

Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat

J Santos, P-C Yang, J D Söderholm, M Benjamin, M H Perdue

Abstract

Background and aims—Stress may be an important factor in exacerbating inflammatory bowel disease but the underlying mechanism is unclear. Defective epithelial barrier function may allow uptake of luminal antigens that stimulate an immune/inflammatory response. Here, we examined the effect of chronic stress on colonic permeability and the participation of mast cells in this response.

Methods—Mast cell deficient Ws/Ws rats and +/+ littermate controls were submitted to water avoidance stress or sham stress (one hour/day) for five days. Colonic epithelial permeability to a model macromolecular antigen, horseradish peroxidase, was measured in Ussing chambers. Epithelial and mast cell morphology was studied by light and electron microscopy.

Results—Chronic stress significantly increased macromolecular flux and caused epithelial mitochondrial swelling in +/+ rats, but not in Ws/Ws rats, compared with non-stressed controls. Stress increased the number of mucosal mast cells and the proportion of cells showing signs of activation in +/+ rats. No mast cells or ultrastructural abnormalities of the epithelium were present in Ws/Ws rats. Increased permeability in +/+ rats persisted for 72 hours after stress cessation.

Conclusions—Chronic stress causes an epithelial barrier defect and epithelial mitochondrial damage, in parallel with mucosal mast cell hyperplasia and activation. The study provides further support for an important role for mast cells in stress induced colonic mucosal pathophysiology.

(Gut 2001;48:630–636)

Keywords: epithelial transport; mast cells; mitochondria; psychological stress; ultrastructure

Although a controversial issue, clinical observations indicate that stress may be an important factor in exacerbating inflammatory bowel disease (IBD).¹ One mechanism by which stress may influence intestinal disorders is by altering epithelial and immune responses in the gut.² Indeed, experimental evidence has demonstrated reactivation or worsening of colonic inflammation after repeated exposure to stress.^{3–5} Epithelial cells participate in the regulation of mucosal inflammation by acting as a physical and functional barrier that limits the uptake of luminal antigens and pathogens. Among immune cells, mast cells play an

important role in the regulation of epithelial transport in both human^{6,7} and rodent intestine,^{8,9} and it is widely accepted that nerve-mast cell interactions are involved in intestinal epithelial dysfunction.^{10,11} We and others have shown that nerves and mast cells participate in the development of colonic epithelial abnormalities induced by acute stress.^{12,13} The mast cell stabiliser doxantrazole abolished acute stress induced ion secretion and permeability enhancement in rat colon,¹² and in a study using mast cell deficient mice, colonic mucin release in response to restraint stress was found to be mast cell dependent.¹³

Experiments involving repeated exposure of rodents to mild stress are commonly used as models of depression (reviewed by Willner¹⁴). These more chronic models better reflect the pattern of daily stress experienced by humans. In a recent study,¹⁵ we developed a rat model of ongoing psychological stress involving repetitive application of a mild stressor, water avoidance stress, for five consecutive days. The stressed rats reduced their food intake and lost weight during the five day period. In addition, jejunal conductance and macromolecular permeability were increased in stressed wild-type rats but not in mast cell deficient rats, suggesting an important role for mast cells in stress induced small bowel mucosal pathophysiology.

A recent clinical study showed that long term stress is more important than acute stressful events for the risk of exacerbation of ulcerative colitis.¹⁶ A possible mechanism could be stress induced deterioration in colonic barrier function. However, the effect of chronic stress on colonic epithelial physiology has not been reported. The specific aims of the present study were: (1) to investigate the effects of chronic stress on epithelial ion secretion and barrier function in rat colon; (2) to determine if mast cell activation plays a role in stress induced colonic mucosal responses; and (3) to investigate if colonic physiological abnormalities were accompanied by ultrastructural changes in colonocytes.

