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Anti-inflammatory effect of acute stress on experimental colitis is mediated by cholecystokinin-B receptors

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Abstract

We aimed to investigate the effects of electric shock (ES) on the course of experimental colitis and the involvement of possible central and peripheral mechanisms. In Sprague–Dawley rats (n = 190) colitis was induced by intracolonic administration 2,4,6-trinitrobenzenesulfonic acid (TNBS). The effects of ES (0.3–0.5 mA) or the central administration of corticotropin-releasing factor (CRF; astressin, 10 μg/kg) or cholecystokinin (CCK_B; 20 μg/kg) receptor antagonists and peripheral glucocorticoid receptor (RU-486; 10 mg/kg) or ganglion (hexamethonium; 15 mg/kg) blockers on TNBS-induced colitis were studied by the assessment of macroscopic score, histological analysis and tissue myeloperoxidase activity. ES reduced all colonic damage scores ($p < 0.05–0.01$), while central CRF ($p < 0.05–0.001$) and CCK_B receptor ($p < 0.05–0.01$) blockers or peripheral hexamethonium ($p < 0.05–0.01$) and RU-486 ($p < 0.05$) reversed stress-induced improvement. ES demonstrated an anti-inflammatory effect on colitis, which appears to be mediated by central CRF and CCK receptors with the participation of hypothalamo-pituitary-adrenal axis and the sympathetic nervous system.
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Keywords: Cholecystokinin (CCK); Colitis; Hypothalamo-pituitary-adrenal (HPA) axis; Stress; Sympathetic nervous system (SNS)

Introduction

A growing body of evidence indicates that stress has a prominent role in the pathophysiology and/or clinical presentation of gastrointestinal conditions (Collins, 2001; Mayer et al., 2001; Söderholm and Perdue, 2001). Any physical or psychological stressor that threatens the homeostasis of an

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organism can initiate a set of behavioral and neuro-endocrinological responses that help the organism to adapt to the altered situation. In conducting the neuro-endocrine responses, hypothalamo-pitutary (HPA) axis and sympatheto-adrenal axis are two major pathways mediating the major components of the stress response (Glavin et al., 1991; Koolhaas et al., 1997). Recent opinions on stress emphasize that in response to stressful events more than one response pattern exists and individual differences in coping with environmental challenges are of vital importance. Depending upon the physical (intensity, frequency, duration) and psychological (predictability and controllability) nature of stressors and individual differences—determined by the characteristics of organism, the experimental and developmental variables—organism may adapt an active (fight/flight, escape or avoidance) or a passive (immobility or crouching) behavioral strategy. These behavioral responses may be accompanied by different neuroendocrine responses ranging from high reactivity of the sympathetic response with increased plasma noradrenaline (seen with active coper) to a predominance in parasympathetic response and a more reactive HPA axis (seen with passive coper) (Glavin et al., 1991; Koolhaas et al., 1997).

The duration of stress and its interaction with several central neurotransmitters including corticotropin-releasing factor (CRF) and cholecystokinin (CCK), also influence the final outcome. It was reported that acute stress causes (a) hyperactivation in many central CRF-containing neurons, particularly found in paraventricular nuclei (PVN) of hypothalamus and amygdala, (b) stimulation in most of the central 5-HTergic and CCKergic systems (Collins, 2001; Dantzer, 1997; Daugé and Léna, 1998; Day et al., 1998; Dunn, 2000; Fink et al., 1998; Frankland et al., 1997). Whereas, chronic stress or chronic infusion of CRF causes (a) a reduction in CRF mRNA level in central amygdala and a slight elevation in PVN, (b) a hypoactivation in most of the central 5-HTergic system, and (c) CRF-hypersensitivity in locus coeruleus (LC) resulting in sympathetic nervous system (SNS) activation (Albeck et al., 1997; Linthorst et al., 1997; Pavcovich and Valentino, 1997). Regarding the peripheral actions of stress-induced central modulation, the net result is either hypo– or hyperactivation of the HPA axis and/or autonomic nervous system with an altered release of adrenocorticotropin (ACTH), glucocorticoids and catecholamines (Koolhaas et al., 1997; Million et al., 1999).

Among many central stress mediators, which modulate responses of HPA axis and SNS, CRF is accepted as the main neuropeptide involved in both physical and emotional stress. The results of a number of studies have suggested that the central CRF exerts a protective role on experimental colitis, possibly through the activation of HPA axis (Million et al., 1999; Gué et al., 1997). Moreover, CCK-like immunoreactivity and CCK_B receptor-mediated amygdaloid regulation of hypothalamus were observed upon stress exposure, suggesting that CCK may participate in the central control of neuro-endocrine response to stress (Daugé and Léna, 1998; Fink et al., 1998). The influence of acute or chronic stress exposure on colitis, as well as mechanisms through which stress alters gut inflammation, including the role of central CCK receptors, have not extensively been investigated.

First aim of the study was to examine the impact of the different stress models—acute or chronic controllable emotional stress, acute uncontrollable physico-psychological stress, acute uncontrollable physico-psychological stress upon chronic controllable emotional stress and the possible interactions of central CRF and CCK_B receptors on the course and modulation of colitis pathogenesis. The second aim was to evaluate the effects of exogenous CCK-8s on the course of colitis and its interaction with central CRF and CCK_B receptors in the modulation of colitis severity. The third aim was to examine the participation of SNS and HPA axis in mediating the inflammatory response of colon to stress, or to central CCK-8s administration.

