THE MODULATION OF intestinal inflammatory response has been an important area of investigation to elucidate the etiology and mechanisms of inflammatory bowel diseases (49). In particular, the regulatory role of the neuroenteric-immune axis in intestinal inflammation is gaining recognition (35). In addition, neuroendocrine-immune interactions can influence processes arising from the brain, and there is evidence that the central nervous system may amplify or modulate aspects of intestinal inflammation through alterations of the autonomic nervous system activity and/or stimulation of the hypothalamic-pituitary axis (HPA) (2, 22, 30). Recent clinical studies support the notion that chronic life stress predicts the outcome of symptom intensity in patients with functional bowel disease (4). Exposure for a few days to stress has been reported to worsen colitis in rats (10, 16). However, the influence of longer exposure to environmental challenges on colitis as well as mechanisms through which stress enhances gut inflammation have been little investigated (16).

A primary mediator of the HPA response to inflammatory and noninflammatory stress stimuli is the 41-residue peptide corticotropin-releasing factor (CRF) (47). CRF in the brain mediates, in addition to the endocrine effector arm, the behavioral, immune, and visceral components of the stress response (11, 45, 47). The activation of CRF receptors in the brain also plays a key role in mediating stress-induced gastrointestinal motor alterations through modulation of the autonomic outflow (28, 45). In contrast, convergent studies indicate that central CRF exerts a protective rather than a causative role against experimental gastric injury induced by acute stress (42, 50). Less is known about the role of brain CRF in stress-related modulation of colitis, although a recent report suggests that central CRF is not involved (16).

The HPA axis response to stress is subject to a genetically determined susceptibility in inbred Lewis and Fischer rat strains, particularly in female rats (33). Lewis rats, compared with the histocompatible Fischer rats, display a blunted HPA axis response to acute and chronic stress, including those of immune origin, that is mainly related to a lower hypothalamic CRF release (8, 12). Therefore, in the present study, we assessed the role of brain CRF in the modulation of colitis by intermittent daily exposure to stress for 1 wk using Fischer and Lewis strains of rats with inborn differences in the HPA axis response to stress. First, we established the susceptibility of female Lewis and Fischer rats to colitis 1 wk after administration of 2,4,6-trinitrobenzenesulfonic acid (TNB) (34). Second, we investigated whether daily intracerebroventricular injection of CRF for 1 wk influences the development of TNB-induced colitis. Third, we examined the effect of daily intermittent stress exposure on TNB-induced colitis and whether intracerebroventricular injections of the newly developed CRF receptor antagonist astressin (17, 37) alter the influence of stress on colitis 1 wk after TNB in these two rat strains. Finally, we compared changes in corticosterone plasma levels induced by TNB alone or combined with chronic stress in both strains at various day intervals to gain insight into the possible influence of the HPA axis activity.

MATERIALS AND METHODS

Animals

Female Lewis (Lew/N) and Fischer (F344/N) rats (10 wk old, Harlan, Indianapolis, IN) weighing 140–170 g were quarantined for 1 wk after shipment, housed in group cages, and maintained in a temperature-controlled room with a 12:12-h light-dark cycle. Animals were then transferred into individual cages and kept under similar conditions with free
access to water and Chow (Purina Rat Chow) for 1 wk before and during the experimental period. Experiments were performed under the Veterans Affairs animal component of the research protocol number 97-057-04.

Treatments

The induction of colitis was performed by intracolonic administration of TNB (80 mg/kg, Fluka Chemical, Ronkonkoma, NY) diluted in equal volume of 50% ethanol in Lewis and Fischer rats deprived of food but not water for 18 h and lightly anesthetized with enflurane. The solution of TNB-ethanol was delivered in a total volume of ~0.2 ml through a PE-60 cannula, the tip of which was positioned ~8 cm past the anus. The cannula was left in place for 1 min to ensure that the TNB-ethanol solution was not expelled immediately. Control groups received vehicle (0.2 ml of 50% ethan/water, vol/vol) under similar conditions of administration.

Intermittent stress exposures involved the psychological stress of water avoidance and the physico-psychological stress of wrap restraint, both known to release CRF in the brain (7, 18). Water avoidance consisted of placing the rat on a plastic platform (height, 8 cm; length, 6 cm, width, 6 cm) located in the middle of a plastic home cage filled with water up to the last 1 cm of the height of the platform (7). The wrap restraint stress model, which restricts the mobility of the forelimbs, was adapted from the previously described method (51). The forelimbs and the upper body were wrapped with gauze and secured by paper tapes to restrict but not to prevent movements. Stress sessions were performed between 10:00 A.M. and 1:00 P.M. to minimize any diurnal variation in their response. Rats were then put back in their home cage with free access to water and food. Control groups were maintained in home cages.

