neurogastroenterology & motility

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Journal:	*Neurogastroenterology and Motility*
Manuscript ID:	Draft
Manuscript Type:	Original Article
Date Submitted by the Author:	
Complete List of Authors:	Jorge, Esther; Universitat Autonoma de Barcelona, Cell Biology, Physiology and Immunology Torres, Rosa; Universitat Autonoma de Barcelona, Pharmacology Vergara, Patri; Universitat Autonoma de Barcelona, Dept.Cell Biology and Physiology Martin, Maria Teresa; Universitat Autonoma de Barcelona, Cell Biology, Physiology and Immunology
Key Words:	small intestinal motility, chronic stress, inflammation, bacterial translocation

FUNCTIONAL CHANGES INDUCED BY PSYCHOLOGICAL STRESS ARE NOT ENOUGH TO CAUSE INTESTINAL INFLAMMATION IN SPRAGUE-DAWLEY RATS

E. Jorge[1], R. Torres[2,3,4], P.Vergara[1,5], & MT. Martin[1,5]

Affiliation

1. Cell Biology, Physiology and Immunology Department, Universitat Autònoma de Barcelona, Bellaterra, Spain

2. Pharmacology Department, Universitat Autònoma de Barcelona, Bellaterra, Spain

3. Pneumology and Respiratory Allergy Department, Hospital Clínic, IDIBAPS, Universitat de Barcelona, Barcelona

4. Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBEREHD). Instituto de Salud Carlos III Mallorca , Spain

5. Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD). Instituto de Salud Carlos III, Barcelona, Spain.

Running title: chronic stress and intestinal inflammation in rats

Address for correspondence:

Maria Teresa Martin

Unitat de Fisiologia (Edifici V)

Universitat Autònoma de Barcelona

08193 Bellaterra

Spain

Telephone no. +34-93-5813834

Fax no. +34-93-5812006

e-mail: maite.martin@uab.cat

ABSTRACT:

BACKGROUND: Although stress seems to modulate the time-course of intestinal inflammation, its role as a trigger of the inflammatory process in absence of other putative contributing factors remains controversial. Our aim was to elucidate whether stress per se can induce a primary gut inflammation in non-prediposed rats.

METHODS: Male Sprague-Dawley rats were divided in sham and stress groups. Chronic stress was induced by submitting animals 1h/day to wrap restraint or water avoidance stress alternatively for 5 consecutive days, as a model of ongoing life stress.

KEY RESULTS: Chronic stress induced a significant decrease in body weight gain without changes in food intake and an increase in frequency of defecation. Electromiografic (EMG) study showed that the duration of the migrating motor cycles (MMCs), but not its frequency, was shortened in stressed animals compared with non-stress conditions. Moreover, stressful stimulus caused mucosal mast cell hyperplasia and a decrease of iNOS mRNA expression. Although more than 50% of animals submitted to stress presented bacterial translocation, no changes in either MPO or COX-2 mRNA expression were observed.

CONCLUSION & INFERENCES: Decreased MMC duration, mast cell hyperplasia and decreased mRNA iNOS expression could be factors implicated in bacterial translocation-induced by chronic stress. However, these changes are not sufficient to induce intestinal inflammation in stress-non susceptible strain of rats.

Keywords: small intestinal motility; chronic stress ; inflammation; bacterial translocation

INTRODUCTION:

It is well known that emotional state influences gastrointestinal function. Changes in motor activity, such as gastric emptying inhibition or increased colonic motility, occur in response to several stressors (1). Moreover, stress has a great impact on epithelial barrier function, causing an increase in intestinal permeability that can result in an enhanced uptake of macromolecules (2). This fact may allow an abnormal presentation of luminal constituents to the mucosal immune system and initiate an inflammatory response.

As stress could act as a modulatory factor of inflammation, several studies have been focused on its contribution at the onset, symptom exacerbation and reactivation of gastrointestinal (GI) disorders, and among them Crohn's disease and ulcerative colitis, the two main pathologies included in the general term of Inflammatory Bowel Disease (IBD). The degree of intestinal inflammation during the course of IBD varies from apparently normal in remission to severe ulceration during relapse. The implication of stress in the reactivation of the illness is indicated in several reports showing a relationship between relapses and previously stressful events suffered by IBD patients (3). Although some recent clinical reports suggest a non-association between stress and relapses (4), studies performed in animal models of IBD demonstrate an increase of several inflammatory parameters in response to different stressors (5;6), supporting the contribution of stress in the reactivation of the inflammatory process.

Besides the possible implication of stressful events in the time-course of IBD, it has also been postulated that stress per se can initiate intestinal inflammation. Salem et al (7) observed an increased prevalence of ulcerative colitis in Bedouin Arabs after they moved to government housing. More recently, it was described that early life stresses may predispose development of IBD (8). However, its is difficult to ascertain in human studies whether stressful events can directly induce intestinal inflammation, as genetic predisposition to suffer IBD is also an important factor contributing to the development of the disease. In this sense,

the main evidence supporting stress per se as a trigger on the inflammatory process comes from animal studies. The intestinal mucosa of rats submitted to chronic stress shows an increase of inflammatory markers together with an altered epithelial barrier function and bacterial translocation (9). Moreover, rats exposed to stressful stimuli prior to the induction of experimental colitis have an enhanced susceptibility to develop intestinal inflammation in comparison to non-exposed rats (10). However, most of these studies have been performed in specific strains of animals, such as Wistar-Kyoto or LEW/N rats that show an increased susceptibility to develop intestinal inflammation in response to stress (11;12).

