Stress-induced enhancement of colitis in rats: CRF and arginine vasopressin are not involved

M. Gué, C. Bonbonne, Jean Fioramonti, C. del Rio-Lachèze, Christine Coméra, Lionel Bueno

▶ **To cite this version:**

M. Gué, C. Bonbonne, Jean Fioramonti, C. del Rio-Lachèze, Christine Coméra, et al.. Stress-induced enhancement of colitis in rats: CRF and arginine vasopressin are not involved. AJP - Gastrointestinal and Liver Physiology, 1997, 272 (35), pp.G84-G91. hal-02696333

HAL Id: hal-02696333
https://hal.inrae.fr/hal-02696333

Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Stress-induced enhancement of colitis in rats: CRF and arginine vasopressin are not involved

MICHÈLE GUÉ,[1] CATHY BONBONNE,[2] JEAN FIORAMONTI,[2] JEAN MORÉ,[2] CHANTAL DEL RIO-LACHÈZE,[2] CHRISTINE COMÉRA,[2] AND LIONEL BUÉNO[2]
[1]*Institut de Recherches Jouveinal, 94265 Fresnes; and* [2]*Department of Pharmacology, Institut National de la Recherche Agronomique, 31931 Toulouse Cédex, France*

Gué, Michèle, Cathy Bonbonne, Jean Fioramonti, Jean Moré, Chantal Del Rio-Lachèze, Christine Coméra, and Lionel Buéno. Stress-induced enhancement of colitis in rats: CRF and arginine vasopressin are not involved. *Am. J. Physiol.* 272 (*Gastrointest. Liver Physiol.* 35): G84–G91, 1997.—Because exacerbation of colitis seems to be associated with stress, we proposed evaluating the influence of stress and the involvement of corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) on experimental colitis in rats. Partial restraint stress was applied during 4 consecutive days, before or after intracolonic 2,4,6-trinitrobenzenesulfonic acid (TNB) instillation (15 mg) in rats. Finally, two groups of rats were centrally injected with α-helical CRF-(9—41) (5 μg) or AVP antagonist (5 μg) before each session of stress. Stress was applied before or right after TNB enhanced colitis, with an increase in macroscopic and histological scores and myeloperoxidase activity. α-Helical CRF-(9—41) or AVP antagonist had no effect on TNB-induced colitis but enhanced the effects of stress on colitis. These results show that stress may exacerbate experimental colitis in rats and that CRF and AVP are not responsible for this effect.

2,4,6-trinitrobenzenesulfonic acid; inflammatory bowel disease; experimental colitis

ULCERATIVE COLITIS and Crohn's disease are inflammatory bowel diseases (IBD) that exhibit an unpredictable clinical course usually characterized by successive exacerbations and remissions of variable intensity and duration. In patients with IBD, emotional stress is clinically associated with exacerbation of IBD (6). In rats, 4 h of cold-restraint stress increases prostaglandin E_2 (PGE_2) and leukotriene C_4 (LTC_4) synthesis in the proximal colon (28). McHugh et al. (18) observed that stress reactivated experimental colitis when it was applied several weeks after its induction in rats. The inflammatory reaction in IBD is characterized by prominent colonic mucosal polymorphonuclear leukocyte infiltration and the presence of LTB_4 (17). Activated neutrophils (9) and macrophages (29) are major components of active lesions in both ulcerative colitis and Crohn's disease (17). In rats, intrarectal instillation of 2,4,6-trinitrobenzenesulfonic acid (TNB) causes a profound colonic inflammation with histological changes and mediator release mimicking that found in human IBD (19).

Exposure to adverse stimuli or stressors modulate various aspects of immune function. For instance, prolonged restraint stress resulted in a decreased incidence and severity of experimental autoimmune encephalomyelitis when applied before but not after its induction (15). Integrative physiological models initially proposed that stress-induced immune suppression was mediated by activation of the pituitary-adrenal axis with the release of the glucocorticoids (27). Now there is no doubt that a counterregulatory feedback loop exists between the immune system and central nervous system (CNS), in which immune or proinflammatory mediators stimulate corticotropin-releasing factor (CRF) activation of the hypothalamic-pituitary-adrenal (HPA) axis (23). The resultant increase in plasma glucocorticoids serves to restrain and limit the intensity of the inflammatory-immune response. However, stress-induced gastrointestinal disturbances are mediated through a mechanism involving the central release of CRF but are not linked to the stimulation of the HPA axis (8).

In the present study, we have examined the ability of partial restraint stress (PRS) applied during 4 consecutive days to modify the course of colitis induced by TNB in terms of macroscopic and histological scores and granulocyte recruitment. Finally, because CRF acts in synergy with arginine vasopressin (AVP) to regulate pituitary adrenocorticotropic hormone (ACTH) secretion and ultimately the activity of the pituitary-adrenal axis (5, 31), we have also attempted to determine the roles of CRF and AVP in the modulation of colitis severity by stress.

METHODS

Animals. Twenty groups of eight male Wistar rats (Centre d'Élevage R. Janvier, Le Genest Saint Isle, France) weighing 225–300 g were used for these experiments. Animals were housed individually in polypropylene cages (37.5 × 17 × 15 cm) and kept in a temperature-controlled room (21 ± 1°C, 50 ± 5% rh) on a 12:12-h light-dark cycle (lights on at 8:00 A.M.). The rats were fed standard laboratory diet and tap water ad libitum. All experimental procedures described in this report were performed in accordance with the guidelines of the local ethics committee for in vivo animal studies (Agreement 94.203 A).

Under general anesthesia with ketamine (Imalgene 1000, Rhône Mérieux, Lyon, France; 100 mg/kg ip), six groups of eight rats were fitted with a small polyethylene catheter (0.3 mm ID, 0.7 mm OD) inserted into a lateral ventricle of the brain with the following coordinates from bregma: anteroposterior −1.3 mm, lateral 1.8 mm, and ventral 3.5 mm. Two screws were implanted in the bone surface, and dental cement secured the catheter.