Methods

ANIMALS

Ws/Ws rats and their +/+ littermate controls were obtained from our breeding colony at McMaster University (original breeders obtained from Dr Y Kitamura, Osaka, Japan).

Abbreviations used in this paper: G, conductance; HRP, horseradish peroxidase; IBD, inflammatory bowel disease; Isc, short circuit current; Ws/Ws, mast cell deficient rats with gene deletion at the “white spotting” (Ws) locus; +/+, normal littermates of Ws/Ws rats.

Intestinal Disease Research Program, Department of Pathology and Molecular Medicine, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada

J Santos
P-C Yang
J D Söderholm
M Benjamin
M H Perdue

Correspondence to: M H Perdue, Intestinal Disease Research Program, HSC-3N5C, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada, L8N3Z5. perdue@fhs.mcmaster.ca

Accepted for publication 21 November 2000

Ws/Ws rats have a 12 base deletion in the tyrosine kinase domain of the *c-kit* gene and by 10 weeks of age no mast cells can be detected in their intestine, whereas +/+ rats have normal numbers of mast cells.¹⁷ Early in life, Ws/Ws rats also have anaemia, but this is largely corrected in rats older than 10 weeks of age.¹⁷ Weight (250–300 g) and age (14–20 weeks) matched rats, individually housed, were maintained on a 12:12 hour (8 am/8 pm) dark/light cycle and provided with food and water ad libitum. The Animal Care Committee at McMaster University approved all procedures.

STRESS PROTOCOL

Four groups of rats were studied: Ws/Ws rats, either stressed or sham stressed, and +/+ rats, either stressed or sham stressed. The rats were handled daily for two weeks by the same examiner and then submitted to water avoidance stress or sham stress daily for one hour over five days. The procedure involved placing the animals on a platform (8×6 cm) in a plastic container (56×50 cm) filled with warm water (25°C) to 1 cm below the platform. Sham stressed rats were placed on the same platform above a waterless container. The procedures were performed between 8:00 am and 10:00 am to minimise the effect of circadian rhythm. In previous experiments, we determined that the effects of acute stress on epithelial function were maximal at 5–7 hours after stress and returned to normal at 24 hours (Saunders and Perdue, unpublished observations). Based on this finding, six hours after the last stress or sham session, rats were killed by decapitation and tissues were obtained for functional and morphological studies.

Additional experiments were performed to assess the duration of stress effects on epithelial physiology. Since only +/+ rats showed stress induced epithelial abnormalities, an additional group of +/+ rats was submitted to the sham or stress protocols (five days) and then returned to their cages where they remained undisturbed. Rats were killed on days 1, 3, and 7 (n=8 for each time point) after completion of sham/stress protocols, and colonic tissue obtained for studies.

USSING CHAMBER STUDIES

Distal colonic segments were immediately immersed in oxygenated Krebs solution, stripped of longitudinal muscle and myenteric plexus, and 2–4 adjacent pieces from each segment were mounted in Ussing chambers (World Precision Instruments, Narco Scientific, Mississauga, Ontario, Canada). The chamber exposed 0.6 cm² of tissue surface area to 8 ml of circulating oxygenated Krebs buffer at 37°C. The buffer contained (in mM) 115 NaCl, 1.25 CaCl₂, 1.2 MgCl₂, 2.0 KH₂PO₄, and 25 NaHCO₃ (pH 7.35). The serosal buffer also contained 10 mM glucose as an energy source, osmotically balanced by 10 mM mannitol in the mucosal buffer. The chambers contained agar-salt bridges to monitor the potential difference across the tissue and to inject the required short circuit current (Isc) to

maintain a zero potential difference as registered via an automated voltage clamp (Narco Scientific). Every five minutes, a pulse of 1 mV was passed through the tissue, and by measuring the change in Isc, the conductance was calculated by Ohm's law. Baseline values for Isc and conductance were recorded at equilibrium, 20 minutes after mounting of the tissues. Segments with signs of poor viability—that is, unstable Isc and/or conductance—were excluded from the study.