Therefore, the main goal of the present study was to assess whether functional changes induced by stress in a non-susceptible strain of animal, i.e., Sprague-Dawley rats, are enough to induce intestinal inflammation. Specifically, this study focused on the effects of chronic stress on 1) intestinal motor activity, considered as a regulator of intestinal bacterial load; 2) mast cells, a cellular type that plays a fundamental role in the epithelial barrier dysfunction induced by stress; 3) bacterial translocation, that can trigger intestinal inflammation and 4) several inflammatory parameters such as myeloperoxidase, iNOS and COX-2 RNA expression in intestinal tissues.

MATERIALS AND METHODS:

Animals

Male Sprague-Dawley rats (Charles River, Lyon, France), 8-10 weeks old and weighting 300-350 g were used. Animals were kept under conventional conditions in an environmentally controlled room (20-21ºC, 60% humidity, 12:12-h light-dark cycle) with tap water and standard laboratory rat chow ad libitum. All experimental protocols were approved by the Ethical Comitée of the Universitat Autònoma de Barcelona.

Experimental model

Rats were handled daily by the same investigator who performed the experiments for 1 week before the study, in order to minimize the stress of unusual contact with humans.

Chronic stress was induced by submitting animals to a heterotypic chronic intermittent stress paradigm involving the physico-psychological stress of wrap restraint (WR) (13) and the psychological stress of water avoidance stress (WAS) (14). During the WR procedure the limbs and the body of animals were wrapped in a cloth harness to restrict, but not to prevent, body movements. Water avoidance procedure (WAS) consisted of placing the rat on a plastic platform (7x7x10 cm) located in the middle of a plastic container (55 cm diameter) filled with warm water (25ºC) to 1cm below the platform . The chronic stress protocol was applied between 9:00 and 12:00 AM to minimize the effect of diurnal circadian rhythm and consisted of submitting animals 1h/ day to WR or WAS alternatively for 5 consecutive days.

Study I

A group of 4 rats was used to study the effects of stress on the small intestinal motor activity. To avoid the stress induced by restraining, we used the radio telemetry technique for the detection and measurement of the jejunum myoelectrical activity in freely moving rats.

Electrodes: A telemetric transmitter measuring biopotentials (PhysioTel Implant TL10M3-F50-EEE, Data Sciences International, St Paul, MN, USA) was used. Electrodes were prepared similarly to previously described (20). Briefly, two 25 mm diameter varnished Ag wires were soldered to the stainless steel wire of the transmitter's leads and used as bipolar electrodes. The Ag wires were stripped of their insulation for 1 cm at the end, stock through a reinforced silicon sheet (5mm x 8mm) with a space of 4,5 mm between them. They were fixed with a second silicon sheet which was glued on top with silicone paste.

Preparation of animals: Under general anesthesia induced by a mixture of ketamine and xylacine (90mg/kg and 10 mg/kg respectively, ip.) a small skin incision between the shoulder blades was performed to fix the telemetry transducer. Electrodes were tunneled subcutaneously to the abdominal cavity to be placed in the jejunum (10 cm distally to the ligament of Treitz) and fixed by suturing the silicon sheet to the serosa. Postoperative pain

was reduced by subcutaneous administration of 2mg/Kg of meloxicam (Metacam, Boehringer Ingelheim, Germany) once daily for 3 days. All rats were monitored and weighed daily during the recovery period (10 days).

EMG recording by telemetry: To record the intestinal electrical activity, EMG signals from the radio telemetry transmitter were collected by a receiver (RCP1, Data Science International) placed under the animal's cage. The receiver was linked through a raw data analog converter (DL10, Data Science International) to a personal computer equipped with a specific data recording system (PowerLab/800). To eliminate possible interfering electric signals, a low-pass filter of 50 Hz was used.

Experimental design: Studies were started after 10 days from the surgery, to allow the recovery of the normal gastrointestinal motility. EMG recordings during both normal (basal) and stress conditions were obtained from the same animal. Two basal EMG recordings of 2,5 hours (separated by 3-4 days) were obtained in rats fasted for 12 hours before applying the stress protocol in order to confirm the total recovery after surgery. Then, the stress protocol was applied to the same rat and myoelectrical activity was recorded during the last stress session, coinciding with the WR stimulus. Records were obtained for 1 h during the stress session plus 1,5 h after. The effects of stress on the electrical activity of the jejunum were evaluated by determining the frequency of the migrating motor complex (MMC) and the duration of phase III of the MMC.

At the end of the study rats were killed by isoflurane overdose. Necropsy of animals was performed in order to confirm the absence of inflammation around the implanted electrodes and transmitters.

Study II

Two groups of animals were used: 1) control group (n=11) of non-stressed rats, that were maintained in their own home cage during the stress procedure, and 2) chronic stress group

(n=11), that was submitted to the stress protocol. Animals were killed after the last stress/control session by isofluorane inhalatory anesthesia and exsanguinated by heart puncture, to obtain intestinal tissue samples.

Animals monitoring

Several parameters were evaluated to confirm the effectiveness of the stress protocol.

Growth: Rats were weighted daily through the stress protocol period and body weight change was expressed as % of weight gain in relation to the first day.

Food intake: Food consumption was recorded daily through the stress protocol period and results were expressed as g/day.