Induction of colitis. Rats (n = 8/group) were randomized into treated groups. After an overnight fast and under general anesthesia (ketamine, 100 mg/kg ip), colitis was induced by intracolonic administration of 0.15 ml of 50% ethanol (vol/vol) containing 15 mg of TNB as previously described (19). A rubber catheter (2 mm OD) was inserted rectally into the colon so that the tip was 8 cm proximal to the anus, approximately at the splenic flexure. The instillation proce-

0193-1857/97 $5.00 Copyright © 1997 the American Physiological Society

Downloaded from www.physiology.org/journal/ajpgi by ${individualUser.givenNames} ${individualUser.surname} (138.102.192.062) on August 31, 2018.
Copyright © 1997 American Physiological Society. All rights reserved.

dure required 30 s to complete. After instillation of the TNB-ethanol solution, the cannula was left in place for a few seconds and then gently removed.

Stress procedure. PRS, a relatively mild nonulcerogenic model of restraint (31), was used in all stress sessions. Animals were lightly anesthetized with ethyl ether, and the foreshoulders, upper forelimbs, and thoracic trunk were wrapped in a confining harness of paper tape to restrict but not to prevent body movements; the animals were then placed in their home cages for 2 h. The rats recovered from ethyl ether within 2–3 min, immediately moved about in their cages, ate, and drank but had restricted mobility of their forelimbs, which prevented them from grooming the face, upper head, and neck. Control sham-stressed animals were anesthetized but were not wrapped. After recovering from ethyl ether anesthesia, control rats diligently groomed the face, head, and abdomen. PRS or sham-stress was applied for 4 consecutive days to mimic a repetitive situation of stress.

Two stress protocols were used as shown in Fig. 1. In *protocol A*, two groups of eight rats were subjected to chronic PRS before either saline (noninflamed group) or TNB instillation at 15 mg. Groups of rats were killed on *day 4* after TNB for assessment of colitis. In *protocol B*, chronic PRS was applied from *days 1* to *4* after instillation of saline or TNB (15 mg). Groups of rats were killed on *day 4* after TNB instillation. For each protocol, a group of eight rats was subjected to sham stress and received saline or TNB (15 mg) as in the corresponding protocol. PRS was always performed between 10:00 and 12:00 A.M., and assessment of damage was always performed between 2:00 and 4:00 P.M. Rats were weighed before intracolonic instillation and the day of colitis assessment.

Effect of CRF antagonist and AVP antagonist. In a last series of experiments, rats were injected intracerebroventricularly with saline alone (10 μl) or containing 5 μg of either α-helical CRF-(9—41) or [deamino-Pen^1,Val^4,D-Arg^8]vasopressin, a synthetic analogue of AVP with antagonist properties, 5 min before each PRS or sham-stress session. The animals were daily submitted to PRS or sham stress during 4 consecutive days and were instilled with TNB (15 mg) as in *protocol A*. The rats were killed on *day 4* after induction of colitis. Doses of CRF antagonist and AVP antagonist were chosen in accordance with previous studies (5, 21).

Assessment of colonic injury and inflammation. The severity of the colitis was assessed in three ways: macroscopic scoring, histological evaluation, and quantification of granulocyte infiltration through measurement of myeloperoxidase (MPO) activity. MPO is an enzyme found in cells of myeloid origin, especially neutrophils, and has been used as a quantitative marker of granulocyte infiltration into gastrointestinal tissues (19).

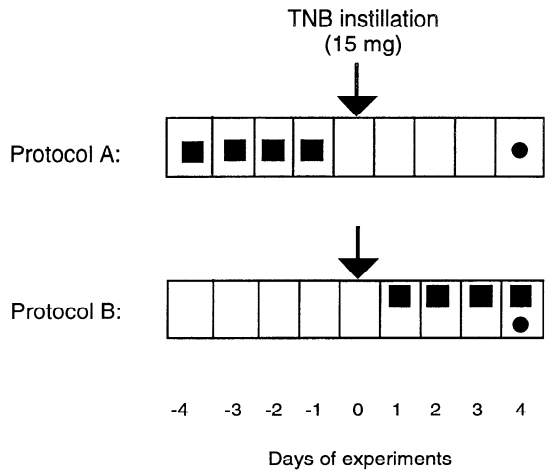

Fig. 1. Schematic representation of stress procedures (*protocols A* and *B*). TNB, 2,4,6-trinitrobenzenesulfonic acid; ■, partial restraint stress (PRS); ●, damage assessment.

Table 1. *Criteria for macroscopic scoring of colonic ulceration and inflammation*

Score	Appearance
0	Normal appearance
1	Focal hyperemia, no ulcers
2	Ulceration with inflammation at 1 site
3	Two or more sites of ulceration and inflammation
4	Major sites of damage extending >1 cm along length of colon
5–10	When an area of damage extended >2 cm along length of colon, score was increased by 1 for each additional cm of involvement

Modified from Ref. 22.

Rats of the randomized treated groups were weighed and killed by cervical dislocation, and the 10 cm of distal colon were removed. The colon was opened by a longitudinal incision, rinsed with saline, and pinned out on a wax block. The macroscopic scoring of colonic damage was performed using the criteria outlined in Table 1 (modified from Ref. 22), which take into consideration the area of damage involvement and the presence or absence of ulcers. The presence or absence of adhesions between the colon and other organs was also noted. Scoring of damage and excision of tissue samples were performed by an observer unaware of the treatment group (C. Bonbonne and M. Gué).

The distal portion of the colon (6–7 cm proximal to anus) was excised in two pieces. One piece was immersed in neutral buffered formaldehyde solution and was then processed by routine techniques before embedding in paraffin. Thin sections (5 μm) were mounted on glass slides and stained with hematoxylin and eosin to reveal structural features. Histological assessment was performed on coded slides to prevent observer (J. Moré) bias by the criteria outlined in Table 2 (modified from Ref. 7). The other piece of tissue sample was frozen on dry ice and stored at −80°C for subsequent measurement of MPO activity no more than 10 days later. MPO activity was measured by the modified technique of Bradley et al. (3). The MPO assays were performed in a blinded fashion on coded tubes. Protein concentration was measured by the modified method of Lowry et al. (16) using the Bio-Rad DC test, and results were expressed as units of MPO assay per gram of tissue protein.

Statistical analysis. Data are expressed as means ± SE. Comparisons among groups of data were made by one-way analysis of variance followed by a Newman-Keuls post hoc test. Differences in the incidence of adhesions between groups were compared with a median test and χ^2. $P \leq 0.05$ was considered significant.

Materials. TNB was obtained from Fluka Chemie (Buchs, Switzerland). α-Helical CRF-(9—41) and [deamino-Pen^1,Val^4,D-Arg^8]vasopressin were obtained from Sigma Chemical (St. Louis, MO). Bio-Rad DC test was obtained from Bio-Rad (Ivry sur Seine, France).