Mucosal to serosal transport of macromolecules was assessed by measuring transepithelial flux of horseradish peroxidase (HRP), a model protein antigen.¹⁸ Fifteen minutes after mounting the tissues, HRP (type VI, Sigma Chemical Co., St Louis, Missouri, USA) was added to the luminal buffer at a final concentration of 10⁻⁵ M and allowed to equilibrate for 30 minutes. Serosal samples (0.5 ml) were obtained at 30 minute intervals for two hours and replaced by 0.5 ml of appropriate buffer solution. HRP activity was determined by a modified Worthington method, as previously described.¹⁸ The mucosal to serosal flux of HRP was reported as the average value of two consecutive stable flux periods (between 30 and 90 minutes), and expressed as pmol/h/cm².

HISTOLOGY

Full thickness segments of colon were fixed in Carnoy's fixative and stained with toluidine blue (Sigma). Overall morphology was assessed. Mast cells were counted in coded sections at 400× magnification using a micrometer grid (0.032 mm² area). For each rat, 12 contiguous non-overlapping mucosal areas above the *muscularis mucosae* were evaluated.

ELECTRON MICROSCOPY

Colonic tissues were obtained from rats immediately after being killed by decapitation and also from chambers two hours after addition of HRP into the luminal compartment. Tissues were immediately fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for two hours at 22°C, rinsed for 18 hours (4°C) with 0.05 Tris buffer (pH 7.6), and washed three times, five minutes each time. Methods for HRP product identification have been described previously.¹⁸ Quantitative analysis of HRP uptake in intracellular endosomes and paracellular HRP transport were performed on coded high magnification photomicrographs, 15 per rat (four rats/group). The total area of HRP containing endosomes within colonocytes was determined in an area of 300 μm² in the apical region of the cells, using a computerised image analysis system (Kontron Mop Videoplan; Kontron, Echting, Germany). Tissues were also examined for evidence of paracellular HRP transport in coded photomicrographs that were randomly selected. In each group, 1000 paracellular regions from four different rats were examined. HRP was identified as electron dense material within the paracellular regions (between lateral cell membranes of two adjacent cells).

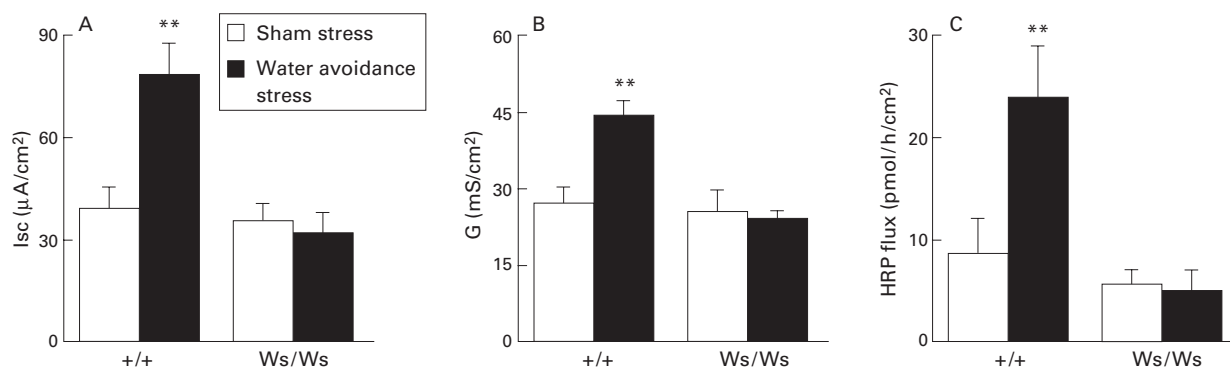


Figure 1 Effect of chronic stress on colonic physiology and macromolecule permeability. Mast cell deficient rats (*Ws/Ws*) and their normal mast cell containing littermates (*+/+*) were submitted to sham stress or water avoidance stress for five consecutive days. (A) Baseline short circuit current (*I*_{sc}), (B) conductance (*G*), and (C) horseradish peroxidase (HRP) flux studied in Ussing chambers six hours after the final sham/stress session. Bars represent mean (SEM); *n*=6 rats/group, with 2–4 tissues averaged per rat. ***p*<0.01 *v* all other groups.