Intracerebroventricular cannulation was performed under ketamine (75 mg/kg ip; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (5 mg/kg ip; Mobay, Shawnee, KS) anesthesia. An intracerebroventricular 26-gauge cannula (Plastics One, Roanoke, VA) was positioned unilaterally by using stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The coordinates were as follows: anteroposterior, −0.8 mm; lateral, 1.2 mm; dorsoventral, 3.4 mm from the bregma, with the incisor bar placed at 3.3 mm below the interaural plane. Cannulas were anchored to the skull by stainless steel screws and dental cement. One week later, intracerebroventricular injections were performed in conscious rats, lightly restrained, using a 33-gauge injection cannula, 1 mm longer than the guide cannula that was connected to a catheter (Plastics One) filled with saline and an air bubble to separate the saline from peptide. Correctness of the intracerebroventricular injections was verified at the end of the experiment by macroscopic visualization of the dye into the lateral ventricle after its intracerebroventricular injection under the same conditions as peptide.

Measurements of Colitis

Colitis was assessed by macroscopic damage scoring, histological examination, and quantification of granulocyte infiltration through the measurement of tissue-associated myeloperoxidase (MPO) activity. To avoid observer bias, quantification of macroscopic scores and histological evaluations were verified by another independent observer unaware of the treatments.

For macroscopic damage scoring, the colon was visually examined for adhesions and gross morphological changes immediately after death. The entire colon was then excised and opened longitudinally to assess inflammation, wall thickness, and the nature of feces. The macroscopic scoring of colonic damage was performed by adapting the method developed by McCafferty et al. (29). Thus ulcerations were rated between 0 and 10. Scores for adhesion (0–2), diarrhea (0–1), and colonic wall thickness (in mm) were added to the ulceration scores as detailed in Table 1.

MPO activity was measured in an area corresponding to 2–8 cm proximal to the anus that was excised and cut in two equal halves along the longitudinal axis of the colon. Part of one-half of the sample was taken for histological evaluation, whereas the remaining sample was used for MPO assay. Samples for MPO assay were immediately rinsed in ice-cold saline (0.9%), blotted, and kept in dry ice (−80°C). MPO activity was measured 24–48 h later as previously described (15). Tissues (200–400 mg) were weighed, homogenized in 10% (wt/vol) of 20 mM phosphate buffer (pH 7.4), and centrifuged at 8,000 g for 20 min at 4°C. The supernatant, which contained <5% of the total MPO activity, was discarded. The pellet was rehomogenized in 50 mM acetic acid (pH 6.0) containing 0.5% hexadecyltrimethylammonium hydroxide. MPO activity was assessed by measuring the H2O2-dependent oxidation of 3,3′,5′-tetramethylbenzidine and expressed as units per milligram wet tissue; one unit is the quantity of MPO present that produces a change of 1 when measured at the absorbency of 655 nm for 1-min period at 37°C.

Histological analysis was performed on samples fixed by overnight immersion in neutral buffered Formalin (4%) and processed for embedding in paraffin wax. Sections (4 µm) were cut and stained with hematoxylin and eosin and examined by light microscopy.

Measure of Food Intake and Body Weight

Individually housed rats had access to Purina Rodent Chow and water ad libitum. Daily body weights and food intakes were monitored for 7 days before and after TNB or vehicle administration.

Measurement of Plasma Corticosterone

Trunk blood was collected in chilled tubes containing EDTA (1.5 mg/ml). Samples were centrifuged at 1,500 rpm at 4°C for 20 min, and plasma was kept at −80°C for subsequent analysis. Plasma levels of corticosterone were assessed by a standard radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA). Detection limits were ~25 ng/ml. Inter- and intra-assay control variabilities were 6.5% and 3.7%, respectively.