Methods

Experiments were performed on adult male Sprague–Dawley rats, weighing 200–270 g that were housed in a light– and temperature-controlled room on a 12:12 h light-dark cycle where the temperature (22 ± 2 °C) and relative humidity (65–70%) were kept constant. The animals were fed a standard pellet lab chow, and access to water was allowed ad libitum up to onset of experiments. Animals were handled daily for a week by the individuals who performed the experimental procedures. The rats were anesthetized with ketamine (100 mg/kg; intraperitoneally, ip) and chlorpromazine (30 mg/kg; ip) and according to the atlas of Paxinos and Watson, stainless steel cerebroventricular guide cannulas (22–gauge; Plastic Products, Roanoke, VA) were inserted into the right lateral cerebral ventricles (Paxinos and Watson, 1996). Experiments were performed at least one week after the cannulation. At the end of the each experiment, methylene blue was injected to verify the correct placement of the cannulas and the animals were then decapitated. All studies including the stress models were approved by the Marmara University, School of Medicine, Animal Care and Use Committee.

Rats were deprived of food, but not water, for 16 h and were lightly anesthetized with ether and a polyethylene catheter was inserted rectally into the colon so that the tip was 8 cm proximal to the anus. The induction of colitis was performed by intracolonic administration of 0.5 ml of 38% ethanol containing 15 mg of 2,4,6-trinitrobenzenesulfonic acid (TNBS, Sigma). On the 3rd day of colitis induction, the rats were decapitated and the colonic tissue was examined macroscopically for the presence of adhesion and gross morphological changes and the removed colonic segments were weighed. Colitis was assessed by macroscopic damage scoring, microscopic evaluation and the degree of granulocyte infiltration estimated by the measurement of tissue myeloperoxidase (MPO) activity. The macroscopic scoring of the damaged segment was performed using the criteria considering number and length of ulceration and inflammation with a maximum score of 10 (Wallace et al., 1989). Following the macroscopic scoring, more than one piece of the colonic segment was immersed in formaldehyde solution (10%) and was then processed by routine techniques before embedding in paraffin wax. Thin sections (5 μm) were cut and stained with hematoxylin and eosin. To avoid observer bias, light microscopic assessment was performed by the other two observers who were unaware of the treatments and the following criteria were considered: score 0, no damage; score 1, mild; score 2, moderate; and score 3, severe damage in following parameters; (a) decrease in size of epithelial layer, (b) mucosal damage, (c) mucosal hemorrhage, (d) interstitial edema, and (e) inflammatory cell infiltration (Modified from Gué et al., 1997). The maximum score that any colonic segment could get was three. The remaining piece of the colonic segment was stored at −80 °C for subsequent measurement of MPO activity was assessed by measuring the H_2O_2-dependent oxidation of o-dianizidine.2HCL and expressed as units per gram wet tissue weight. One unit of enzyme activity was defined as the amount of the MPO present that produces a change in absorbance of 1.0 ml/min at 460 nm and 37 °C (Bradley et al., 1982).

Stress models

In the present study, two groups of stress models were used; an uncontrollable physico-psychological partial restraint stress (PRS) and a controllable psychological electric shock (ES) stress. Stress sessions were performed between 10^{00}–12^{00} a.m. to minimize any diurnal variation. The animals were returned to their home cages after shock exposure.

In acute PRS, the forelimbs and upper body were wrapped with gauze and secured by paper tapes for 2-h to restrict, but not to prevent movements. In acute controllable ES, each rat was placed in a plexiglass chamber for 30 min where a series of 20 random electric foot shocks were supplied to the grid floor by a pulse–generated scrambler (Northel, Istanbul). Each foot shock, within the range of 0.3–0.6 mA lasted for 5 s and produced discomfort but not pain. In the specifically designed chamber equipped with a photocell in a corner, rats received shocks only when the animals were detected by the photocell. That is, after 4–5 exposures, they learned to escape from shock (stress control) by hiding in the opposite corner.

To reinforce the involvement of limbic system structures in the modulation of stress-induced pathophysiological changes in the gastrointestinal system, ES model was modified to induce chronic stress. After the first day of shock application, rats were put in the same boxes for 3 more days at times when they received first-day-shock; but they received no electric shock. In order to induce acute stress upon chronic stress exposure, on the 4th day, the 2-hour PRS was applied to animals that had "no shock" chronic stress model.

Administration of drugs

All intracerebroventricular (icv) injections were administered, by using a Hamilton syringe, in a 5–μl volume over a period of 1 minute at least a week after the icv cannulation. The solutions of CCK-8s (Sigma; St Louis, USA) and astressin (CRF receptor antagonist; kindly provided by Jean Rivier, The Clayton Foundation Lab. for Peptide Biology, San Diego, California) were prepared in saline, while CI-988 (CCK_B receptor antagonist; Parke-Davis, Neuroscience Res. Centre, Cambridge) was dissolved in 3% dimethyl sulfoxide (DMSO; Sigma). A 10-min interval was given between the injection of the antagonists or the vehicle (saline or 3% DMSO) and icv agonist injection or stress induction.

In the last series of experiments, to determine the participation of SNS and HPA axis in mediating the colonic inflammatory effects of stress or exogenous CCK-8s, some of the animals received an intraperitoneal (ip) injection of 10 mg/kg (in a 1 ml volume) RU-486 (the glucocorticoid antagonist, Sigma) dissolved in 25% ethanol. The others were treated with the ganglion blocker hexamethonium (15 mg/kg; ip in a volume of 0.3 ml/rat, Sigma) dissolved in saline.

Experimental design

Experiments were performed, at least a week after the icv cannulation following a recovery and acclimatization period. This study was designed as two main parts: stress and central CCK-8s administered groups. In each subgroup, at least 6 animals (n = 6–13) were used and to minimize any diurnal variation in their response, all procedures in these groups were performed between 10^{00}–12^{00} a.m. and 02^{00}–04^{00} p.m. Same doses of the central agonist or antagonists were also administered intraperitoneally to determine whether the effects were central or peripheral. Colitis was induced by the intracolonic administration of TNBS on the 4th hour after the last stress exposure or after the last injection of the agonist. On the 3rd day of colitis induction, the rats were decapitated, the colonic segments were removed and weighed, damages were assessed by tissue MPO activity, macroscopic and microscopic evaluation.

