A. Average clinical scores evaluating arthritis severity in forepaws and hindpaws of adjuvant injected LEW/N and F344/N rats, treated and untreated with antalarmin. Each point is the mean ± SEM of each group of 8 rats. Statistical analysis by MANOVA revealed that the clinical scores were significantly higher in vehicle treated than antalarmin treated LEW/N rats, and over time, $p < 0.05$.

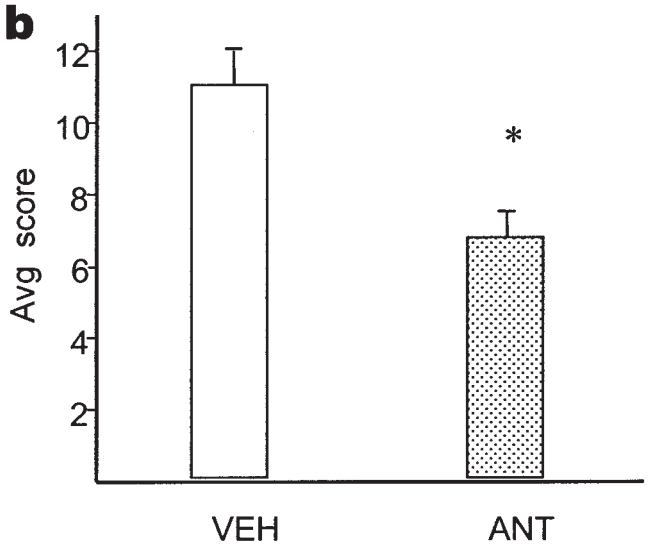
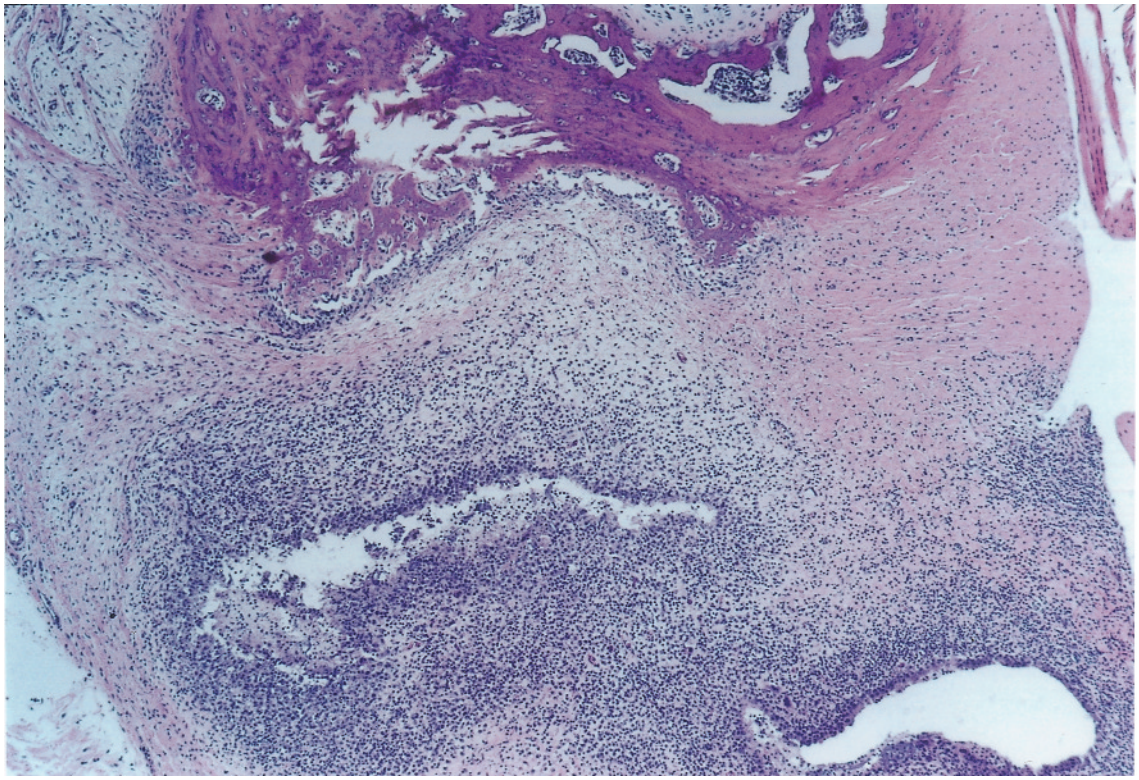


Figure 3B. Histopathology scores representing the sum score of all 4 paws of each animal, mean ± SEM of antalarmin or vehicle treated adjuvant injected LEW/N rats (n = 8 for each group). *$p < 0.02$ antalarmin vs vehicle treated values (Mann-Whitney nonparametric test).