RESULTS

Induction of colitis in prestressed rats (protocol A). Figure 2 shows the severity of colitis expressed as mean body weight change over 4 days, the colitic damage

Downloaded from www.physiology.org/journal/ajpgi by ${individualUser.givenNames} ${individualUser.surname} (138.102.192.062) on August 31, 2018.
Copyright © 1997 American Physiological Society. All rights reserved.

Table 2. *Criteria for histological scoring of colonic mucosa*

Variables	Severity of Changes			
	0	1	2	3
Ulceration	No ulcer	Erosion or single ulceration not exceeding lamina muscularis mucosae	Multifocal ulcerations not exceeding the submucosa	Ulcerations exceeding the submucosa
Mucous cell depletion	Preserved mucous cell	Mild depletion in a few cells	Moderate depletion (>50% of cells)	Severe depletion or complete disappearance of mucosa
Mucosal atrophy	Normal thickness	Mild atrophy (>10%)	Moderate atrophy (10–50%)	Severe atrophy (>50%)
Edema (submucosa)	Normal thickness	Mild edema (submucosal expansion >10%)	Moderate edema (submucosal expansion, 10–100%)	Severe edema (submucosal expansion >100%)
Inflammatory cell infiltration	No inflammatory cell infiltration	Mild inflammatory cell infiltration; few scattered cells	Moderate: distributed but not dense inflammatory cell infiltration	Dense: inflammatory cell infiltration
Vascular dilatation	Normal blood vessels	Mild dilatation of single blood vessels	Moderate: dilatation of several blood vessels	Severe: dilatation of several blood vessels

Modified from Ref. 7.

scores, and MPO activity in colonic tissue in nonstressed rats and after chronic PRS applied during 4 consecutive days. Chronic PRS applied in healthy rats had no effect on the mean weight gain or colitic aspect, since no change was detected macroscopically or histologically, even though chronic PRS reduced by 66% the MPO activity compared with control (sham-stressed) animals (35 ± 3 vs. 104 ± 8 U/g protein).

Four days after TNB administration, rats exhibited a drastic colitis characterized by severe hyperemia and ulceration extending along the distal colon up to 2 cm. The colitic damage scores reached a value of 5.6 ± 0.8. Adhesions between the affected portion of the colon and other organs, usually the small intestine, were observed in 39% of TNB-treated rats (Table 3). When they existed, adhesions were invariably located very close to a site of ulceration. Histological observations showed mucosal extended ulceration and moderate-to-severe mucin cell depletion in addition to mucosal atrophy and edema (10–30%), dilated vessels, and inflammatory cell infiltration into the submucosa (Fig. 3, Table 3). MPO activity was increased by approximately three times for TNB-treated rats over that of saline controls (293 ± 31 vs. 104 ± 8 U/g protein). Colitis observed in rats treated with TNB was paralleled by changes in body weight. Rats in the TNB-treated group lost an average of 21 g over a 4-day period.

With the chronic PRS applied during 4 consecutive days before induction of colitis, administration of TNB resulted in a significant increase of severity of colitis over that of nonstressed rats, assessed both macroscopically, with the areas of ulceration extended up to 5–6 cm instead of 2 cm, and by the colonic damage score (9.6 ± 0.5 vs. 5.6 ± 0.8); furthermore, the incidence of adhesions was 84%. Histological examination of tissues provided results complementary to the macroscopic data (Fig. 3, Table 3). This consisted of mucosal ulcerations that did not exceed the superior third of the lamina propria. The edema was moderate, and the mucosal atrophy was ~50%. The mucous cell depletion was severe as was the vascular dilatation (Fig. 3). In all rats, there was marked inflammation in the colon as shown by the significant difference between sham-stressed and prestressed rats submitted to colitis (Fig. 2, Table 3). In addition, the colonic tissue of these rats exhibits a significant increase by 118% in MPO activity compared with the TNB-treated group. Chronic PRS applied after induction of colitis slightly reduced the change in body weight, even though there was no significant difference compared with the sham-stress group (Fig. 2).

Effect of chronic PRS applied after induction of colitis (protocol B). Applied during 4 consecutive days after induction of colitis, PRS increased the colitic damage score in rats treated with TNB (8.8 ± 1.2 vs. 4.6 ± 1.4 in nonstressed rats), which paralleled an increase in the incidence of adhesions (66 vs. 36%). Furthermore, PRS applied after TNB instillation increased MPO activity by 38% (403 ± 35 vs. 293 ± 31 U/g protein; Fig. 4). Histologically, there was mucosal ulceration with severe mucous cell depletion, mucosal atrophy was ~70%, and the edema with 100% of submucosal expansion (Table 3). However, chronic PRS had no significant effect on the changes in body weight that accompanied the development of colitis (Fig. 4).

Central injection of α-helical CRF-(9—41) and AVP antagonist. Intracerebroventricular injection of either α-helical CRF-(9—41) or AVP antagonist performed before each session of PRS had no effect per se on MPO activity in saline-treated rats.

In rats injected with α-helical CRF-(9—41) or AVP antagonist before each session of sham stress, the severity of colitis was not significantly different compared with the group treated intracerebroventricularly with saline, as assessed by the colitic damage score, the histological score, the weight body change, and the MPO activity (Fig. 5, Table 4).

In rats injected with α-helical CRF-(9—41) (5 μg intracerebroventricularly) before each session of PRS, the colitis was much more severe when measured 4 days after TNB (15 mg) instillation (Fig. 5). Indeed, MPO activity significantly ($P < 0.05$) increased compared with the PRS group (1,429 ± 347 vs. 702 ± 31 U/g protein), as did the macroscopic score (9.8 ± 1.1 vs. 5.9 ± 0.8). At the same time, rats lost an average of 45 ± 6 vs. 8 ± 3 g in PRS (Fig. 5). In one of eight

Downloaded from www.physiology.org/journal/ajpgi by ${individualUser.givenNames} ${individualUser.surname} (138.102.192.062) on August 31, 2018.
Copyright © 1997 American Physiological Society. All rights reserved.

animals, cicatricial tissue was observed, whereas seven of eight rats presented ulcerations exceeding the submucosa, severe edema and mucosal atrophy, inflammatory cell infiltration in the submucosa, and severe dilatation of blood vessels (Fig. 6). Similarly, AVP antagonist (5 μg/kg) centrally injected before the stress session significantly increased the MPO activity and the loss of weight in rats treated with TNB and increased the severity of colitis assessed macroscopically (Table 4) and histologically (Fig. 6, Table 4).