Stress groups

In the present study, in order to examine the effects of stress models with different nature (controllable or uncontrollable, acute or chronic, physico-psychological or psychological) a) acute ES b) acute PRS and c) acute PRS upon chronic ES stress models were used (Fig. 1). In a second set of stress experiments, to determine the central and peripheral mechanisms involved in stress-induced modulation of experimental colitis, 10 min before acute ES session, rats were injected (icv; in 5 μl) with astressin (10 μg/kg) or CI-988 (20 μg/kg). Among these dosage regimens, astressin were selected based on previous reports (Pavcovich and Valentino, 1997). The effective dose of CI-988 on stress-induced damage score was determined by using different doses: 10, 20 and 40 μg/kg. The first dose did not cause any significant change in stress-induced macroscopic damage score, while the two higher doses were equally effective. In another group of rats, either hexamethonium (15 mg/kg; ip, 30 min before and 24 after) or RU-486 (10 mg/kg; ip, 12—and 1-hour before and 24 h after) were given before and after stress application (Casadevall et al., 1999; Meddings and Swain, 2000).

Central CCK-8s administered groups

In preliminary experiments, different treatment protocols with different doses of CCK-8s (40, 80 and 160 ng/kg) applied at various schedules were tried to find the effective doses. Among these, icv injection of CKK-8s at doses of 80 and 160 ng/kg administered at 10^{00} a.m. and 03^{00} p.m. on the first day, at 10^{00} a.m. on second day, significantly changed stress-induced colitis damage score. Similar to acute ES groups, rats were injected icv with the same specific receptor antagonists 10 min before the icv injection of CCK-8s (80 ng/kg). Lastly, either hexamethonium (30 min before each injection), or RU-486 (on the first day 12—and 1-hour before and on the second day 1-hour before) was given ip before the injection of central CCK-8s (80 ng/kg).

Statistical analysis

The results are expressed as means ± SE. Both parametric and non-parametric tests were used. For comparison of paired results, Student's *t*-test or Mann-Whitney U test and for multiple

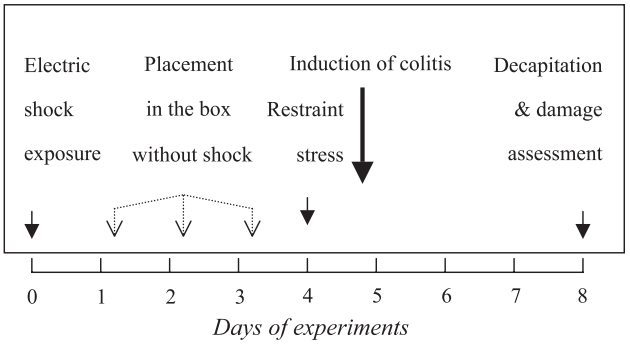


Fig. 1. Schematic representation of acute partial restraint stress upon chronic electric shock stress procedures applied before colitis induction.

comparisons, one-way analysis of variance (ANOVA) or Kruskal-Wallis test was used. Differences were considered statistically significant if $P < 0.05$.

Results

Evaluation of TNBS-induced tissue injury

Intracolonic application of TNBS induced a typical colonic injury characterized by hyperemia, inflammation and ulceration extending up to 2 cm in length. When compared with the macroscopic damage score (2.6 ± 0.49) and tissue MPO activity (102.06 ± 17.24 u/g) of the vehicle (ethanol) group, administration of TNBS significantly increased both parameters (4.75 ± 0.52 and 162.62 ± 10.49 u/g, respectively; $p < 0.05–0.01$) (Fig. 2). In contrast to nearly normal appearance seen in the vehicle group with a minute microscopic damage score (0.15 ± 0.05), histological observation in the colitis group showed severe epithelial and glandular damage accompanied by interstitial edema, severe hemorrhage, and inflammatory cell infiltration extending to submucosa. The microscopic damage score (Fig. 3a and b) was significantly higher (2.9 ± 0.01, $p < 0.01$).

Stress groups

When compared with the non-stressed colitis group, acute PRS stress or chronic ES alone had no significant effect, while both acute ES and acute PRS upon chronic ES decreased MPO activity and macroscopic scores ($p < 0.05–0.01$, Fig. 2). Regarding the mean macroscopic ($F = 3.015$; $p < 0.05$) and microscopic ($F = 7.52$; $p < 0.01$) scores, triple comparisons among chronic ES, acute PRS and acute PRS upon chronic ES revealed that there were significant variations. Furthermore, when compared to acute PRS or chronic ES alone, a significant reduction was observed in the microscopic damage score of acute PRS upon chronic ES group ($p < 0.05$). Histological examination of colonic tissues taken from acute ES group revealed reduced hemorrhage and damage in epithelial and glandular structures (Fig. 3c) with a microscopic score of 2.31 ± 0.53 ($p < 0.05$). In the acute PRS upon chronic ES group the amount of reduction was much more evident, noticed as normal epithelial and glandular structures in most of the observed areas with narrow hemorrhagic regions and moderate leukocyte infiltration (Fig. 3d) and a lower microscopic score was obtained (1.9 ± 0.17, $p < 0.05$). In acute ES group, the reductions in each of the above-mentioned parameters were abolished by the treatments with CI-988 or astressin ($p < 0.05–0.001$, Fig. 3d and Fig. 4). The degrees of epithelial and glandular damages, hemorrhage, edema and leukocyte infiltration in all of the antagonist-treated groups were similar and not different than those seen in the non-stressed colitis group (Fig. 3 b and 3e). However, ip injection of these antagonists at the same doses did not significantly change acute ES-induced increases in macroscopic scores and MPO activity.