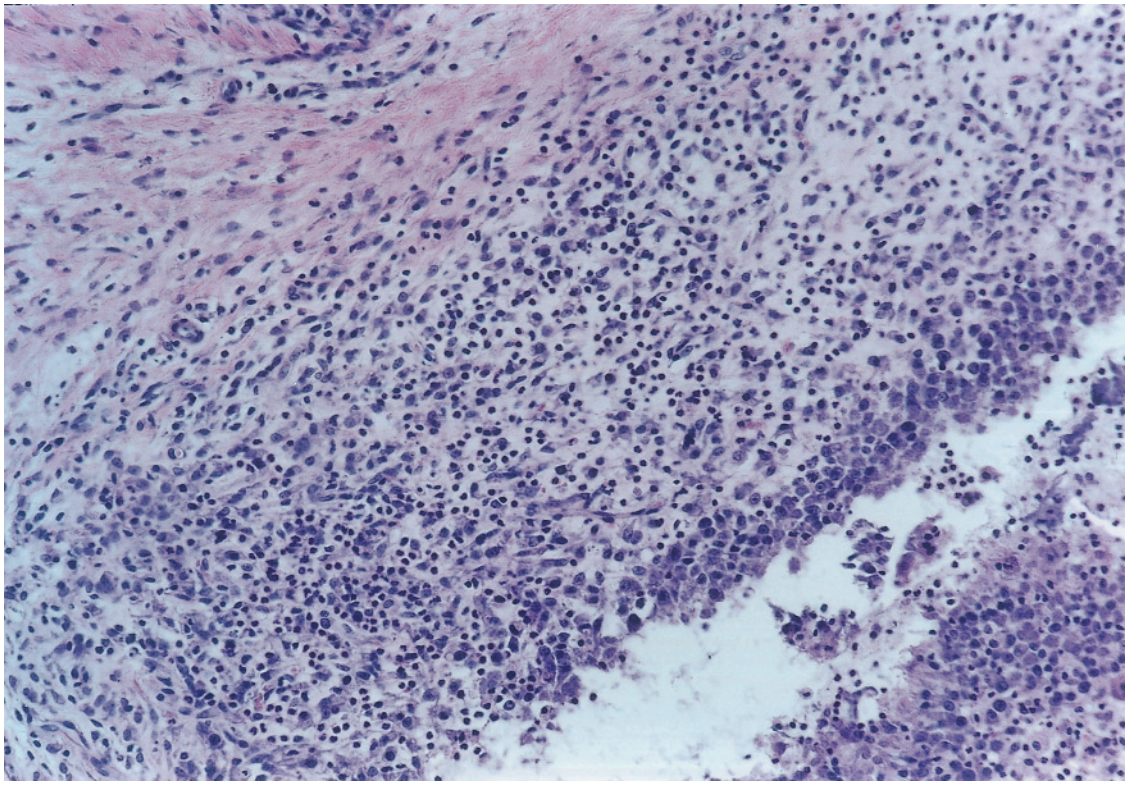
treated F344/N rats. Thus, these data are also consistent with our findings[4,5] regarding differential HPA axis responsiveness to inflammatory stimuli in these 2 strains.

The sympathetic nervous system also plays an important role in regulation of inflammation and arthritis. Recently, a proinflammatory role for epinephrine in acute local inflammation was reported in CRH deficient mice. Epinephrine and CRH appeared to be additive, as the combination of antalarmin plus propranolol was more effective than either agent alone in reducing carrageenan induced subcutaneous inflammation in wild-type mice[11]. Further, Lorton and colleagues[30] observed that denervation of sympathetic innervation to arthritic joints and secondary lymph nodes reduced arthritis, while 6-hydroxydopamine (6-OHDA) lesioning, sparing sympathetic hind limb innervation, exacerbated arthritic symptoms in rats. The amelioration of arthritis upon sympathetic denervation of the hind limbs may be explained by the loss of noradrenergic stimulation of proinflammatory vascular and immune cell responses. Further evidence for the dual role of both HPA axis and sympathoneuronal pathways in inflammatory arthritis has been reported in juvenile RA[31,32]. Chronic antalarmin treatment reduces both social stress induced increases in plasma epinephrine and norepinephrine in monkeys[20], and acute antalarmin administration reduced restraint stress induced increases in plasma norepinephrine in Sprague-Dawley rats[33]. Thus, it is tempting to speculate that the amelioration of adjuvant induced arthritis in the LEW/N rats treated with antalarmin results from the combined effects of antalarmin in decreasing sympathetic outflow as well as blocking local immune CRH.