Table 1. Criteria for macroscopic scoring of colitis

<table>
<thead>
<tr>
<th>Type of Changes</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulceration</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>1</td>
</tr>
<tr>
<td>Focal hyperemia, no ulcers</td>
<td>2</td>
</tr>
<tr>
<td>Ulceration without hyperemia or bowel wall thickening</td>
<td>3–6</td>
</tr>
<tr>
<td>Major damage &gt;2 cm</td>
<td>7–10</td>
</tr>
<tr>
<td>Inflammation and ulcerations up to 2 cm</td>
<td>Plus</td>
</tr>
<tr>
<td>Adhesions</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>Minor</td>
<td>2</td>
</tr>
<tr>
<td>Major</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>X</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Wall thickness, mm</td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td></td>
</tr>
</tbody>
</table>
Experimental Protocols

Effect of daily intracerebroventricular injections of CRF on TNB-induced colitis. Four groups of Lewis and four groups of Fischer rats with chronically implanted intracerebroventricular cannula were used. Two groups of rats in each strain received intracolonic administration of vehicle (vol/vol: 50% ethanol-water) or TNB (80 mg/kg) and thereafter no further treatment. The remaining two groups of each strain were treated with TNB and 24 h later were injected intracerebroventricularly (5 µl) daily with either rat/human CRF (1 µg/rat, Peptide Biology Laboratory, Salk Institute, La Jolla, CA) or vehicle (0.9% saline) for 6 consecutive days. The intracerebroventricular dose of CRF was based on previous studies showing that 0.3–10 µg/rat induces a significant dose-related gastrointestinal motor alteration similar to those induced by stress (25, 51). All rats were killed 24 h after the last intracerebroventricular injection, and colitis was assessed by macroscopic damage score, microscopic examination, and MPO activity.

Effect of chronic stress on TNB-induced colitis, body weight, and food intake. Four groups of Lewis and four groups of Fischer rats received intracolonic administration of either vehicle (vol/vol: 50% ethanol-water) or TNB (80 mg/kg) and were subjected either to no further treatment or were exposed 24 h later to daily intermittent stress (3 h/day water avoidance stress or wrap restraint alternatively for 6 days). The two stressors were selected to delay adaptation of the HPA that might arise as a result of continuous exposure to a single type of stressor (24). Daily food intake and body weight were recorded for a period of 1 wk before and after intracolonic treatment in all the groups. All rats were killed 24 h after the last stress session, and colitis was assessed by macroscopic damage score, microscopic examination, and MPO activity.

Effect of intracerebroventricular astressin on chronic stress-induced alterations of TNB colitis. Four groups of Lewis and four groups of Fischer rats with chronically implanted intracerebroventricular cannula received an intracerebroventricular administration of TNB (80 mg/kg). One group in each strain did not receive further treatment. Twenty-four hours after TNB, the three remaining groups were exposed to daily intermittent stress (3 h/day exposure to water avoidance stress and on the alternate day wrap restraint for 6 days) that was combined in each strain with either intracerebroventricular daily injection of vehicle (distilled water) or astressin (2 µg/rat, Peptide Biology), 10 min before the onset of each stress session or no intracerebroventricular injection. The intracerebroventricular dose of astressin was selected based on a report showing that chronic intracerebroventricular injections of astressin at 2.5 µg/rat prevented the biological action of endogenous CRF (9). All rats were killed 24 h after the last treatment (7 days after TNB administration), and colitis was assessed by macroscopic damage score, microscopic examination, and MPO activity.

Influence of TNB alone or with stress on plasma corticosterone levels. Essentially the same protocol outlined above for the effect of stress on TNB colitis was used. Groups of TNB- or vehicle-treated Lewis and Fischer rats were subjected or not to daily intermittent stress (3 h/day exposure to water avoidance stress and on the alternate day to wrap restraint) for 1, 3, or 6 consecutive days. Twenty minutes after the end of the stress session, rats were killed by decapitation, and blood was collected for determination of plasma corticosterone levels.

Statistics

Data are expressed as means ± SE. Values were analyzed by one-way ANOVA followed by the Student-Newman-Keuls multiple-comparison test for comparison between groups. A two-way ANOVA was used to analyze changes in corticosterone plasma levels with days of treatment, strain, and stress as main factors. P values <0.05 were considered significant.