Ultrastructural epithelial damage was evaluated in colonocytes by the presence of mitochondrial changes such as enlargement, swelling, disorientation, and disappearance of cristae. The mean area of individual mitochondria within the apical region of the colonocytes was measured in four rats per group, 15 sections per rat, using a computerised image analysis system as described above.

Mast cells were evaluated for signs of activation by electron microscopy and image analysis. Patterns of mast cell activation/degranulation were assessed as: (a) piecemeal degranulation, defined as loss of granule density without granule-cell membrane fusion,¹⁹ and (b) anaphylactic degranulation, defined as solubilisation of granular contents with fusion of intergranular and granule-cell membranes.²⁰ Fifteen sections containing mast cells from each tissue (four rats per group) were randomly selected, the number of intracytoplasmic granules counted, and each granule analysed for loss of density and perigranular vacuolisation.

FAECAL OUTPUT

At the end of each experimental session (stress or sham stress), the number of faecal pellets expelled was counted and recorded. Changes in faecal pellet output has previously been shown to be an index of changes in colonic transit time during stress in rats.²¹

STATISTICS

Results are expressed as mean (SEM). Results were analysed by two way ANOVA with contrasts to show the simple effects of the following main factors: treatment group (stress *v* sham) and strain (*Ws/Ws v +/+*). A between-within multivariate ANOVA, where the third

factor was a repeated measures factor (time), was used to compare means at different days in strain and treatment groups. The unpaired Student's *t* test was used to compare post-treatment differences (at each time) on epithelial parameters in sham and stress groups, with Bonferroni correction for multiple testing. The Mann-Whitney U test and Fisher's exact test were also used when appropriate. *p*<0.05 was considered significant.

Results

EFFECTS OF STRESS ON ELECTROPHYSIOLOGY

After sham stress, baseline *I*_{sc} was similar in colonic tissues from *+/+* and *Ws/Ws* rats. Stress doubled the *I*_{sc} in *+/+* rats but had no effect in *Ws/Ws* rats compared with the respective non-stressed controls (fig 1A). The increased *I*_{sc} in *+/+* rats was significant, as shown by the treatment by strain interaction ($F_{1,20}=8.7$; *p*<0.01).

Similarly, conductance was not different in sham stressed *+/+* and *Ws/Ws* rats whereas stress markedly increased colonic conductance in *+/+* rats ($F_{1,20}=19.3$; *p*<0.001) but not in *Ws/Ws* rats (fig 1B)

EFFECTS OF STRESS ON MACROMOLECULAR TRANSPORT

HRP flux across colonic tissues was similar after sham stress in *+/+* (8.9 (3.3) pmol/h/cm²) and *Ws/Ws* (5.9 (1.4) pmol/h/cm²) rats. Stress enhanced HRP flux across the colonic epithelium in *+/+* rats threefold to 24.3 (4.9) pmol/h/cm² ($F_{1,20}=8.1$; *p*<0.01) (fig 1C) whereas no increase was found in *Ws/Ws* rats.

On electron microscopy, HRP was evident within endosomes in colonocytes of both stressed and control rats. However, the number and size of HRP containing endosomes were significantly higher in stressed *+/+* rats compared with the three other groups, as indicated by the HRP-endosome area (table 1, fig 2A, B). In addition, HRP was observed within colonic tight junctions and paracellular regions only in stressed *+/+* rats, where 30% of paracellular spaces contained HRP (fig 2B).