Central CCK-8s administered groups

When compared with the non-stressed colitis group, in rats injected icv with CCK-8s (80 ng/kg) both macroscopic score and tissue MPO activity were decreased ($p < 0.05–0.01$, Fig. 5), whereas peripheral (ip) administration of CCK-8s at the same doses had no significant effect on macroscopic score (4.63 ± 0.75) or MPO activity (151.37 ± 12.86 u/g). Microscopic evaluation revealed a reduction in colonic

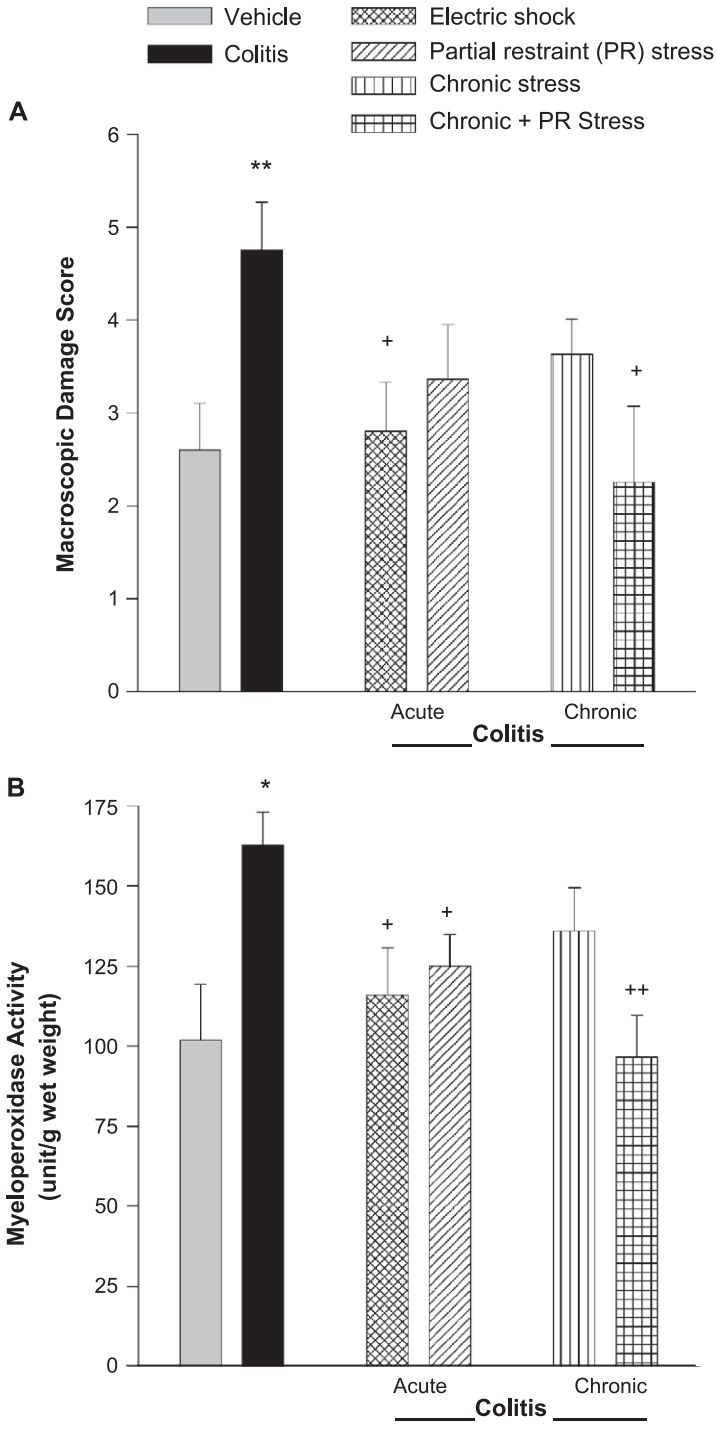


Fig. 2. Effect of different types of stress on trinitrobenzene sulfonic acid-induced colitis as assessed by macroscopic damage score (A) and tissue myeloperoxidase activity (B). *$p < 0.05$ and **$p < 0.01$, compared to vehicle (38% ethanol). +$p < 0.05$ and ++$p < 0.01$, compared to non-stressed colitis group.