RESULTS

Effect of Intracerebroventricular Injections of CRF on TNB-Induced Colitis

In both Lewis and Fischer rats implanted with a chronic intracerebroventricular cannula, intracolonic administration of TNB induced severe inflammation of the distal colon compared with vehicle treatment as assessed 7 days later. This was characterized by adhesions with the adjacent segments and organs, thickened walls, and mucosal lesions ranging from small scars to ulcers of >1 cm. The TNB-induced colonic lesions corresponded to macroscopic damage scores of 7.7 ± 1.0 vs. 3.2 ± 0.8 (P < 0.05 vs. vehicle) in Lewis and 8.5 ± 1.3 vs. 3.0 ± 0.5 (P < 0.05 vs. vehicle) in Fischer rats (Fig. 1A). Vehicle score included mainly

![Image](http://ajpgi.physiology.org/)

**Fig. 1.** Effect of daily intracerebroventricular (ICV) injection of corticotropin-releasing factor (CRF) on macroscopic damage score (A) and myeloperoxidase (MPO) activity (B) 7 days after 2,4,6-trinitrobenzenesulfonic acid (TNB) induction of colitis in female Lewis and Fischer rats. Vehicle or TNB (80 mg/kg) was instilled into colon, and groups of rats with intracerebroventricular cannulas were injected intracerebroventricularly for 6 consecutive days starting 24 h after TNB with either saline (10 µl·rat⁻¹·day⁻¹) or CRF (1 µg·rat⁻¹·day⁻¹); other groups with intracerebroventricular cannulas received no further treatments. Each column represents mean ± SE of 10–15 rats/group. *P < 0.05 vs. respective strain treated with intracolonic vehicle. #P < 0.05 vs. respective TNB-treated strain injected with intracerebroventricular saline.
the wall thickness, which is taken into account in the macroscopic damage scoring method (Table 1), and in some cases focal hyperemia. TNB induced a significant increase in MPO activity in both rat strains (Lewis: TNB, 19.0 ± 4.0 vs. vehicle, 3.8 ± 0.7 U/mg tissue (P < 0.05); Fischer: TNB, 17.0 ± 3.0 vs. vehicle, 2.3 ± 0.9 U/mg tissue (P < 0.05)) (Fig. 1B). There were no significant differences between Lewis and Fischer rats in the above parameters of colitis assessed 1 wk after TNB (Fig. 1).

Saline injection (5 µl·rat⁻¹·day⁻¹ icv for 6 days) did not modify colitis induced by TNB compared with chronically intracerebroventricular cannula implanted groups with no injection (Fig. 1). CRF (1 µg·rat⁻¹·day⁻¹ for 6 days) reduced TNB-induced colitis compared with intracerebroventricular saline-treated group in both strains. TNB-induced macroscopic damage scores in intracerebroventricular CRF-injected groups were significantly lower (5.0 ± 0.9 in Lewis and 4.5 ± 0.5 in Fischer rats) than in intracerebroventricular saline-treated groups (Lewis, 8.0 ± 1.1; Fischer, 8.5 ± 1.0) (Fig. 1A). Likewise, MPO activity values were decreased from 19.2 ± 4.0 to 11.0 ± 3.8 U/mg tissue (P = 0.05) in Lewis groups and from 17.5 ± 3.1 to 6.5 ± 2.9 U/mg tissue (P < 0.05) in Fischer TNB-treated rats (Fig. 1B). In intracerebroventricular CRF-treated Fischer rats, MPO values and macroscopic damage scores were no longer significantly different from the vehicle (ethanol)-treated group (Fig. 1). There was also no strain difference in the protective effect of intracerebroventricular CRF on TNB-colitis (Fig. 1). Microscopic examination revealed reduction of TNB-induced colonic lesions and inflammatory cell infiltration after chronic intracerebroventricular injections of CRF as illustrated in Fischer rats (Fig. 2B) compared with intracerebroventricular saline injection (Fig. 2A).

Effect of Chronic Stress on TNB-Induced Colitis, Body Weight, and Food Intake

TNB produced a severe inflammation of the distal colon as monitored by macroscopic damages and MPO activity 7 days after intracolonic application (Fig. 3). Microscopic examination revealed erosions and ulceration of the mucosa, sometimes extending to the muscular layer, edematous submucosa, and massive infiltration of inflammatory cells (polymorphonuclear cells and some scattered mononucleated cells such as macrophages) particularly in the submucosal layer and granuloma formation in Lewis (Fig. 4B) and Fischer rats (Fig. 4C). There were no significant differences between Lewis and Fischer rats in TNB-induced changes in the above parameters (Fig. 3).