Defecation: Defecation, as an indirect index of colonic propulsive activity (15) was determined at each session. Pellets expelled during 2 hours (1 hour stress/sham session plus 1 hour after) were counted and expressed as the number of fecal pellets/2h.

Effects of stress on mucosal mast cells

Distal ileum samples were fixed in 4% buffered formaldehide and processed for embedding in paraffin wax. Tissue sections were cut (3 µm) and immunodetection of rat mast cell protease II (RMCP II) was carried out using a monoclonal antibody (1:500; Moredun Animal Health, Edinburgh, UK). Detection was performed with avidin/peroxidase (Vectastain ABC kit; Vector Laboratories, Burlingame CA, USA). Sections were counterstained with haematoxylin and counted at x 400 magnification. Positively stained mast cells were counted in three to five sections per animal. Seven to ten well-oriented villus-crypt units (VCU) were examined per section. Analysis of all morphological data was performed blinded to prevent observer bias. Estimation of mast cell numbers was expressed as cells per VCU.

Bacterial Translocation

Bacterial translocation from the lumen of the intestine was determined by detection of viable enteric bacteria in mesenteric lymph nodes (MLN) (16). MLN from the ileocaecal region of each rat were removed aseptically immediately after being killed. Specimens were frozen in liquid nitrogen, powdered in a mortar kept at -80ºC, and weighted. Samples were homogenized in ten parts of milk (Difco, Detroit, MI) and incubated under aerobic conditions onto blood agar and McConkey agar for 48 h at 37ºC. After incubation, colonies were identified by studying their morphological and biochemical properties. Bacterial translocation data were expressed as the number of positive cultures of the total number of samples in each group.

RT-PCR studies.

Distal ileum samples of each rat were taken immediately after the animal was killed. One additional rat exposed to LPS 15 mg/kg ip (Lipopolysaccharide 0111:B4 (*Escherichia coli*) dissolved in saline solution; Sigma, St. Louis, MO, USA) was used as a positive control for iNOS (17). Tissue segments were frozen in liquid nitrogen and stored at -80ºC until use.

RNA extraction. Isolation of total RNA was performed using TRI Reagent (Ambion, Madison, WI, USA) according to the manufacturer's instructions and treated with DNA-free (Ambion) for 30 min at 37ºC to remove any genomic DNA contamination.

RT-PCR. First-strand cDNA was synthesized from 5 µg total RNA in a reaction mixture of 50 µL containing 0.5 µg of oligo18 (dT) primer (Ambion), 2mM dNTP (Ecogen, Barcelona, Spain), and 10 units Moloney murine leukemia virus (Ambion). The resultant cDNA was amplified in a total volume of 50 µL with 1 unit of *taq*DNA (Ecogen), 1mM dNTP mixture, and 1.2 µM primers (Proligo). The sequences of sense and antisense primers for rat iNOS, COX-2, and GAPDH (a housekeeping gene used as an internal control) are listed in Table 1. Thermal cycling conditions were as follows: denaturation for 4 min at 95 ºC, then 30 (COX-2) or 35 cycles (iNOS, GAPDH) PCR with denaturation at 95ºC for 1 minute, annealing at 62ºC (iNOS), 53 ºC (COX-2) or 50 ºC (GAPDH) for 1 minute, and extension at 72ºC for 1 minute, and a final extension of 72ºC for 5 minutes. Amplified products were electrophoresed on

1.5% agarose gel in TAE buffer, stained with ethidium bromide, photographed under ultraviolet light, and quantified using image-analyzing software (PC-BAS 2.0). For semiquantification, the ratio of the optical density of each PCR product and GAPDH was determined.

Tissue Myeloperoxidase (MPO) Determination

Proteins were extracted from the ileal tissue of each animal using a lysis buffer containing protease inhibitors (Minicomplete tablet, Roche Diagnostics, Mannheim, Germany). MPO concentration was determined using a specific enzyme-linked immunosorbent assay (HyCult Biotechnology, Uden, The Netherlands), with a minimal detectable concentration of 1 ng/mL.

Statistical Analysis

Data are expressed as means ± SEM. Statistics were performed using Graph Pad Prism v.4.0 software (San Diego, CA, USA). Effects of stress on frequency and duration of MMC were determined using a paired student's *t*-Test. In the study II, differences between groups in relation to the progression of animal growth and food intake were assessed by a Two-way analysis of variance (ANOVA). Results from RT-PCR studies and MPO determination were analyzed using an unpaired student's *t*-Test. Bacterial translocation were determined by a Chi-square test. In all cases, results were considered to be statistically significant when $p<0.05$.

RESULTS:

Study I

Effects of stress on the inter-digestive myoelectric activity of the small intestine

Animals were closely monitored to evaluate the recovery after the transmitter implantation. After the abdominal surgery, all animals recovered well and tolerated the transmitter. Basal

records started 10 days after the surgical procedure and both frequency and duration of MMC were similar to that previously reported using non-radio telemetry systems (18).

To evaluate the effects of stress on intestinal motility, records during basal and stress conditions were obtained from the same animal. In these animals, the effectiveness of the stress protocol was determined as for study II, obtaining similar results to that reported below (see animals monitoring in study II). Figure 1A shows a typical record of the electrical activity of the jejunum in a rat submitted to the stress protocol. The inter-digestive motility pattern present in a stressed animal was similar to that recorded during basal conditions, with the presence of typical MMC cycles. However, although no changes were observed in the frequency of MMC, exposure to stress caused a significant shortening in its duration (Figure 1B,C).