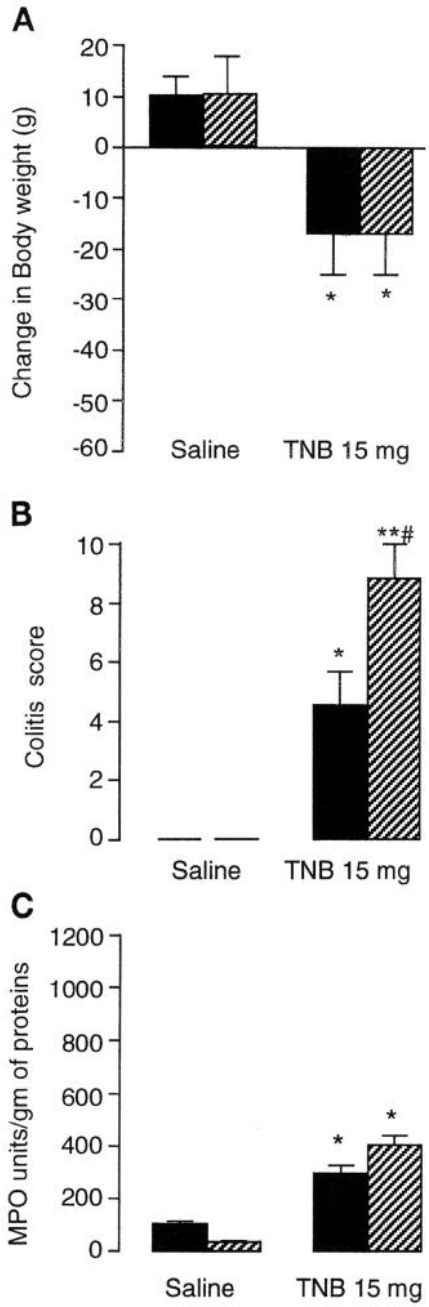

Fig. 2. Body weight changes (*A*), colitic score (*B*), and myeloperoxidase (MPO) activity (*C*) in sham-stressed (solid bars) and PRS (hatched bars) rats 4 days after saline or TNB (15 mg) instillation. PRS was applied before induction of colitis after *protocol A*. $^{*}P < 0.05$, significantly different from corresponding saline values. $^{**}P < 0.01$, significantly different from corresponding saline values. $\#P < 0.05$, significantly different from corresponding sham-stress values. $n = 8$ rats/group.

Table 3. *Histologically assessed damage and incidence of adhesions induced by TNB: effects of PRS before (protocol A) or after (protocol B) induction of colitis with TNB*

Treatment	Days After TNB	Histological Damage Score	Incidence of Adhesions, %
Protocol A			
Sham stress + TNB	4	1.58 ± 0.65	39
PRS + TNB	4	2.69 ± 0.47*	84*
Protocol B			
TNB + Sham stress	4	1.49 ± 0.52	36
TNB + PRS	4	2.53 ± 0.51*	66*

Histological damage scores are given as means ± SE. TNB, 2,4,6-trinitrobenzenesulfonic acid; PRS, partial restraint stress. $^{*}P < 0.05$, significantly different from corresponding TNB + sham-stress values (Newman-Keuls test).

DISCUSSION

Our findings show that PRS applied during 4 consecutive days considered as a chronic stress increases the severity of experimental colitis in rats. These changes were expressed by an increased colitic damage score, an aggravation of microscopic aspect, and increased granulocyte recruitment assessed by MPO activity. Second, our present study shows that central injection of either CRF antagonist or AVP antagonist before PRS enhanced the colitis already increased by stress. Our results reinforce the hypothesis that stress may be involved in exacerbation of colitis, at least in the experimental conditions described herein.

Intrarectal administration of TNB in ethanol results in acute inflammation, with ulcers, that evolves into chronic inflammation of the distal colon in rats. When TNB binds to tissue proteins, it elicits cell-mediated immune responses and induces an inflammation of the gut comparable to human Crohn's disease. In particular, the histologically observed infiltration of lymphocytes and histiocytes is similar to that described for the human disease (19). Various inflammatory mediators such as PGE_2, thromboxane A_2, prostacyclin, LTB_4, LTC_4, platelet-acivating factor (PAF), and interleukin (IL) may be involved in TNB colitis as in human IBD (25). Among various inflammatory mediators in TNB-induced colitis, IL-1 is considered to be the most significant indicator of mucosal inflammation because its level correlates with MPO activity (20).

Peripherally generated inflammatory mediators and immune cytokines derived from various inflammatory and immune cells activate the HPA axis at some or all of its levels, which include hypothalamic CRF neurons, pituitary corticotrophs, and the adrenal cortex (23). Included among these substances are mast cell-derived PAF, lymphocyte-derived γ-interferon, IL-2, IL-6, macrophage-derived IL-1, tumor necrosis factor (TNF), and other interleukins. In addition, the CNS contains neuronal pathways and receptors for cytokines such as IL-1, especially in the hypothalamus, an area of the brain that is important in the mediation of acute-phase

Downloaded from www.physiology.org/journal/ajpgi by ${individualUser.givenNames} ${individualUser.surname} (138.102.192.062) on August 31, 2018.
Copyright © 1997 American Physiological Society. All rights reserved.