In addition to these effects on arthritis, antalarmin had differential effects in suppressing corticosterone vs ACTH. In our study, treatment with antalarmin resulted in more robust reductions in plasma corticosterone than ACTH, in agreement with earlier findings in CRH-R1 knockout mice[28,34], and in Sprague-Dawley rats in response to immo-



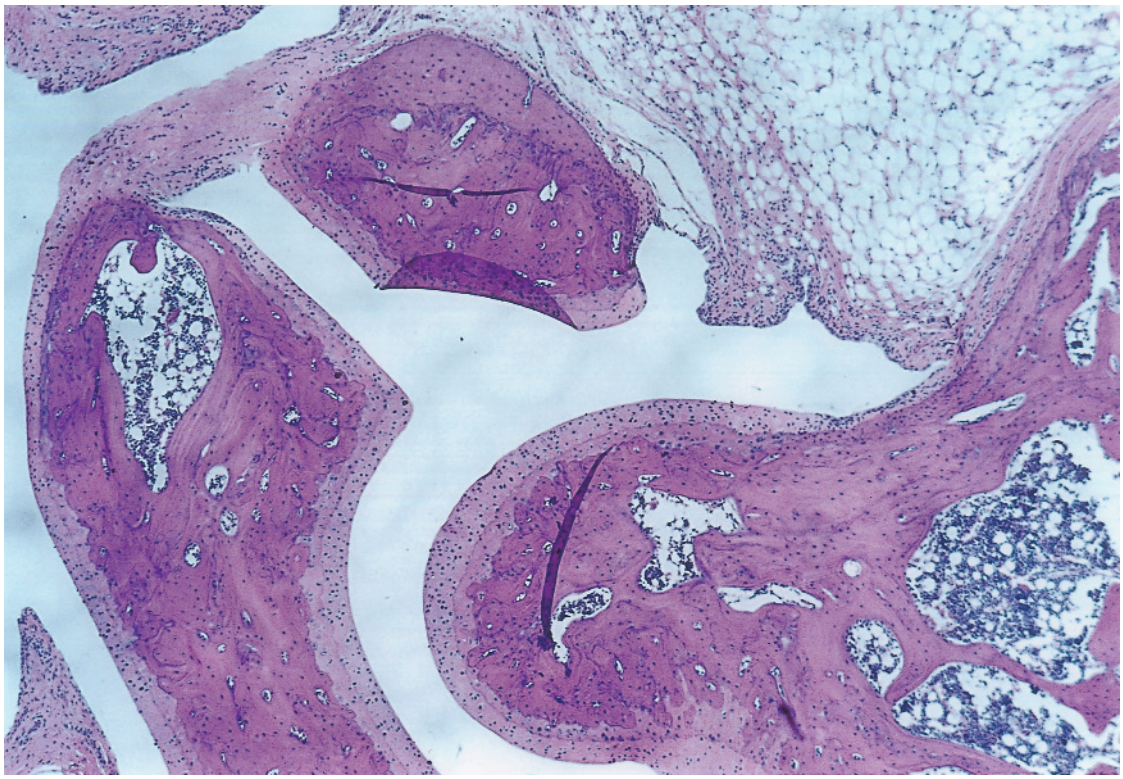
A



B

Figure 4. Representative histopathology findings of joint space from hind paw in antalarmin and vehicle injected adjuvant injected Lewis rats. A. Maximal inflammatory reaction to adjuvant induced arthritis in rats administered placebo (original magnification ×15). B. The same tissues at higher magnification (×60); note the hyperplastic synovium with intense inflammatory cell infiltration.