Study II

Animal monitoring

During the experimental period, stressed rats showed a significant reduction in body weight gain, whereas control animals continued to grow. As shown in figure 2, this effect was not related to differences in food intake, as food consumption was similar in both control and stressed animals.

Moreover, chronic stress significantly increased fecal pellet output when compared with rats not submitted to the stressful stimuli (Figure 3). The response to stress exposure was stable over the 5-day duration of the stress protocol, with the rate of defecation of stressed rats being three times greater than the mean rate of defecation of unstressed rats.

Effects of stress on mucosal mast cells

The detection of RMCP II, a chymase predominantly expressed by rat intestinal mucosal mast cells, by immunochemistry is a usual method used to determine mucosal mast cell count. As figure 4 shows, exposure to chronic stress was associated with a significantly

higher number of mast cells in the mucosa of the ileum when compared to the control group. Moreover, this increase in the mucosal mast cell count was observed in all rats included in the stress group.

Bacterial Translocation

Results of aerobic bacteria isolated from MLN are summarized in Table 2. As shown, chronic stress exposure was associated with bacterial translocation. While neither of control animals presented bacterial translocation, more than 50% of animals submitted to the stress protocol showed remarkable positives bacterial cultures in MLN, to either *Enteriobacteriaceae*, *Lactobacillus* or *Streptococcus/Enterococcus* spp.

RT-PCR studies

Using specific primers for iNOS, COX-2 and GAPDH, as an internal standard, in RT-PCR studies, single band for each cDNA at the expected size were observed.

Figure 5 shows the results of iNOS/GAPDH mRNA expression obtained in both control and stressed rats. In control animals, a low but clear expression of iNOS RNA was detected in the ileum. Exposure to chronic stress significantly decreased RNA expression of this enzyme when compared to the non-stressed group. Regarding COX-2, also a low expression of this enzyme was detected in ileal tissues obtained from control rats. In contrast to the effect induced by stress in the RNA expression of iNOS, no significant changes were observed in COX-2 expression in stressed animals when compared to the control group (0.50 ± 0.04 in control group vs 0.59 ± 0.05 in stress group).

Tissue Myeloperoxidase (MPO) Determination

As a biochemical inflammatory parameter, MPO was evaluated in ileal tissue samples. MPO is an enzyme found in granulated cells and previously shown to be a reliable index of inflammatory activity (19). The data in Figure 6 show that in Sprague-Dawley rats that were submitted to chronic stress, assessment of MPO levels one hour after the last

stress session did not indicate the presence of an inflammatory process, as the results obtained were similar to that found in control animals.

DISCUSSION:

This study was designed to evaluate the impact of chronic stress on Sprague-Dawley rats, a strain not especially susceptible to stress. We showed that chronic stress induced an important bacterial translocation in healthy animals. This finding was associated to small intestinal motility disturbances, mucosa mast cell hyperplasia as well as a decreased iNOS mRNA expression. Although chronic stress induces an important passage of bacteria from the gut to mesenteric lymph nodes, animals did not show any signs of small intestinal inflammation, as COX-2 mRNA expression and MPO levels were similar to non-stressed animals.

It is well known that chronic stress causes a reduction in body weight gain, as has been reported in studies using several rat strains (20;21) or stress protocols (22;23). The stress protocol used in our study also caused a significant decrease in body weight gain of Sprague-Dawley rats. This effect does not seem to be related to changes in food consumption, as food intake was similar in control and stressed animals. Previous studies also describe a non-relationship between food intake and body weight gain (24), although an association between these two parameters has also been reported (25). These different results can be attributed to the different stress protocols used in these studies. In our experimental model, the stress-induced inhibition of weight gain could be mediated by metabolic changes such as stimulation or inhibition of the release of catabolic (cathecholamines, glucocorticoids) and anabolic (thyrotrophic and growth hormone) hormones, respectively (26). On the other hand, as previously reported (27;28), we observed an increase on the defecation frequency in rats submitted to the stress protocol. Taking these results together we can confirm the effectiveness of the stress protocol used in our study. Moreover, there is no adaptation to stress as the effects on body weight gain and defecation frequency persisted without improvement over the 5-day stress period

Several reports have demonstrated that stress induces alterations of gastrointestinal motility. However, these alterations have been described mainly for the upper (stomach) and lower (colon) segments (1). To evaluate whether changes occur also in small intestine we used a radio-telemetry system, that allowed us to exclude distress related to handling and restraining rats (29). As previously described (18), our results obtained from rats under control conditions were similar to that obtained using conventional methods not involving radio telemetry, confirming the usefulness of this technique. Rats submitted to chronic stress showed a similar inter-digestive motility pattern (MMCs) to that observed under non-stress-conditions, but some differences were detected. Whereas chronic stress did not modify the frequency of the MMC cycle, a significant reduction in the duration of phase III was found. The inter-digestive intestinal motility pattern, and specifically the regular occurrence of phase III of migrating myoelectric motor complex (MMC) is considered to be one of the physiological mechanisms that prevents bacterial overgrowth and translocation in the intestine (30). One may therefore speculate that decreased MMC duration induced by stress can reverberate in a less effective propelling of the residual food, debris, secretions and bacteria cells. This could facilitate bacterial overgrowth and hence, the bacterial translocation that we observed in rats submitted to chronic stress.