EFFECTS OF STRESS ON EPITHELIAL MORPHOLOGY

On light microscopy, no colonic mucosal abnormalities were observed in either rat group after exposure to stress. However, using

Table 1 Electron microscopy findings in the colonocytes of mast cell deficient rats (*Ws/Ws*) and normal littermates (*+/+*) after chronic stress or sham stress.

	<i>+/+</i> sham	<i>+/+</i> stress	<i>Ws/Ws</i> sham	<i>Ws/Ws</i> stress
HRP-endosome area (μm ² /300 μm ² cell area)	1.7 (0.2)	6.1 (0.8)*	1.6 (0.1)	1.8 (0.2)
Mitochondrial area (μm ²)	0.21 (0.04)	0.45 (0.06)*	0.20 (0.04)	0.18 (0.03)

Data are shown as mean (SEM); *n*=4 rats/group, 15 sections/rat.

Endosomal area is expressed as total area of horseradish peroxidase (HRP) containing endosomes in an area of 300 μm² in the apical region of the colonocytes.

Mitochondrial area is expressed as mean area of individual mitochondria within colonocytes.

**p*<0.05 compared with sham stressed and mast cell deficient rats.

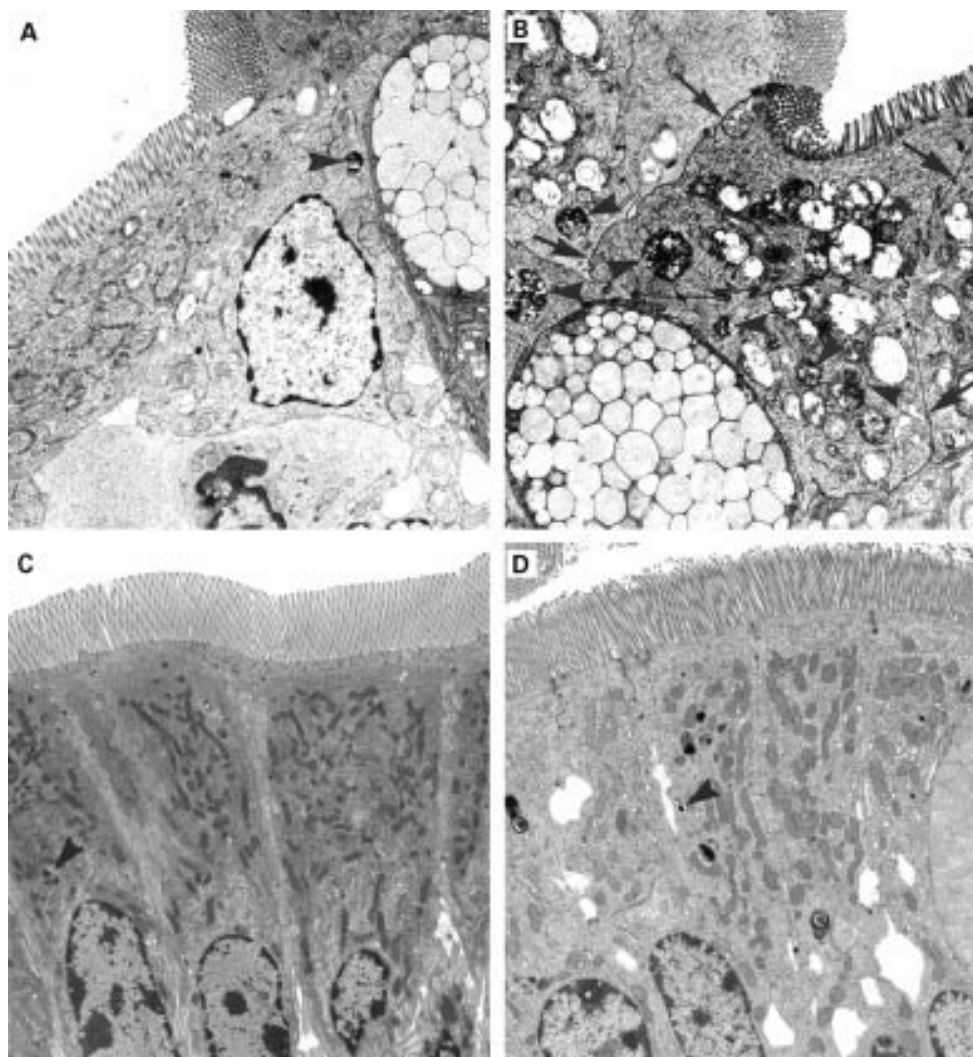


Figure 2 Effect of chronic stress on horseradish peroxidase (HRP) transport and epithelial mitochondria. Mast cell deficient rats (Ws/Ws; left) and their normal mast cell containing littermates (+/+; right) were submitted to water avoidance stress (top photomicrographs) or to sham stress (bottom photomicrographs) for five consecutive days. (A) Photomicrograph showing an HRP filled endosome (large arrowhead) in a colonocyte of a stressed Ws/Ws rat. Paracellular passage of HRP and mitochondrial abnormalities were not observed in Ws/Ws rats. (B) Larger and more numerous HRP filled endosomes (arrowheads) as well as HRP within tight junctions and paracellular spaces between colonocytes (arrows) were observed in the colon of stressed +/+ rats, as shown in this photomicrograph. Epithelial damage is demonstrated by swollen mitochondria with loss of cristae. (C, D) Colonocytes with small endosomal uptake of HRP (arrowheads) and normal mitochondria from sham stressed Ws/Ws (C) and +/+ rats (D), respectively. Mitochondrial surface area was significantly increased in stressed +/+ rats compared with the three other groups (table 1). Magnification: $\times 3000$. Each photomicrograph is representative of four rats/group and 15 sections/colonic tissue.