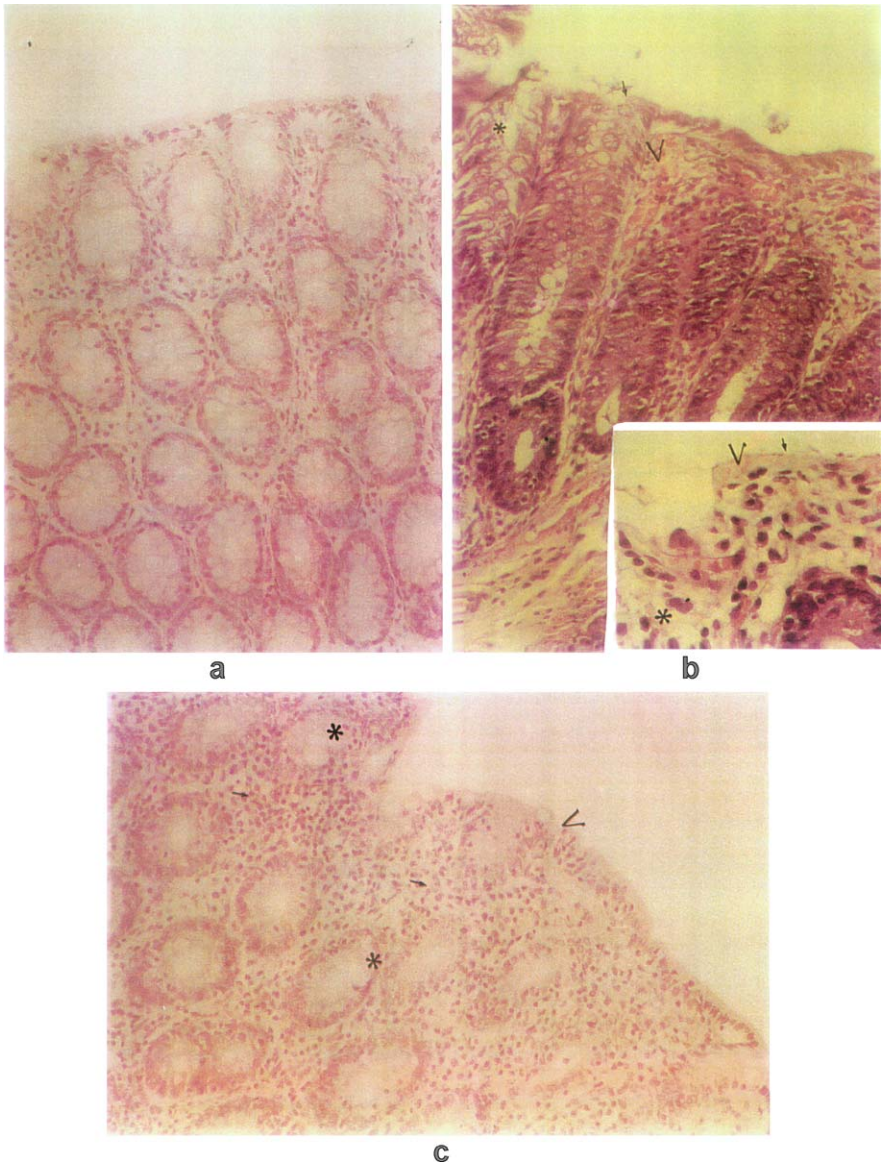


Fig. 3. Micrographs demonstrate **(a)** normal colonic mucosa morphology in the ***vehicle group***, **(b)** epithelial desquamation (→), disrupted glands (*), hemorrhage areas (>) in the ***colitis group***. Inset: epithelial desquamation (→), mucosal leukocyte infiltration (*) and hemorrhage areas (>), **(c)** epithelial desquamation and degeneration (>), disrupted glands (*) and extensive mucosal leukocyte infiltration (→) in the ***acute electric shock stress*** + ***colitis group***, **(d)** mild epithelial degeneration (→), and mucosal leukocyte infiltration (*) in the ***chronic*** + ***partial restraint stress applied colitis group,*** **(e)** extensive mucosal degeneration and submucosal leukocyte infiltration (→) in the ***CCK_B antagonist*** + ***acute electric shock given acute colitis group***, **(f)** mild epithelial degeneration (→) in the ***CCK-8s*** + ***colitis group***. Inset: disrupted glands (>) and extensive leukocyte infiltration (*). Magnification 66 X, inset 132 X; Hematoxylene and Eosin. (Note: Since the histological appearance is similar to that in the ***CCK_B antagonist group,*** the micrograph of the ***CRF antagonist*** + ***acute electric shock given acute colitis group*** is not included.).

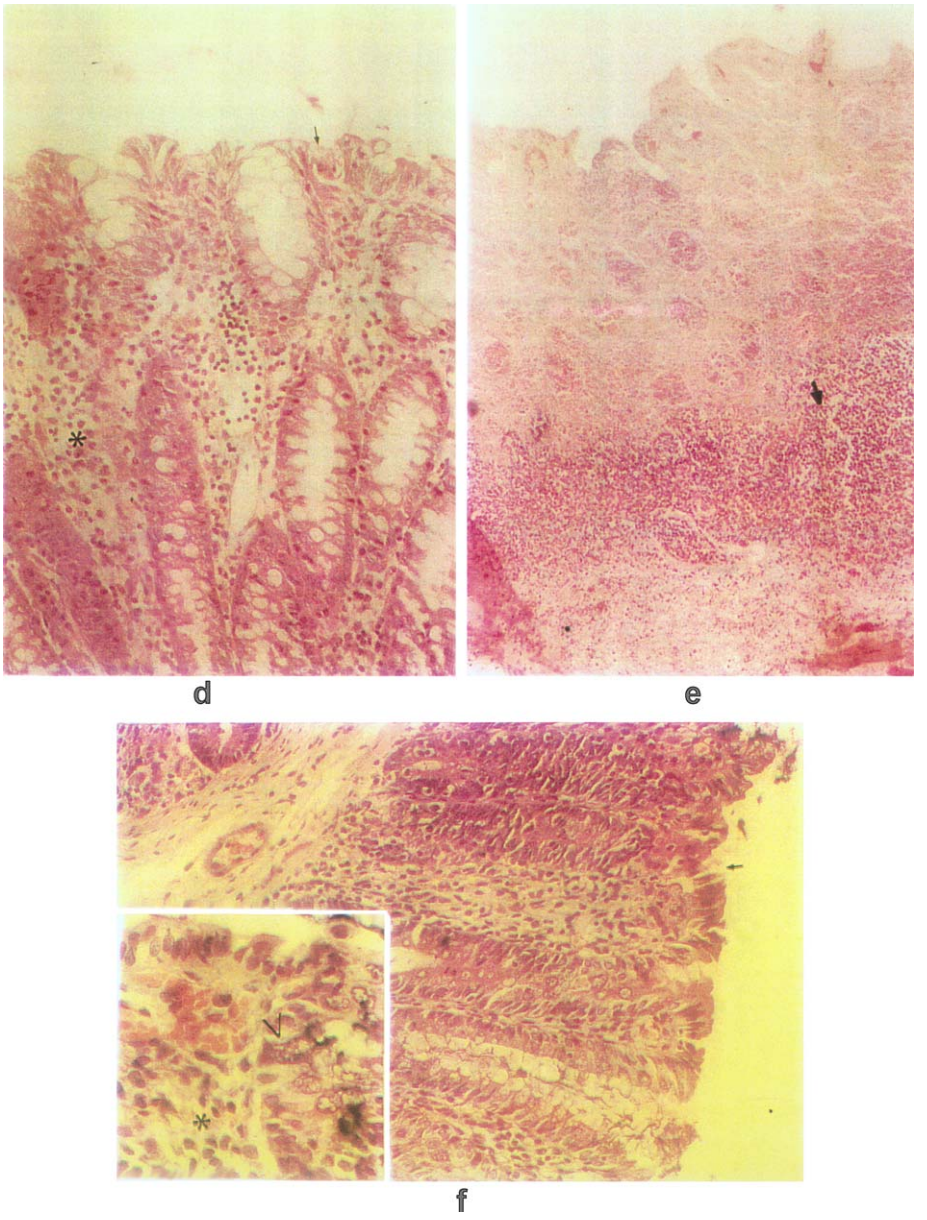


Fig. 3 (*continued*).