Exposure for 3 h/day to water avoidance and, on the alternate day, to wrap restraint stress for 6 consecutive days enhanced TNB-induced colitis in both strains. Macroscopic scores were increased from 7.1 ± 0.7 to 12.0 ± 1.0 (P < 0.05) in Lewis and from 7.5 ± 0.8 to 9.6 ± 0.5 (P < 0.05) in Fischer rats (Fig. 3A). MPO activity also increased from 19.3 ± 3.9 to 33.0 ± 3.3 U/mg tissue (P < 0.05) in Lewis and from 20.5 ± 1.0 to 25.1 ± 2.0 U/mg tissue (P < 0.05) in Fischer rats (Fig. 3B). Stress-induced worsening of TNB-colitis assessed by macroscopic score and MPO activity was significantly higher in Lewis (69 and 71% increase, respectively) than in Fischer rats (28 and 22% increase, respectively) (Fig. 3). Figure 5 illustrates the enhanced microscopic lesions, which included more ulcerations, infiltration with polymorphonuclear cells and macrophages in all layers, and granuloma formation in Lewis (A) compared with Fischer rats (B) treated with TNB and exposed daily to the intermittent stress regimen.

TNB induced a significant decrease in food intake and body weight gain in both Fischer and Lewis rats (Table 2). The mean decrease in daily food intake was more severe for the first 1–3 days in both Lewis (77.0 ± 6.3%, n = 10) and Fischer (81.2 ± 9.2%, n = 10) rats than the reductions in the last 2 days (20.4 ± 2.2% in Lewis and 23.0 ± 3.1% in Fischer rats, n = 10 in each group). The cumulative 7-day food intake was reduced by 52.4 ± 4.2% in TNB-treated Lewis rats compared with the 7 days before treatment, and this was further significantly decreased by intermittent daily stress to reach 70.3 ± 3.3% (Table 2). The 57.3 ± 3.5% decrease in the 7-day food intake induced by TNB in Fischer rats was not significantly enhanced by stress exposure (Table 2). Body weight gain was almost completely suppressed during the 7 days after TNB in both Fischer and Lewis rats (Table 2). Daily intermittent stress
exposure in TNB rats, which in itself did not alter significantly body weight gain in intracolonic vehicle-treated rats, induced a drop in the initial weight after 7 days in Lewis and Fischer rats (Table 2).

In Lewis and Fischer rats treated with intracolonic administration of vehicle, stress did not influence macroscopic damage scores or MPO activity as well as body weight or food intake (Fig. 3, Table 2).

Effect of Intracerebroventricular Astressin on Chronic Stress and TNB-Treated Rats

As in the previous experiment, rats implanted with a chronic intracerebroventricular cannula and treated with TNB with or without daily intracerebroventricular injection of vehicle (water), stress induced an increase in MPO that was higher ($P < 0.05$) in Lewis than Fischer rats (Fig. 6B). However, macroscopic scores did not show a strain difference to stress and TNB under these conditions (Fig. 6A).

Daily intracerebroventricular injection of the CRF receptor antagonist astressin (2 $\mu$g·rat$^{-1}$·day$^{-1}$ for 6 consecutive days) before exposure to stress aggravated the effect of daily intermittent stress on colitis in Lewis rats. Macroscopic scores in intracerebroventricular astressin-injected stressed group reached 13.7 ± 0.3 compared with 11.3 ± 0.3 in intracerebroventricular vehicle-treated stressed rats ($P < 0.05$, Fig. 6A), and MPO activity (in U/mg tissue) was 43.0 ± 2.5 compared with 34.8 ± 2.0 in intracerebroventricular vehicle-treated stressed group ($P < 0.05$, Fig. 5B). There was 16.6% mortality between 4 and 7 days after treatments. In Fischer rats, astressin injected intracerebroventricularly worsened the general conditions, causing 50% mortality between 4 and 7 days and, in the surviving
rats (n = 6), there was an increase in MPO activity (in U/mg tissue: TNB + astressin intracerebroventricularly + stress, 35.5 ± 5.2 vs. TNB + vehicle intracerebroventricularly + stress, 28.0 ± 3.5; P < 0.05) with no additional elevation in macroscopic score damage (Fig. 6).