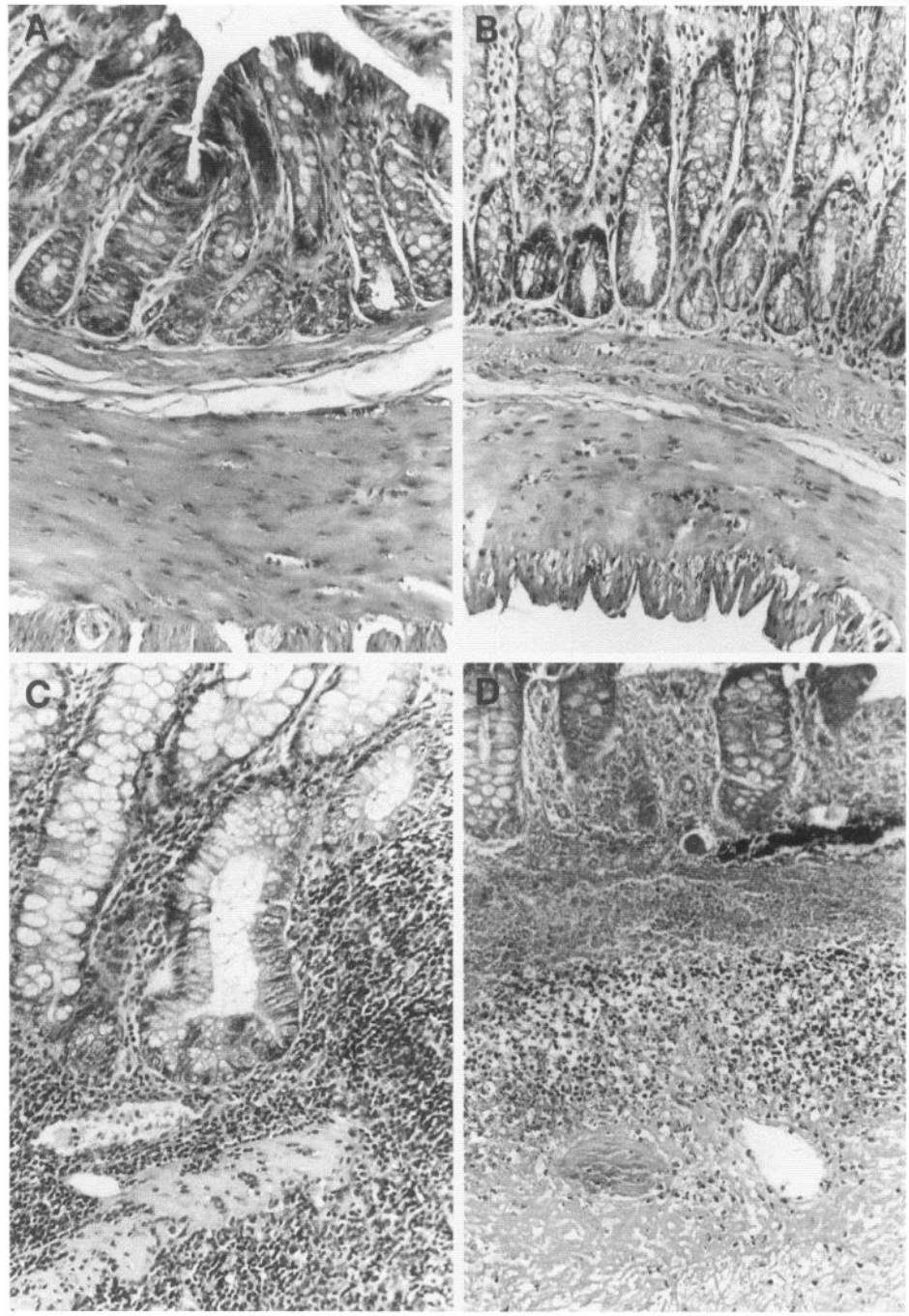

Fig. 3. Light micrographs of colon corresponding to *protocol A*. Appearance of normal colon in control (*A*) and stressed rats (*B*): observe mild erosion of surface epithelium. Inflammation and injury of colonic mucosa 4 days after intracolonic instillation of TNB (15 mg): in nonstressed rat (*C*), note infiltration of mucosa and submucosa by inflammatory cells; in stressed rat (*D*), mucosa is shrunken and partially necrotic.

response (4). It is not known whether peripheral IL-1 interacts with CNS IL-1, these two systems are independent, or peripheral IL-1 crosses the blood-brain barrier to exert its effect. Whatever the mechanism of action, IL-1 stimulates the HPA axis and then the release of glucocorticoids, which are anti-inflammatory and block the production and action of several lymphokines, such as IL-2 and γ-interferon, as well as IL-1 production by macrophages. In fact, there exists a feedback glucocorticoid-associated immunoregulatory system that may exert a continuous surveillance of the immunological state. However, when overstimulation of the pituitary-adrenal axis by cytokines produced by immune-inflammatory cells occurs, it leads to pathological states.

The principal effectors of the stress response include the CRF and locus ceruleus-norepinephrine systems in the CNS. CRF is widely distributed in many brain regions, including the paraventricular nucleus of the hypothalamus, brain stem, limbic system, and cortex. CRF was initially isolated as the principal hypothalamic stimulus to the pituitary-adrenal axis (30). CRF is involved in behavioral and physiological responses to stress. These responses include HPA axis activation, sympathetic nervous system (SNS) activation, anorexia, and changes in motor activity (30).

Increased levels of CRF have been implicated in the stress-induced suppression of immune function (10). In the present study, we show that exposure to PRS

Downloaded from www.physiology.org/journal/ajpgi by ${individualUser.givenNames} ${individualUser.surname} (138.102.192.062) on August 31, 2018.
Copyright © 1997 American Physiological Society. All rights reserved.

during 4 consecutive days induces a decrease of MPO activity in noninflamed rats. Because MPO level is related to neutrophil infiltration (3), this result is in accordance with the knowledge of stress-suppressive action on the immune system (12), even though studies in adrenalectomized rats have shown that this response is independent of glucocorticoids (22). Although the essential pathophysiology of stress-induced immunosuppression has not been determined, there is considerable evidence demonstrating that CRF is an essential agent in stress-induced impairment of immune function (13).

Our study shows the well-recognized stress-induced suppression of the immune system in intact rats but also provides evidence that chronic PRS has a proinflammatory effect in inflamed rats. Indeed, the MPO activity and the colitic damage score were higher in animals stressed before TNB instillation and, to a lesser extent, in rats stressed after induction of colitis. Cytokines appear to play a role in the response to stress and may mediate the release of hormones from the HPA axis. For instance, IL-6, which is not only produced by the cells in immune tissues but also by the cells in neuroendocrine