lesion score (2.13 ± 0.13) following icv administration of CCK-8s (Fig. 3f). Treatment with CI-988 (20 μg/kg, icv) abolished the reduction in colitis damage scores of CCK-8s-injected rats. Astressin treatment reversed the effect of CCK-8s on MPO activity, but colitis damage scores were not significantly changed (Fig. 5). Without icv agonist administration, central injection of either of the two antagonists alone (followed by icv saline) had no effect on TNBS-induced colitis (macroscopic scores as 4.29 ± 0.75 in astressin and 4.71 ± 0.78 in CI-988 groups).

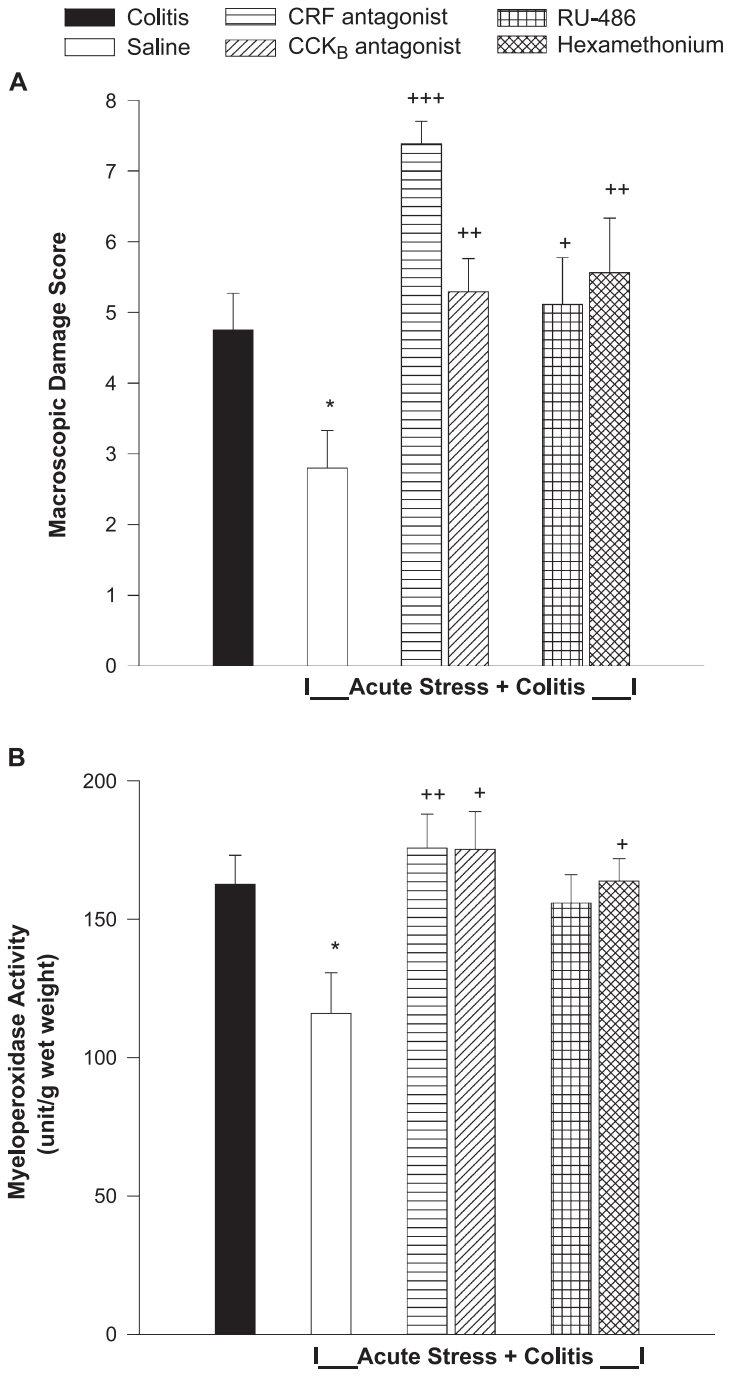


Fig. 4. The effect of corticotropin (CRF; 10 μg/kg, icv), cholecystokinin (CCK_B; 20 μg/kg, icv), glucocorticoid (RU-486; 10 mg/kg, ip) receptor antagonists and ganglion blocker hexamethonium (15 mg/kg, ip) on acute electrical shock-induced improvement of experimental colitis, as assessed by macroscopic damage score (A) and tissue myeloperoxidase activity (B). *$p < 0.05$, compared to non-stressed colitis group. +$p < 0.05$, ++$p < 0.01$ and +++$p < 0.001$, compared to acute stress group.