electron microscopy, ultrastructural signs of epithelial damage were observed in colonic tissues from all stressed +/+ rats. Colonocytes in these rats showed enlarged mitochondria with loss of cristae (fig 2C, D) whereas this abnormality was not found in cells of sham

stressed +/+ or Ws/Ws rats or in cells of stressed Ws/Ws rats (table 1).

EFFECTS OF STRESS ON MUCOSAL MAST CELLS

Mast cells were completely absent in the colon of Ws/Ws rats. In +/+ rats, stress exposure for five days was associated with the appearance of a significantly higher number of mast cells in the mucosa of the colon compared with the sham stressed group (table 2). In addition, as assessed by electron microscopy, a higher proportion of mucosal mast cells (~30%) in tissues from stressed +/+ rats had granules with signs of activation (table 2) whereas changes in granule morphology were observed only in a minority of mast cells (6%) in the mucosa of sham stressed rats. Piecemeal-type degranulation, indicated by loss of granular density, was the predominant pattern observed in activated mast cells, while ultrastructural evidence of

Table 2 Effects of stress on mast cell numbers and activation

	+/+ sham	+/+ stress	Ws/Ws sham	Ws/Ws stress
Mast cell numbers (cells/mm ²)	81.2 (18.5)	445 (39)*	0 (0)	0 (0)
Mast cell activation (% granules with signs of activation)	6.0 (2.1)	31.8 (5.5)*	NA	NA

Mast cells were evaluated for signs of activation by electron microscopy and image analysis. Patterns of mast cell activation/degranulation were assessed as: (a) piecemeal degranulation, defined as loss of granule density without granule-cell membrane fusion and (b) anaphylactic degranulation, defined as solubilisation of granular contents with fusion of intergranular and granule-cell membranes. Fifteen sections containing mast cells from each tissue (four rats per group) were randomly selected, the number of intracytoplasmic granules counted and each granule analysed for loss of density and perigranular vacuolisation.

Data are shown as mean (SEM).