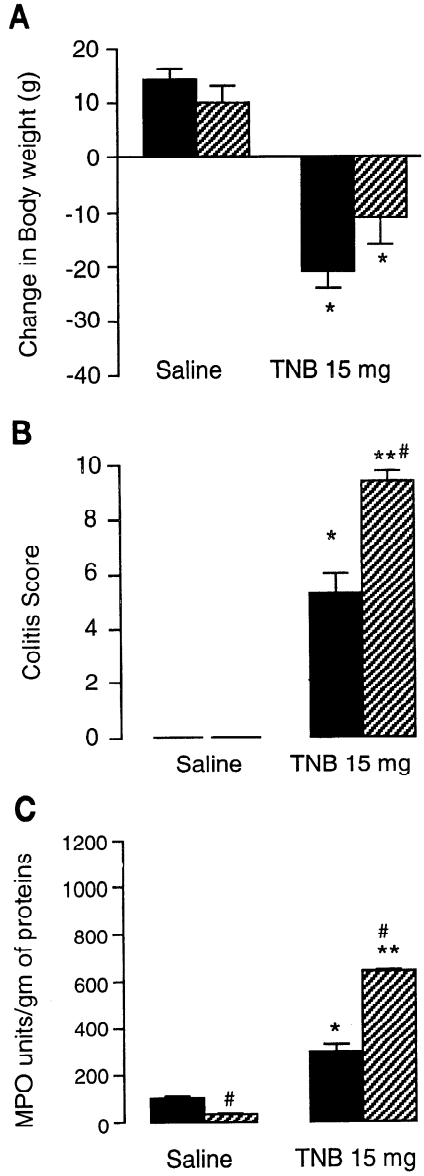

Fig. 4. Effects of PRS applied from *days 1* to *4* after induction of colitis on body weight changes (*A*), MPO activity (*C*), and colitic score (*B*) in sham-stressed (solid bars) and PRS (hatched bars) rats 4 days after saline or TNB (15 mg) instillation. These results correspond to *protocol B*. $*P < 0.05$, significantly different from corresponding saline values. $**P < 0.01$, significantly different from corresponding saline values. $\#P < 0.05$, significantly different from corresponding sham-stress values. $n = 8$ rats/group.

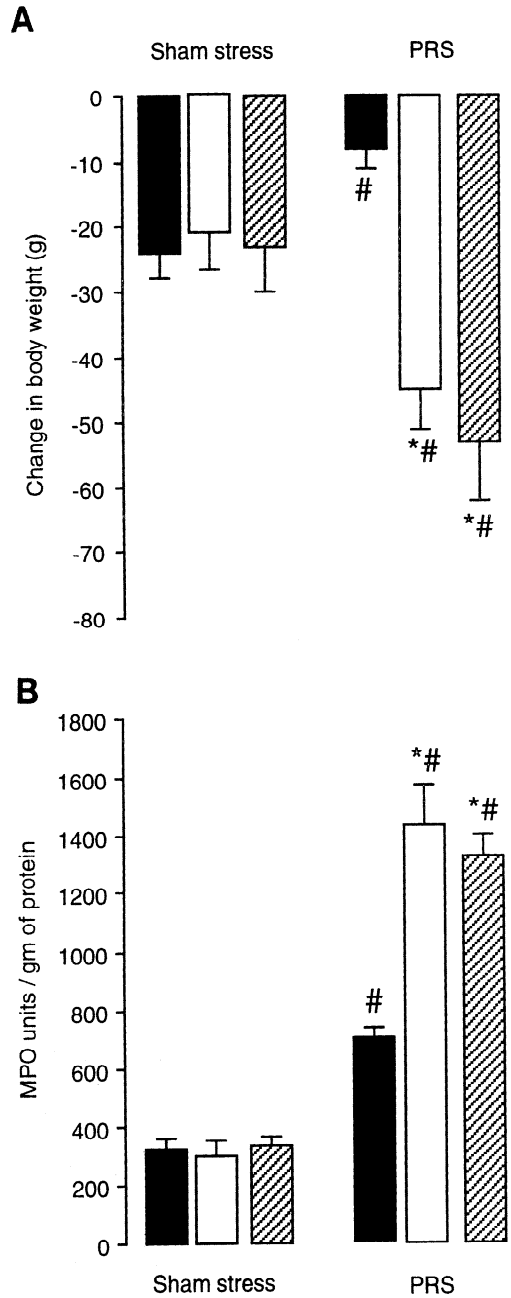

Fig. 5. Effects of α-helical corticotropin-releasing factor (CRF)-(9—41) [5 μg intracerebroventricularly (icv); open bars] and arginine vasopressin (AVP) antagonist (5 μg icv; hatched bars) injected before each session of sham stress or PRS on body weight (*A*) and MPO activity (*B*) 4 days after rectal instillation of TNB (15 mg). Solid bars, saline (10 μl icv). Rats were submitted to same stress procedure as in *protocol A*. $*P < 0.05$, significantly different from corresponding saline values. $\#P < 0.05$, significantly different from corresponding sham stress values. $n = 8$ rats/group.

Downloaded from www.physiology.org/journal/ajpgi by ${individualUser.givenNames} ${individualUser.surname} (138.102.192.062) on August 31, 2018.
Copyright © 1997 American Physiological Society. All rights reserved.

Table 4. *Colitis score and histologically assessed damage induced by TNB instillation: effects of α-helical CRF-(9–14) and anti-AVP injected before each session of sham stress or PRS*

Treatment	Colitis Score	Histological Score
Saline icv + sham stress + TNB	6.1 ± 1.2	1.56 ± 0.53
Saline icv + PRS + TNB	10.3 ± 1.1*	2.61 ± 0.42*
CRF-(9–14) + sham stress + TNB	5.9 ± 0.9	1.42 ± 0.41
CRF-(9–14) + PRS + TNB	14.3 ± 1.6*†	2.98 ± 0.39*†
Anti-AVP + sham stress + TNB	6.2 ± 0.8	1.52 ± 0.52
Anti-AVP + PRS + TNB	15.1 ± 1.4*†	2.89 ± 0.36*†

Values are means ± SE. icv, Intracerebroventricular; CRF-(9–14), α-helical corticotropin-releasing factor (5 μg icv); anti-AVP, arginine vasopressin antagonist (5 μg icv). *$P < 0.05$, significantly different from corresponding sham-stress values (Newman-Keuls test). †$P < 0.05$, significantly different from PRS + TNB values (Newman-Keuls test).

and endocrine tissues, such as the hypothalamus (24), anterior pituitary (26), and adrenal cortex (11), is increased after tissue injury, infection, and inflammation in which there is activation of the HPA axis and SNS. However, in rats exposed to a mild psychological stressor, blood IL-6 increased through a mechanism independent of endotoxemia, tissue injury, or inflammation (14). Recently, Zhou et al. (32) reported that physical or psychological stressors elevated plasma levels of IL-6 in a manner resembling that of corticosterone. Consequently, we can hypothesize that PRS stimulates the release of IL-6, which in turn stimulates and sensitizes the tissues that respond by an increase of colitis after TNB instillation. Of course, this hypothesis needs to be confirmed in further experiments.