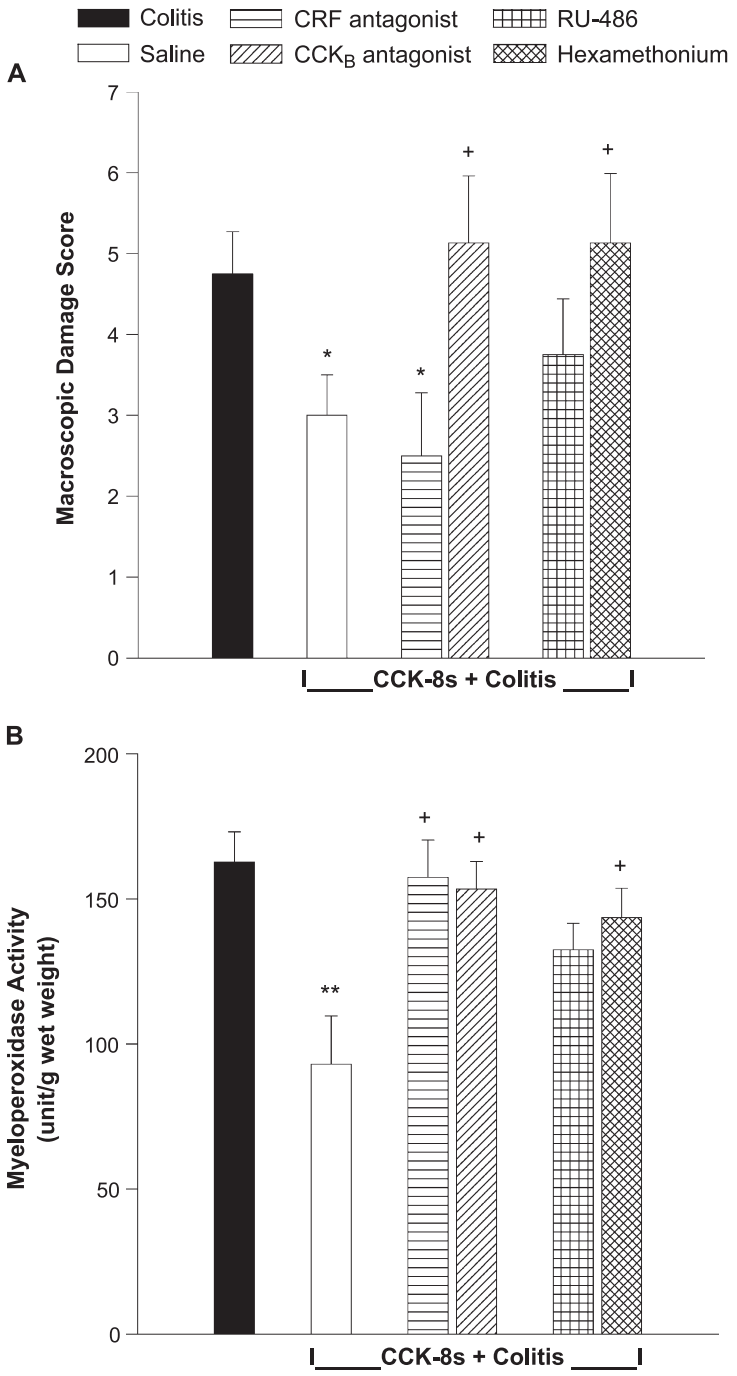


Fig. 5. The effect of corticotropin (CRF; 10 μg/kg, icv), cholecystokinin (CCK$_B$; 20 μg/kg, icv), glucocorticoid (RU-486; 10 mg/kg, ip) receptor antagonists and ganglion blocker hexamethonium (15 mg/kg, ip) on exogenous CCK-8s-induced (80 ng/kg icv) improvement of experimental colitis, as assessed by macroscopic damage score (A) and tissue myeloperoxidase activity (B). *$p < 0.05$ and **$p < 0.01$, compared to colitis group. +$p < 0.05$, compared to CCK-8s group.

The effect of glucocorticoid receptor and sympathetic ganglion blockade

Considering the macroscopic damage score and MPO activity, blockade of the sympathetic ganglia by the ip injection of hexamethonium reversed the anti-inflammatory effects provided by stress or CCK-8s, while RU-486 had a significant effect only on the stress but not on CCK-8s group ($p < 0.05–0.01$, Figs. 4, 5). While ip injection of hexamethonium alone (without stress induction or icv agonist injection) had no effect on TNBS-induced colitis, ip administration of RU-486 alone, surprisingly, reduced both macroscopic score (2.21 ± 0.48, $p < 0.01$) and tissue MPO activity (120.82 ± 14.48 u/g, $p < 0.05$).

Discussion

In contrast to previous studies regarding the relationship between chronic stress and experimental colitis (Million et al., 1999; Gué et al., 1997), our findings, for the first time, showed that acute ES reduced the severity of TNBS-induced colitis, as evidenced by a decreased macroscopic score, an improvement in the histological appearance, and a decreased granulocyte recruitment. These data also provide the first evidence that, besides the known effects of CRF receptors, CCK_B receptors may also participate in the stress-induced modulation of experimental colitis.

In the first report based on chronic stress, Gué et al. (1997) observed that chronic PRS increased the severity of TNBS-induced colitis which was further exaggerated following the injection of CRF receptor antagonist; α-helical CRF-(9–41). This enhancement of the severity in colitis seen after CRF receptor antagonist administration was explained with the hypothesis that CRF might not be responsible in stress-induced exacerbation of experimental colitis. Although this explanation brings about other possible mechanisms, including the involvement of other central neurotransmitters/neuropeptides in the central modulation of colitis, it is not strong enough to exclude the participation of CRF in the exacerbation of chronic stress-induced colitis. For example, in another study, Million et al. (1999) used two different rat strains having hypo– (Lewis) and hyper– (Fischer_344/N) CRF responses to stress and they showed that chronic stress–induced aggravation of colitis was more pronounced in the Lewis rats. In other words, Fischer rats, which displayed a significant increase in plasma corticosterone levels in response to chronic stress, had lower stress-induced worsening of colitis, while the Lewis rats, which failed to mount plasma corticosterone levels, exhibited a higher aggravation of colitis by stress.