Nevertheless, factors other than motility should also be involved in the stress-related bacterial translocation. Several studies from various groups have highlighted the importance of mucosal mast cells in stress-induced mucosal changes and epithelial barrier dysfunction (21;31;32). As reported for other stress protocols (9;31), mucosal mast cell hyperplasia was observed in rats submitted to chronic stress. As a result, with the large number of reports supporting a relationship between mucosal mast cells and stress-induced breakdown of the intestinal epithelial barrier, resulting in an enhanced passage of both small molecules and macromolecules (12), it is reasonable to hypothesize that mast cell hyperplasia could also be a mechanism implicated in stress-related bacterial translocation in our experimental model.

To our knowledge, this is the first report documenting a stress-induced inhibition in iNOS mRNA expression in ileum mucosa. In contrast we did not detect changes in iNOS RNA

expression in colonic tissues samples obtained from stressed animals when compared to the control group (data not shown). Some controversy exists about the effects of stress on iNOS RNA expression in the colonic segment. Whereas some studies have reported similar results to those obtained in the present study (10), others have described an increase of iNOS in response to stress (33). On the other hand, our data seems to indicate that specific organ response differences may exist with regard to expression of iNOS in response to stress. These differences could be due to the fact that although corticosteroids are known to inhibit iNOS in different cell types (34) including epithelial cells (35), they seem to be ineffective in colonic epithelial cells (36). Finally, it has been described that NO acts as part of the nonspecific host defense mechanisms against microorganisms contributing to mucosal protection (37;38). It therefore follows that the stress-induced reduction in iNOS levels of the ileal segment could cause a lowering of the protection provided by the epithelial barrier against microorganisms, contributing to the bacterial translocation.

It has been suggested that bacterial translocation may activate mucosa-associated immune cells leading to an inflammatory response. In our study, stress did not modify the levels of inflammatory parameters as COX-2 and MPO. Although Söderholm *et al* (9) have demonstrated intestinal inflammation due to stress in healthy animals, our study is in agreement with other authors who have described non inflammation (6;13). Therefore, the present study showed that stress-induced bacterial translocation was not associated with small intestinal inflammation in healthy Sprague-Dawley rats. This discrepancy may be explained by the strain rat susceptibility, as in Söderholm *et al.* study, Wistar Kyoto, a hyperresponsive to stress rat strain, was used.

In view of the results obtained in this study we can conclude that stress per se cannot be considered as a trigger of intestinal inflammation. However, as stress causes significant gastrointestinal changes, it could act as a contributing factor to induce inflammation when others factors are also present. In fact, the convergence of stress and bacterial gastroenteritis increases the risk of developing postinfective IBS (39). Moreover, decreased intestinal barrier integrity plus stressful stimuli can result in the onset or relapse of IBD (40).

In summary, this study shows that although chronic stress causes bacterial translocation, this is not enough to trigger intestinal inflammation in healthy non-predisposed animals. Moreover, this bacterial translocation could be facilitated by both small intestinal disturbances and mucosal mast cell hyperplasia induced by stress.

ACKNOWLEDGMENTS AND DISCLOSURES

The authors are grateful to A. C. Hudson for the editorial revision of the manuscript and to A. Acosta for the care of the animals.

This work was supported by FPU grant AP-2004-5574 from Ministerio de Ciencia e Innovación (financial support to E. Jorge), Grant BFU2007-62794 from Dirección General de Investigación, Ministerio de Ciencia e Innovación. CIBEREHD is funded by the Instituto de Salud Carlos III.

Competing interests: the authors have no competing interests.

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TABLES

Table 1. Primer sequences for RT-PCR

Gene	Primer Sequence	Amplicon lenght (bp)
COX-2		
Sense	5'-CAACAGCCCATCTCTCAA-3'	483
Antisense	5'-CCCACTAACTGACTCTGT-3'	
iNOS		
Sense	5'-ACAACAGGAACCTACCAGCTCA-3'	651
Antisense	5'-GATGTTGTAGCGCTGTGTGTCA-3'	
GAPDH		
Sense	5'-CCGCCCCTTCCGCTGATGCC-3'	140
Antisense	5'-ATGAGCCCTTCCACGATGCC-3'	

Table 2. Presence of bacterial translocation for a specific microorganism in control rats and rats submitted to 1h/day of WR/WAS alternatively for 5 consecutive days. Data are expressed as number of positive cultures of the total animals of each group. * $p<0.05$ compared to control-non stressed group. n = 11 in each group.

	Control	Chronic stress
Bacterial translocation	6/11**	0/11
Enterobateriaceae	1/11	0/11
Enterococcus/Streptococcus spp.	3/11	0/11
Lactobacillus spp.	3/11	0/11

FIGURES

FIGURE AND TABLE LEGENDS

Fig. 1. A representative EMG recording of the myoelectric activity of jejunum from an animal submitted to chronic stress. The recording was obtained using radiotelemetry **(A)**. Effect of chronic stress on the frequency **(B)** and duration **(C)** of the migrating myoelectric complex (MMC) in jejunum. Values are means ± SEM. * $p<0.05$.n = 4

Fig 2. Effect of chronic stress during the course and at the end of the stress protocol on body weight gain **(A1, A2)** and food intake **(B1, B2)**. Data are expressed as means ± SEM.

* $p<0.05$, ** $p<0.01$. n = 11 animals/group.