When animals received central administration of either CRF antagonist [α-helical CRF-(9—41)] or AVP antagonist before each PRS session applied before induction of colitis, these treatments enhanced the level of inflammation already increased by stress. Indeed, the level of MPO was much higher than that observed in rats intracerebroventricularly injected with vehicle. The central injection of either α-helical CRF-(9—41) or AVP antagonist blocked the stress-induced stimulation of HPA axis and therefore the subsequent glucocorticoid release. Thus the colonic inflammation related to TNB was exacerbated, as shown by increased MPO activity. These results provide evidence that, during a stress situation, CRF and AVP exert protective effects against inflammation and avoid overinflammation.

With regard to body weight during colitis, we observed that central administration of α-helical CRF-(9—41) or AVP antagonist before a stress session increased the loss in body weight, suggesting that CRF and AVP prevented the worsening of inflammation-induced weight loss. These observations reinforce the hypothesis that, during stress, CRF and AVP act to minimize the influence of general colitis, at least through the stimulation of HPA axis. Indeed, the physiologically relevant ACTH secretagogues are CRF and AVP (31). AVP alone has a weak stimulatory effect on ACTH secretion but strongly potentiates the ACTH-releasing capacity of CRF (13) and seems to modify the inhibitory effect of glucocorticoids on pituitary corticotroph cells (2).

Stress-induced alterations of gastrointestinal functions such as intestinal (31) and colonic (8) motility are directly linked to the central release of either CRF or

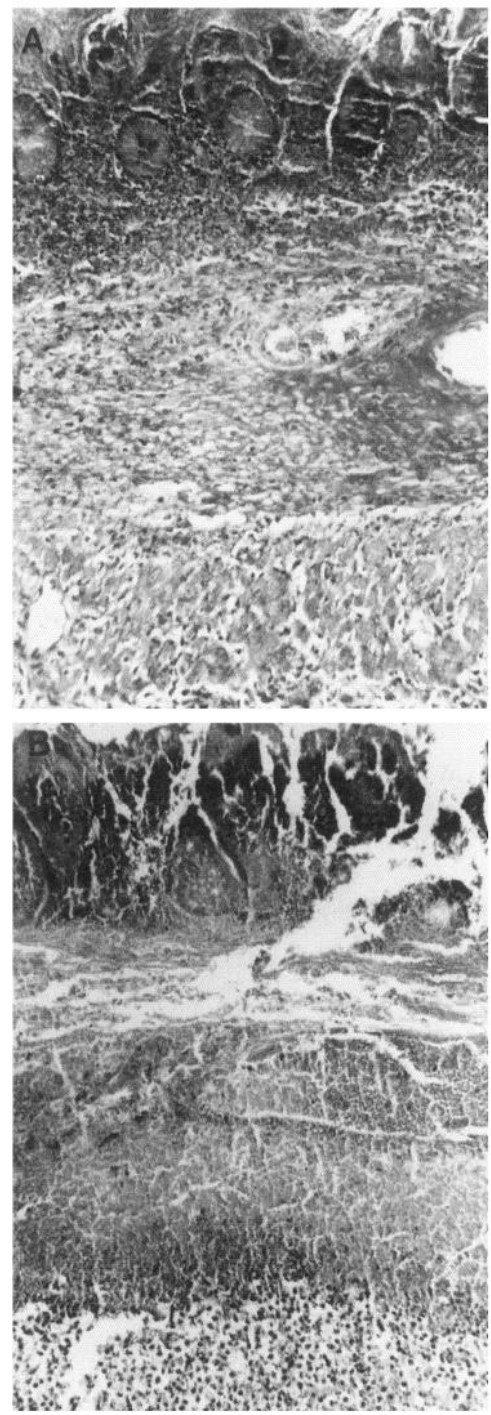

Fig. 6. Histology of TNB colitis tissue obtained from α-helical CRF-(9—41) and AVP antagonist-pretreated rats before each session of stress. *A*: colonic mucosa 4 days after induction of colitis in α-helical CRF-(9—41)-pretreated rat. *B*: colonic mucosa 4 days after induction of colitis in AVP antagonist-pretreated rat. Note in both cases invasion of whole colonic wall by inflammatory cells and necrosis of mucosa.

Downloaded from www.physiology.org/journal/ajpgi by ${individualUser.givenNames} ${individualUser.surname} (138.102.192.062) on August 31, 2018.
Copyright © 1997 American Physiological Society. All rights reserved.

AVP (16) but are not linked to the stimulation of the HPA axis (8, 23). Consequently, we could not reject the hypothesis that CRF and AVP may act on other pathways than the HPA axis to decrease the severity of colitis during stress.

In conclusion, this study provides evidence that stress may enhance experimental colitis; however, the mediators and the mechanisms involved in such a response need to be determined.

The authors thank L. Ressayre for skillful technical assistance.

This work was presented in part at the 95th Annual Meeting of the American Gastroenterological Association, May 14–17, 1995, San Diego, CA.

Address for reprint requests: M. Gué, Dept. of Pharmacology, Institut National de la Recherche Agronomique, 180 Chemin de Tournefeuille, BP3, 31931 Toulouse Cédex, France.

Received 10 April 1996; accepted in final form 30 August 1996.