Table 2. Effect of TNB-colitis with or without chronic intermittent stress on food intake and body weight gain in Lewis and Fischer rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Food Intake, g/7 days</th>
<th>Body Weight Gain, g/7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lewis</td>
<td>Fischer</td>
</tr>
<tr>
<td>Naive</td>
<td>117.1±9.2</td>
<td>85.6±5.1*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>109.2±8.0</td>
<td>78.4±7.2*</td>
</tr>
<tr>
<td>Vehicle+stress</td>
<td>103.7±9.5</td>
<td>76.2±6.6*</td>
</tr>
<tr>
<td>TNB</td>
<td>55.5±5.0*</td>
<td>36.6±3.8*</td>
</tr>
<tr>
<td>TNB+stress</td>
<td>34.6±4.5*</td>
<td>28.7±4.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE of food intake (g/7 days) and body weight gain (g/7 day); n = 10 in each group except naive group, which corresponds to pooled (n=40 for each parameter in each rat strain) means ± SE of 7-day values before rats were subjected to any treatment. Rats treated with intracolonic vehicle or 2,4,6-trinitrobenzenesulfonic acid (TNB, 80 mg/kg) were exposed 24 h later, all groups except two were injected daily intracerebroventricularly with either sterile water (10 µl·rat⁻¹·day⁻¹) or astressin (2 µg·rat⁻¹·day⁻¹) 10 min before each session of stress for 6 days. One group was submitted to stress without intracerebroventricular injection, and another had no further treatment after TNB. Each column represents mean ± SE of 8 rats/group, except astressin Fischer group (n = 6). *P < 0.05 vs. respective strain injected with TNB alone. †P < 0.05 vs. TNB plus stress pretreated with intracerebroventricular sterile water. ‡P < 0.05 vs. corresponding Fischer groups.

Corticosterone Response to TNB Alone or in Combination With Stress in Lewis and Fischer Rats

On day 1 after administration of vehicle into the colon, plasma corticosterone levels were 190.0 ± 26.1 and 171.0 ± 15.2 ng/ml in Lewis and Fischer rats, respectively. Plasma corticosterone had similar levels at 1, 3, or 6 days after intracolonic vehicle administration, and no strain difference was observed (Fig. 7).
TNB increased significantly plasma corticosterone by 1.5- and 1.4-fold in Lewis and Fischer rats, respectively, compared with vehicle as monitored 24 h after intracolonic administration. Thereafter (days 3 and 6), values were no longer different between vehicle- and TNB-treated groups in both strains.

Exposure to water avoidance stress for 3 h, on day 1 after vehicle instillation into the colon, increased significantly plasma corticosterone as measured 20 min after the end of the stress session. This response was higher (P < 0.05) in Fischer (632.5 ± 22.1 ng/ml) than in Lewis rats (365.0 ± 30.6 ng/ml). By day 3 of daily exposure to water avoidance/wrap restraint, the response to stress was lower than day 1 but significantly (P < 0.05) higher than in the nonstressed groups. Plasma corticosterone levels were similar in both strains (265 ± 30.6 ng/ml in Lewis and 260.5 ± 40.2 ng/ml in Fischer rats). After 6 consecutive days of intermittent stress exposure, both strains of rats no longer showed elevated plasma corticosterone (Fig. 7).

In TNB-treated Fischer rats, exposure to stress further elevated (P < 0.05) plasma corticosterone levels on days 1 and 3 compared with TNB alone. In contrast, in Lewis rats, stress did not further enhance the corticosterone response induced by TNB treatment alone at all times (Fig. 7).

**DISCUSSION**

In this study, we showed that both Lewis and Fischer rats exhibit a similar increase in tissue damage (255 and 240%, respectively) and MPO activity (503 and 400%, respectively) as well as a decreased 7-day cumulative food intake (52 and 57%, respectively) and suppression of body weight gain (92 and 93%, respectively) 7 days after TNB administration. These findings extend to other rat strains the experimental model of colitis previously reported in Wistar and Sprague-Dawley rats (10, 20, 34). Although Lewis rats have been well established as a model of inflammatory-prone animals in response to challenges targeted to the skin (carrageenan), joint (streptococcal cell wall), or pancreas (cerulein) (1, 33), the present results indicate that Lewis and Fischer rats are equally susceptible to TNB-induced colitis as assessed 1 wk later by MPO, histology, macroscopic lesions, and associated suppression of food intake and body weight gain. Likewise, with the use of intramural intestinal application of peptidoglycan polysaccharide, no difference in the inflammatory response was observed between Lewis and Fischer rats after 1 wk. However, in Lewis rats there was an acute enterocolitis peak response and spontaneous, long-lasting, reactivation after 12–17 days that were not observed in Fischer rats (31, 39). As the colonic damage was evaluated on day 7 after TNB administration, the present results do not preclude the possibility that strain differences in the development of colitis may occur at other time intervals after TNB as reported in the peptidoglycan polysaccharide colitis model (39).