C



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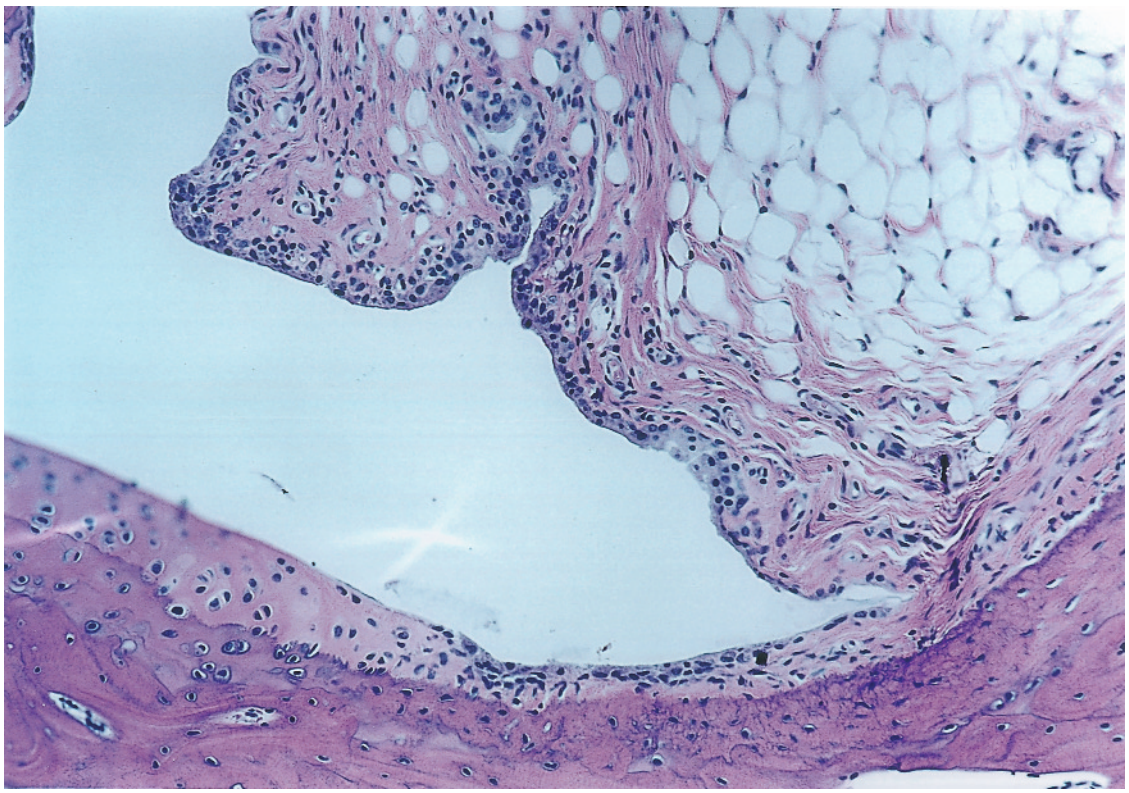

Figure 4. C. Representative section from an antalarmin treated adjuvant induced arthritis animal (original magnification ×15). D. The same tissues at higher magnification (×60); the membranes show no evidence of a reactive cellular response and no inflammation.

bilization stress following intracerebroventricular administration of a peptide antagonist[35]. ACTH stimulation of steroidogenesis is modulated by splanchnic nerve and vascular connections to the adrenal cortex[22], thus the suppression of adrenocortical responsiveness by antalarmin is likely to be dually mediated through the HPA axis and the sympathetic nervous system. In summary, the CRH-R1 antagonist predominantly blocked the peripheral proinflammatory effects of immune CRH, more than the indirect antiinflammatory glucocorticoid mediated effects of hypothalamic CRH in an experimental arthritis model in rats. These results support a major proinflammatory role for CRH-R1 receptor in peripheral inflammation. From a practical perspective, the observation that CRH-R1 antagonist treatment did not increase the susceptibility of the resistant Fischer animals to develop inflammation suggests that circulating glucocorticoids are not the sole factor governing disease vulnerability or resistance in these strains, but that multiple factors and pathways, in addition to cortisol levels, play a role in arthritis resistance in F344 rats. Indeed, this is consistent with genetic studies showing that over 20 different regions on 15 chromosomes are associated with inflammatory arthritis susceptibility, as well as with our previous studies showing multiple neuroendocrine and neuronal deficits in LEW/N compared to F344/N rats, including blunted sympathoneuronal responses[38].

These findings, although preliminary and based on a preclinical model of RA, point to a possible therapeutic role of CRH-R1 antagonists in the treatment of often intractable autoimmune disorders such as RA that frequently coexist with depression. Antalarmin is a prototype of other medications being developed for treatment of neuropsychiatric disorders. Thus, the finding that a "psychiatric" medication is possibly efficacious for treatment of an immune disorder carries the field of neuroendocrine immunology into the clinical arena. This observation should lead to future investigations into the action of other neuroimmune modulators in other unrelated disease states.

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