Fig. 3. Effects of exposure to chronic stress on fecal pellet output. Data are expressed as means ± SEM. ***, p<0.001. n = 11 animals/group

Fig. 4. Bar diagram representing the number of mucosal mast cells per villus-crypt unit (VCU) in ileum of each group. Three to five sections were counted per rat, 7-10 units well-oriented VCU were examined per section. Values are means ± SEM. * p<0.05. n = 10-11 animals/group.

Fig. 5. (A) Representative photographs of agarose gels showing RT-PCR products for inducible isoforms of nitric oxide synthase (iNOS) mRNA in ileum. C-: negative PCR control; C+: positive control (LPS group); CTRL: control animal and STRESS: stress animal. **(B)** Bar diagram showing semiquantitative analysis by RT-PCR of iNOS mRNA expression. Values are means of n=11 rats in each group ± SEM. * p<0.05

Fig. 6. Bar diagram showing average MPO concentration in each group. Bars represent the mean ± SEM; n=6 rats/group.

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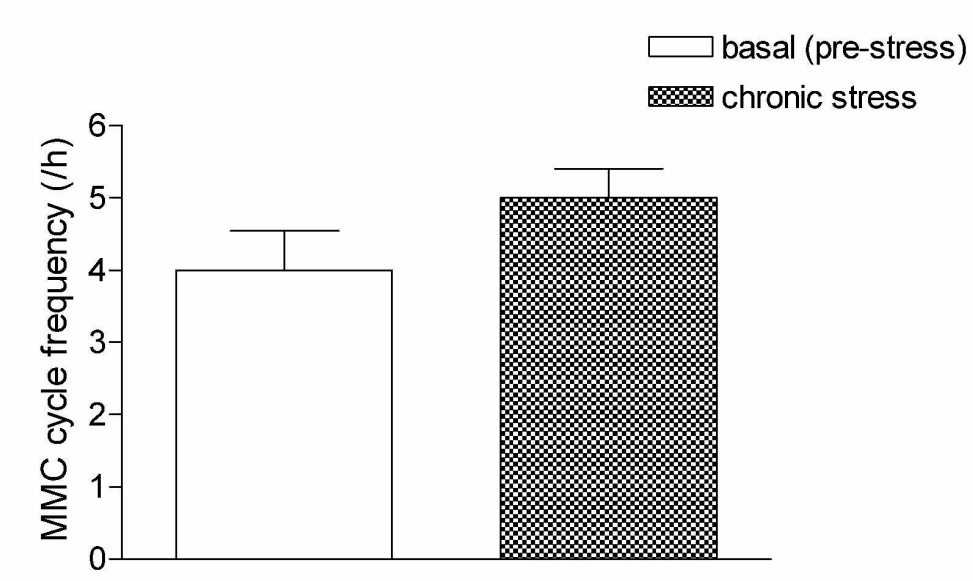

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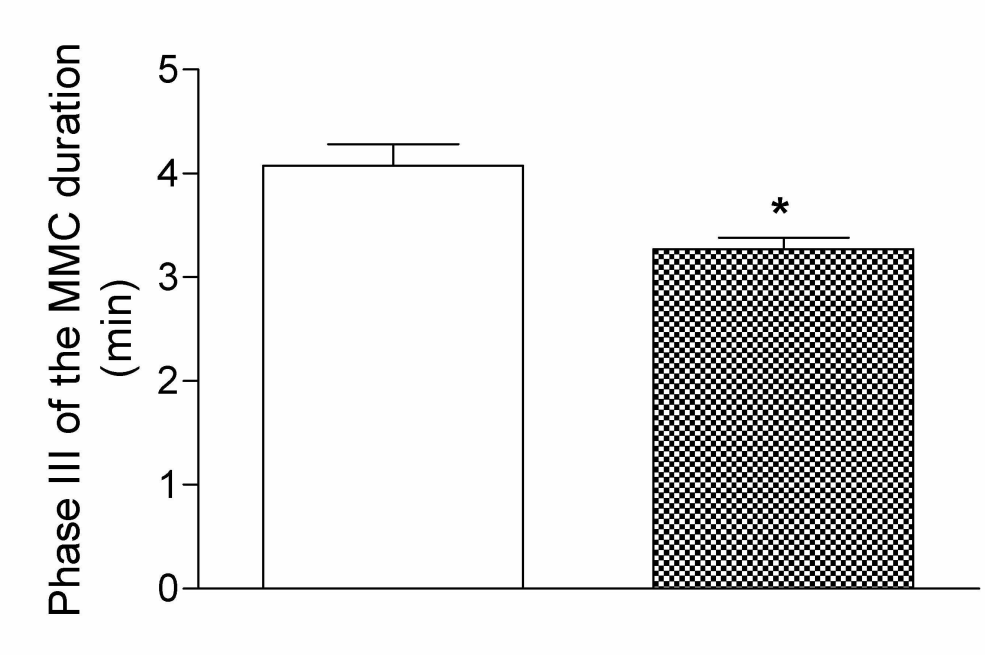