However, the present study shows that chronic stress-induced aggravation of colitis is more pronounced in Lewis than in Fischer rats. When intermittent (3 h/day) psychological (aversion to water) and physico-psychological (partial restraint) stress was repeated in alternance over a 6-day period, TNB-induced colitis was aggravated, resulting in a 71% further increase in MPO activity in Lewis compared with a significant lower response (22%) in Fischer rats. Likewise, stress, when superimposed on the ongoing colitis, exerts a more pronounced effect on clinical signs of colonic inflammation, such as a further significant decrease in
food intake in Lewis (38%) compared with Fischer (22%) rats. The worsening of colitis manifestations represents a potentiating effect of chronic stress as a similar regimen of stress exposure in ethanol-treated rats failed to increase macroscopic damage scores and MPO activity or to decrease significantly food intake or body weight gain in both strains. Despite numerous clinical reports suggesting that psychological disturbances are risk factors in ulcerative colitis (26, 46), the influence of stress on the development of experimental colitis has only recently been examined and was limited to a shorter period of exposure (10, 16). In male Wistar rats, restraint applied for 2 h daily during the first 4 days after TNB enhanced the development of acute colitis (16). Restrained stress applied for 4 days before or at 42 days after TNB also resulted in worsening of biochemical markers of inflammation in male rats (10, 16). Other species, such as the cotton-topped tamarin, develop a high frequency of spontaneous enteritis/colitis in captivity, which has been related to the chronic stress of captivity and genetic susceptibility since other types of tamarins did not develop such colitis (39, 44). The present findings in rodents also support the notion that interactions between chronic environmental stress and genetic factors impact on the development of colitis.

The study also provides convergent evidence that stress-induced aggravation of TNB-colitis is not mediated by brain CRF which, in contrast, exerts beneficial effects in this experimental model of inflammatory bowel disease. First, daily intracerebroventricular injections of CRF inhibit the development of colitis in both rat strains rather than mimicking the effect of stress as assessed 1 wk after TNB administration. Second, blockade of central CRF receptors by daily intracerebroventricular injections of astressin, the potent CRF receptor subtype 1 and 2 antagonist (17, 28, 37), before each stress exposure worsened stress-induced aggravation of colitis with chronic inflammation characteristics. A previous report indicates that another CRF antagonist, α-helical CRF-(9—41), which is more selective for CRF receptor subtype 2 (13, 23), injected intracerebroventricularly before restraint further aggravates the effect of intermittent stress exposure in an acute model of colitis (16). Finally, female Lewis rats that are known to have a blunted hypothalamic CRF response to various stressors including restraint or swim stress (43) exhibit more susceptibility to stress-induced aggravation of colitis. Taken together, these findings clearly establish that activation of brain CRF receptors play a protective rather than causative role in stress-induced worsening of colitis as assessed 1 wk after TNB. The mechanisms through which chronic stress aggravates colitis are still to be determined. It may be mediated by the enhanced proinflammatory mediators such as interleukin-1, which are released by restraint independently from adrenocorticotropic hormone and corticosterone release (32) and noncentral CRF-dependent autonomic alterations of intestinal epithelial cell function (5).

It may be speculated that the stimulation of corticosterone secretion is part of the mechanisms through which exogenous or endogenous central CRF restraints the proinflammatory effect of stress (40). The end effect of HPA activation, glucocorticoid, represses the immune and inflammatory responses (6) through their potent inhibitory action on nuclear factor-κB, a pivotal transcription factor for the expression of many cytokine genes in chronic inflammation (3). In the experimental model of colitis induced by TNB, endogenous and exogenous glucocorticoids decrease MPO activity and histological scores as assessed 24 h after intracolonic administration (48). In the present study, daily intracerebroventricular injection of CRF, known to induce a sustained hyperactivity of the HPA axis and corticosterone secretion in rats (27), markedly reduced TNB-induced colitis. In addition, Fischer rats, which displayed a significant increase in plasma corticosterone levels in response to chronic intermittent stress exposure as monitored on days 1 and 3, had lower stress-induced worsening of colitis compared with Lewis rats. Conversely, Lewis rats, which failed to mount plasma corticosterone levels on days 1 and 3 during daily exposure to stress, exhibited higher aggravation of colitis by stress. The different patterns of HPA activity in response to stress in these two inbred strains has been well characterized (14, 33, 43).