A number of reports provide evidence that CRF exerts beneficial effects on inflammatory events. In two of those studies, central CRF exerted a protective role against TNBS-colitis (Million et al., 1999) and experimental gastric injury induced by acute stress (Shibasaki et al., 1990; Wang et al., 1996). In another study, central injection of CRF abrogated lipolysaccharide-induced expression of ICAM-1 expression and increased leukocyte recruitment measured by leukocyte rolling, adhesion and emigration, which were reversed by the blockade of endogenous glucocorticoids (Casadevall et al., 1999). In the present study, the abolishment of acute ES-induced anti-inflammatory effects on TNBS colitis by central CRF receptor antagonist did not only support the participation of CRF receptors in the pathophysiology of colitis, but also offered a potential explanation for the involvement of CRF and HPA axis on the modulation of colitis. Acute controllable ES and acute PRS upon chronic ES, but not acute uncontrollable PRS, caused anti-inflammatory effects on TNBS-induced colitis as demonstrated

by reduced MPO activity, decreased macroscopic and microscopic scores, which were reversed by the blockade of central CRF receptors and by peripheral glucocortioid receptors. Comparison among three types of stress- chronic ES, acute PRS and acute PRS upon chronic ES—showed that, macroscopic and microscopic damage scores were significantly lower in acute PRS upon chronic ES than the other two models. Histological examination of distal colon in the acute ES group revealed reduced hemorrhage and damage in epithelial and glandular structures. In the acute PRS upon chronic ES, the amount of reduction in damage was more prominent, with normal epithelial and glandular structures in most of the observed areas with narrow hemorrhagic regions and moderate leuokocyte infiltration. In our stress models, in contrast to previous findings based on chronic stress exposure, chronic stress caused a sensitization in the CNS, which led to an anti-inflammatory effect on experimental colitis. These controversial effects may be explained by the nature of stressors. In the present study, instead of an uncontrollable physiopsychological chronic stress model as used in previous studies, we used a controllable psychological one with some modifications to reinforce the involvement of upper brain structures including the limbic system. In fact, this chronic stress model is somewhat similar to the paradigm for conditioned fear, in which the animal is conditioned to context.

Among the studies which have investigated the relation between stress and immune system, a number of reports indicate that inescapable shock, but not the escapable one, caused an impairment in the immune system -e.g. decreased cellular immune response and natural killer activity (Dantzer, 1997; Jessop et al., 1989). Taken together with the previous studies which show that 1) central CRF exerts a protective effect, 2) chronic uncontrollable stress worsens TNBS-induced colitis, while controllable stress improves it, and 3) a deficient central CRF response enhances the deleterious effects of stress on colitis, a strong support is provided for the hypothesis that chronic ''non-overcome stress'' may cause CRF hyporesponsiveness in the CNS which is accompanied by an impaired HPA axis and reduced plasma glucorticoid level, thereby increasing the susceptibility and stimulating the reactivation of colitis. Nevertheless, this hypothesis does not exclude other possible mechanisms, which may include CRF-dependent (but HPA axis–independent) and CRF-independent mechanisms.

The results of the present study provide direct evidence about the role of CRF in stress-induced modulation of experimental colitis, since icv injection of CRF reversed the anti-inflammatory effect induced by acute ES in TNBS colitis. Moreover, the results of this study demonstrate for the first time that not only central CRF receptors but also central CCK_B receptors may participate in stress-induced central regulation of experimental colitis. Immunohistochemical and radioreceptors studies revealed the presence of CCK and CCK_B receptors in the area, including AP, DMN, NTS, hypothalamus and limbic structures where central modulation of gastrointestinal function is driven out (Fink et al., 1998). In one of the functional studies, central CCK was shown to block emotional stress– or CRF-induced colonic motor acceleration (Gué et al., 1994). However, regarding our data no interaction was evident between the central CCK and CRF systems. Central administration of CCK_B receptor antagonist abolished the acute ES-induced reduction in MPO activity, macroscopic and microscopic damage scores. Furthermore, in rats injected with icv CCK-8s (80 and 160 ng/kg) both macroscopic score and tissue MPO activity were decreased, while peripheral administration had no significant effect. Histological evaluation of icv CCK-8s group, demonstrated moderate–to–severe leukocyte infiltrations, moderate glandular damage, interstitial edema and hemorrhage, mild-to-moderate epithelial damage. In the groups designed to study the interactions among central CRF and CCK, CCK_B receptor antagonists abolished the reduction in colitis damage scores of CCK-8s-injected rats, while in the CKK-8s group treated with CRF receptor antagonist, colitis damage scores were not significantly changed.

There are two well-known pathways involved in the transportation of central response to the effectors of the periphery. The central activation of HPA axis and SNS either by neuronal stimulation or stress induction—including the used stress models: electric shock and PRS—is accompanied by increased plasma ACTH, corticosterone and catecholamine levels and these may contribute to stress-induced modulation of colonic inflammation (Glavin et al., 1991; Koolhaas et al., 1997; Schouten and Wiegant, 1997; Million et al., 1999; Melia et al., 1994; Riverst and Rivier, 1994). The present study demonstrates for the first time that ip injection of RU-486 or hexamethonium abolished acute ES-induced reductions in macroscopic damage score and leukocyte infiltration in colitis, while CCK-8s-induced changes were affected only by the blockade of sympathetic ganglia. These observations provide strong support for the hypothesis that, during stress—at least in the acute controllable ES—central CRF and CCK act to minimize the influence of colitis through the stimulation of HPA axis and/or SNS. In other words, central CRF-dependent and/or CRF-independent autonomic alterations may be responsible from stress-induced modulation of experimental colitis and our study provides evidence that, central CCK may be one of the candidates involved in the CRF– and HPA-independent modulation of colonic inflammation.

Conclusion

The results of the present study demonstrate that acute psychological stress reduces the severity of experimental colitis, possibly via the stimulation of HPA axis and/or SNS. Moreover, our results also provide the first evidence that CCK_B receptors participate in the stress-induced modulation of colitis.

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