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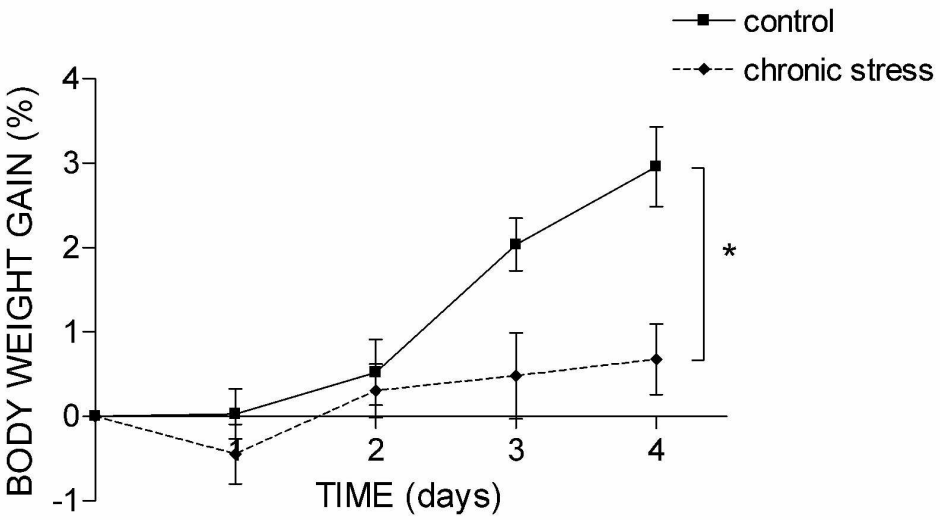

* p<0.05 stress 2-way ANOVA

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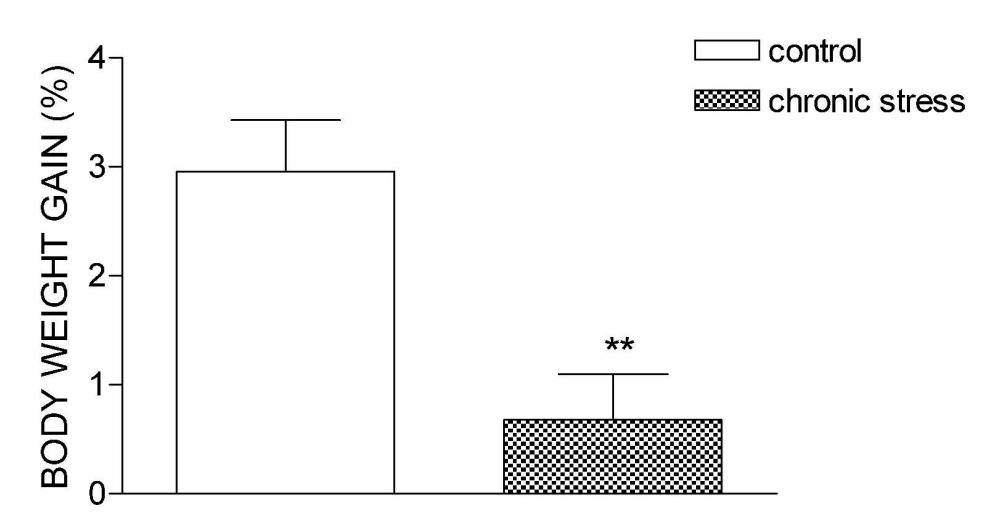

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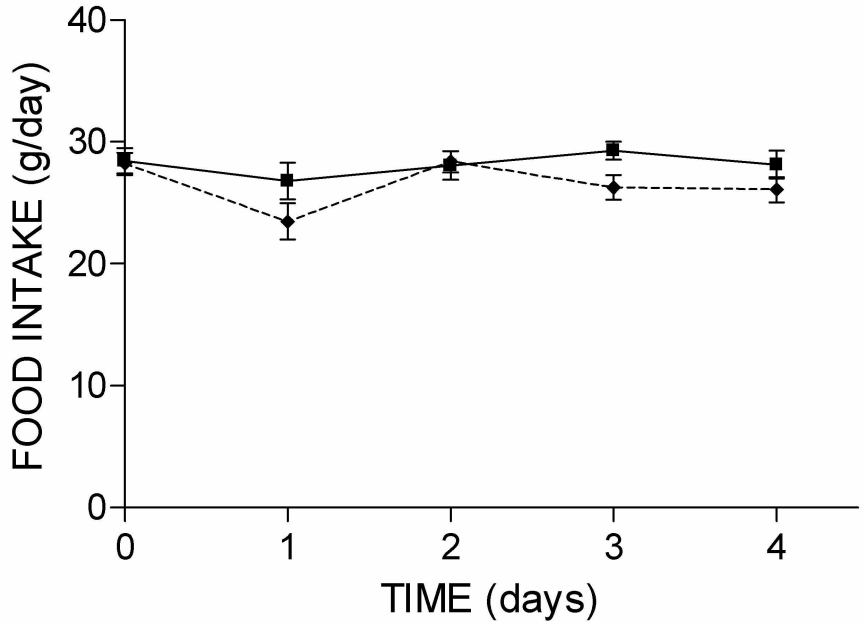

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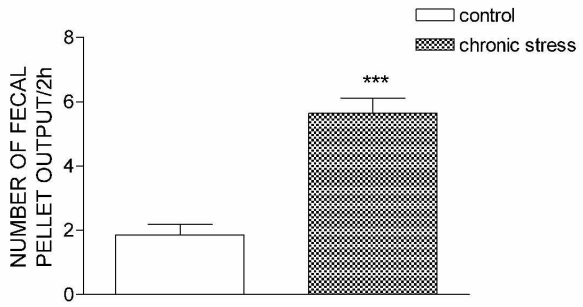