REFERENCES

1. **Antoni, F. A.** Hypothalamic control of adreno-corticotropin secretion: advances since the discovery of 41-residue corticotropin releasing factor. *Endocrinol. Rev.* 7: 351–381, 1986.
2. **Bilezikjian, L. M., A. L. Blount, and W. Vale.** The cellular actions of vasopressin on corticotrophs of the anterior pituitary: resistance to glucocorticoid action. *Mol. Endocrinol.* 1: 451–458, 1987.
3. **Bradley, P. P., D. A. Priebat, R. D. Christensen, and G. Rothstein.** Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J. Invest. Dermatol.* 78: 206–209, 1982.
4. **Breder, C. D., C. A. Dinarello, and C. B. Saper.** Interleukin-1 immunoreactive innervation of the human hypothalamus. *Science Wash. DC* 240: 321–324, 1988.
5. **Buéno, L., M. Gué, and C. Del Rio.** CNS vasopressin mediates emotional stress and CRH-induced colonic motor alterations in rats. *Am. J. Physiol.* 262 (*Gastrointest. Liver Physiol.* 25): G427–G431, 1992.
6. **Collins, S. M.** Is the irritable gut an inflamed gut? *Scand. J. Gastroenterol.* 27, *Suppl.* 192: 102–105, 1992.
7. **Fabia, R., A. Ar'rajab, M. L. Johansson, R. Willen, R. Anderson, G. Molin, and S. Bengmark.** The effect of exogenous administration of *Lactobacillus reuteri* R2LC and oat fiber on acetic acid-induced colitis in the rat. *Scand. J. Gastroenterol.* 28: 155–162, 1993.
8. **Gué, M., J. L. Junien, and L. Buéno.** Mental stress in rats enhances colonic motility through the central release of corticotropin-releasing factor. *Gastroenterology* 100: 964–970, 1991.
9. **Hallgren, R., J. F. Colombel, R. Dahl, K. Fredens, A. Kruse, N. O. Jacobsen, P. Venge, and J. C. Rambaud.** Neutrophil and eosinophil involvement of the small bowel in patients with celiac disease and Crohn's disease: studies on the secretion rate and immunohistochemical localization of granulocyte granule constituents. *Am. J. Med.* 86: 56–64, 1989.
10. **Irwin, M.** Stress-induced immune suppression: role of brain corticotropin releasing hormone and autonomic nervous system mechanisms. *Adv. Neuroimmunol.* 4: 29–47, 1994.
11. **Judd, A. M., and R. M. MacLeod.** Adenocorticotropin increases interleukin-6 release from rat adrenal zona glomerulosa cells. *Endocrinology* 130: 1245–1254, 1992.
12. **Keller, S. E., J. M. Weiss, S. J. Schleifer, N. E. Miller, and M. Stein.** Suppression of immunity by stress: effect of a graded series of stressors on lymphocyte stimulation in the rat. *Science Wash. DC* 213: 1397–1400, 1981.
13. **Kort, W. J.** The effect of chronic stress on the immune response. *Adv. Neuroimmunol.* 4: 1–11, 1994.
14. **LeMay, L. G., A. J. Vander, and M. J. Kluger.** The effects of psychological stress on plasma interleukin-6 activity in rats. *Physiol. Behav.* 47: 957–961, 1990.
15. **Levine, S., R. Strebel, E. Wenk, and P. Harman.** Suppression of experimental allergic encephalomyelitis by stress. *Proc. Soc. Exp. Biol. Med.* 109: 294–298, 1962.
16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275, 1951.
17. **MacDermott, R. P., and W. F. Stenson.** Inflammatory bowel disease. In: *Immunology and Immunopathology of the Liver and Gastrointestestinal Tract*, edited by S. R. Targan and F. Shanahan. Tokyo: Igaku-Shoin, 1990, p. 459–486.
18. **McHugh, K., H. P. Weingarten, I. Khan, R. Riddell, and S. M. Collins.** Stress-induced exacerbation of experimental colitis (Abstract). *Gastroenterology* 104: A803, 1993.
19. **Morris, G. P., P. L. Beck, M. S. Herridge, W. Depew, M. R. Szewczuk, and J. L. Wallace.** Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 96: 795–803, 1989.
20. **Rachmilewitz, D., P. L. Simon, L. W. Schwartz, D. E. Griswold, J. D. Fondacaro, and M. A. Wasserman.** Inflammatory mediators of experimental colitis in rats. *Gastroenterology* 97: 326–337, 1989.
21. **Rivier, C., and W. Vale.** Modulation of stress-induced ACTH release by corticotropin releasing factor, catecholamines and vasopressin. *Nature Lond.* 305: 325–327, 1983.
22. **Saperstein, A., H. Brand, T. Audhya, D. Nabriski, B. Hutchinson, S. Rosenzweig, and C. S. Hollander.** Interleukin 1β mediates stress-induced immunosuppression via corticotropin-releasing factor. *Endocrinology* 130: 152–158, 1992.
23. **Sapolsky, R., C. Rivier, G. Yamamoto, P. Plotsky, and W. Vale.** Interleukin-1 stimulates the secretion of hypothalamic corticotropin releasing factor. *Science Wash. DC* 238: 522–524. 1987.
24. **Schobitz, B., D. A. M. Voorhuis, and E. R. De Kloet.** Localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. *Neurosci. Lett.* 136: 189–192, 1992.
25. **Sharon, P., and W. F. Stenson.** Enhanced synthesis of leukotriene B_4 by colonic mucosa in inflammatory bowel disease. *Gastroenterology* 86: 453–460, 1984.
26. **Spangelo, B. L., R. M. MacLeod, and P. C. Isakson.** Production of interleukin 6 by anterior pituitary cells in vitro. *Endocrinology* 126: 582–586, 1990.
27. **Stein, M., R. C. Schiavi, and M. Camerino.** Influence of brain and behavior on the immune system. *Science Wash. DC* 191: 435–440, 1976.
28. **Stein, T. A., L. Keegan, L. J. Auguste, B. Bailey, and L. Wise.** Stress induced experimental colitis. *Mediators Inflammation* 2: 253–256, 1993.
29. **Tanner, A. R., M. J. P. Arthur, and R. Wright.** Macrophage activation, chronic inflammation and gastrointestinal disease. *Gut* 25: 760–783, 1984.
30. **Vale, W., J. Spiess, C. Rivier, and J. Rivier.** Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science Wash. DC* 213: 1394–1397, 1981.
31. **Williams, C. L., R. G. Villar, J. M. Peterson, and T. F. Burks.** Corticotropin-releasing factor directly mediates colonic response to stress. *Am. J. Physiol.* 253 (*Gastrointest. Liver Physiol.* 16): G582–G586, 1987.
32. **Zhou, D., A. W. Kusnecov, M. R. Shurin, M. dePaoli, and B. S. Rabin.** Exposure to physical and psychological stressors elevates plasma interleukin 6: relationship to the activation of hypothalamic-pituitary-adrenal axis. *Endocrinology* 133: 2523–2530. 1993.

Downloaded from www.physiology.org/journal/ajpgi by ${individualUser.givenNames} ${individualUser.surname} (138.102.192.062) on August 31, 2018.
Copyright © 1997 American Physiological Society. All rights reserved.