Female Lewis rats exposed to various acute or chronic stressors exhibit a reduced or blunted corticosterone response compared with Fischer rats (12, 43). In contrast, they have similar basal corticosterone levels under resting conditions as observed previously (12, 14). Finally, central injection of astressin, which prevents the activation of the HPA axis during stress (17), further aggravated the influence of stress on colitis and is associated with mortality in 16% of Lewis and 50% of Fischer rats treated with TNB. It is unlikely that intracerebroventricular injections of astressin-induced mortality in stress rats treated with TNB was related to a nonspecific toxic action of the peptide since repeated intracerebroventricular injections of astressin in doses ranging from 2.5 to 15 µg/rat had no side effects in rats under basal conditions (9). However, astressin has been shown in vitro to be 100-fold more potent than α-helical CRF-(9—41) to block CRF receptor subtype 1 (17, 37) involved in pituitary ACTH release (47), and astressin injected intracerebroventricularly at 15 µg inhibited ACTH response to peripheral injection of CRF while the first CRF antagonist developed α-helical CRF-(9—41) injected at 25 µg had no effect (38). Therefore, the mortality induced by central administration of astressin would be consistent with other reports showing that removal of the HPA response in the presence of inflammatory conditions results in lethality due to the lack of appropriate glucocorticoid release (21). A possible explanation for the higher mortality of Fischer compared with Lewis rats after daily intracerebroventricular injections of astressin could be that Lewis rats, although known to have a defective CRF-HPA axis system in response to stress stimuli including immunological stimuli (14, 43), have a higher hypothalamic and circulating arginine vasopressin response than Fischer rats (36). Because arginine vasopressin has a regulatory role in the HPA axis activity (41), it was suggested that the higher level of arginine vasopressin in Lewis
may represent a compensation for the global insufficiency of their CRF-producing neurons and to enable them to maintain a minimum HPA axis activity (36). It is thus possible that Fischer rats are highly dependent on their robust CRF-related HPA axis activity in response to stress (43), and blockade of the CRF pathways makes them more vulnerable to a combined adaptive response of chronic stress and colitis.

TNB alone induced a significant rise in corticosterone as measured 24 h after the administration in both strains as previously observed in Wistar male rats (48). The similar corticosterone response to TNB in both Fischer and Lewis rats contrasts with the blunted rise in corticosterone monitored 20 min after the end of restraint/water avoidance stress in Lewis rats or to other stressors (12). However, we cannot infer based on the evidence of one time point whether the profile of corticosterone response to TNB will be similar in both strains throughout the 24-h period after TNB. Alternatively, it may indicate that such a visceral stimulus recruits different pathways regulating corticosterone release (19) that are not altered in Lewis rats. The time course of corticosterone changes after 1, 3, and 6 days clearly showed that there is an adaptation as reported during other chronic stress regimens (12, 14), since the increase in corticosterone is no longer observed at day 6 after TNB. Taken together, these data suggest that the weaker anti-inflammatory axis in Lewis rats may contribute to the greater deleterious effect of stress on TNB-colitis compared with Fischer rats. However, additional mechanisms may also exist related to CRF actions on CRF receptor subtype 2, which needs to be further investigated.

In summary, these results show that both Lewis and Fischer rats develop similar intensity of colitis on day 7 after TNB and that intermittent chronic stress exposures consistently worsen colitis more in rats with weaker HPA axis response to stress (Lewis). The data also highlight the protective role of brain CRF in stress-related aggravation of colonic inflammation. This is supported first by the demonstration that intracerebroventricular injection of CRF inhibits colitis induced by TNB in both strains. Second, daily intracerebroventricular injection of the CRF receptor antagonist astressin further aggravates the potentiating effect of stress on colitis and induces mortality. Finally, Lewis rats, known to have a genetically impaired CRF-HPA axis response to stress and which showed a blunted corticosterone response to restraint/water avoidance stress, had a higher degree of exacerbation of colitis and food intake suppression than Fischer rats. Taken together, the protective action of intracerebroventricular CRF while chronic stress worsened TNB-induced colitis and that a deficient central CRF response enhanced the deleterious effect of stress on colitis indicate that endogenous brain CRF restrains the proinflammatory effect of chronic stress. Moreover, we showed that strain differences in the HPA axis responsiveness to stress have a bearing in the outcome of experimental colitis. Whether central CRF is delaying the colitis response or providing long-term protection beyond initial treatment needs to be further established based on these findings.

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REFERENCES

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