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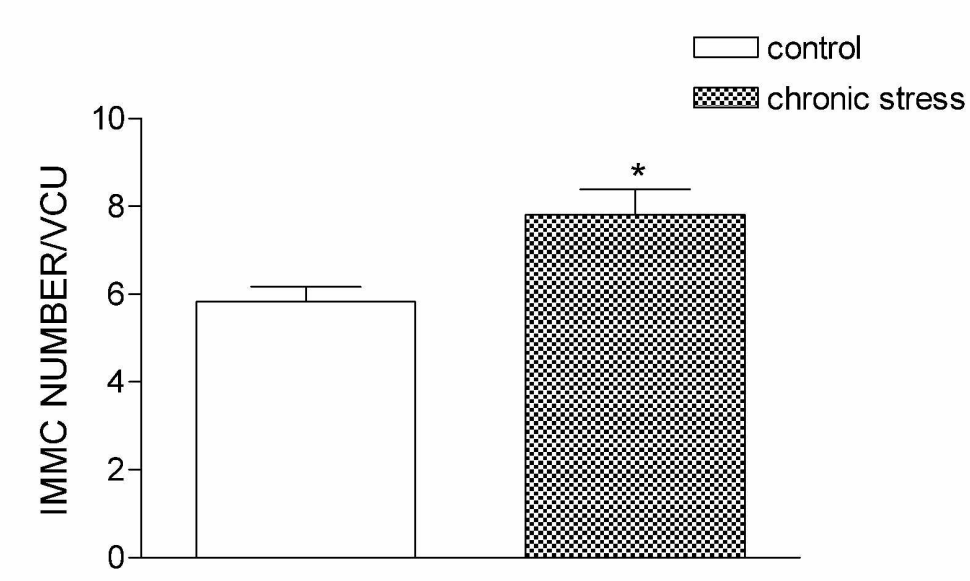

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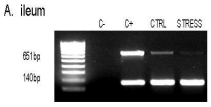
FIGURE 5:



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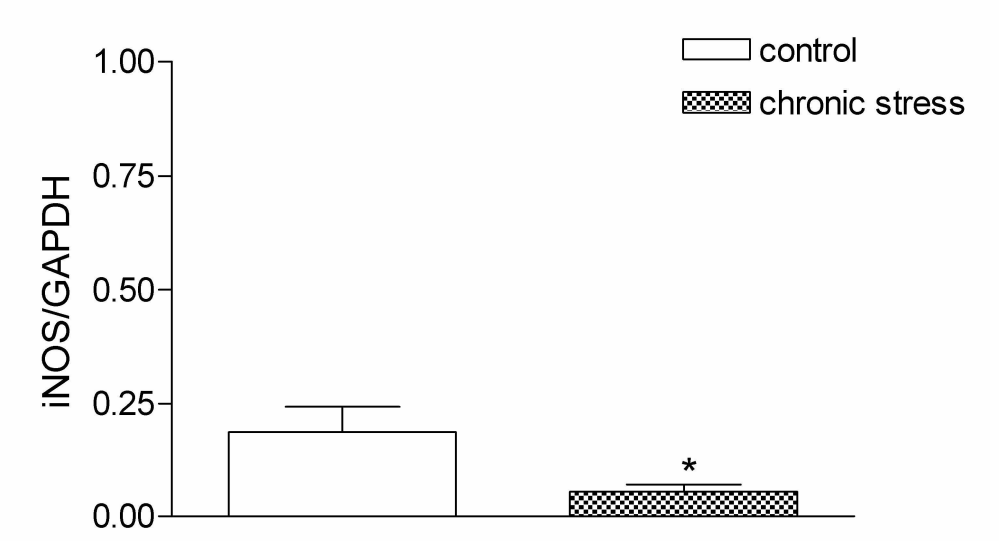

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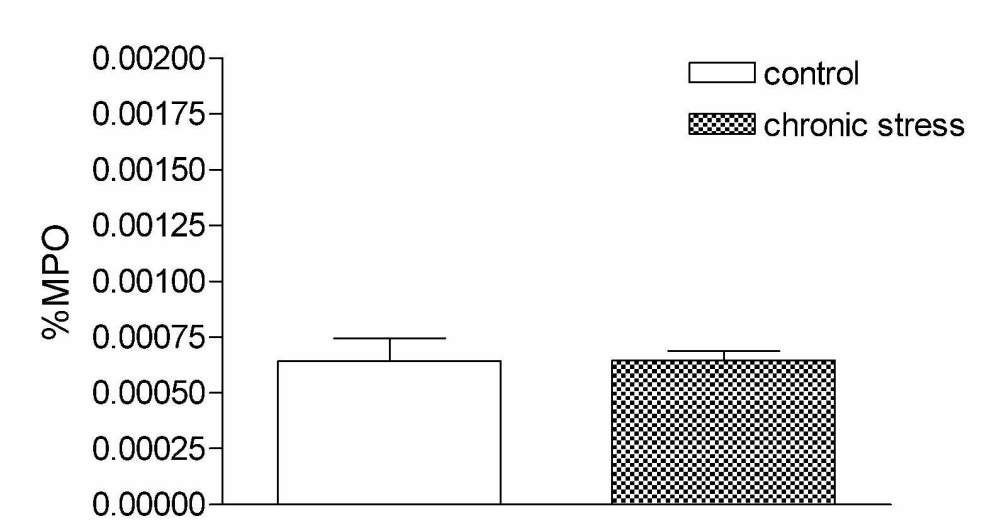

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