* $p < 0.05$ compared with sham stressed rats.

NA, not available.

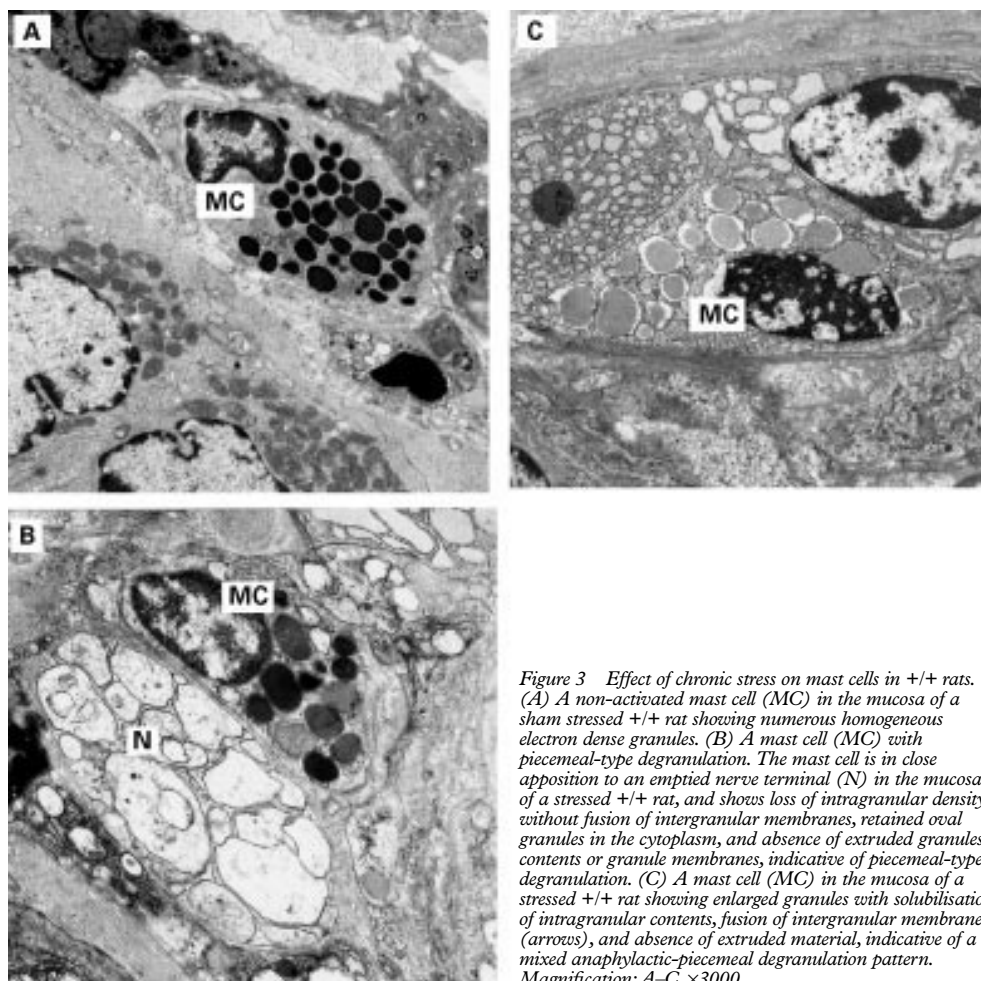


Figure 3 Effect of chronic stress on mast cells in +/+ rats. (A) A non-activated mast cell (MC) in the mucosa of a sham stressed +/+ rat showing numerous homogeneous electron dense granules. (B) A mast cell (MC) with piecemeal-type degranulation. The mast cell is in close apposition to an emptied nerve terminal (N) in the mucosa of a stressed +/+ rat, and shows loss of intragranular density without fusion of intergranular membranes, retained oval granules in the cytoplasm, and absence of extruded granule contents or granule membranes, indicative of piecemeal-type degranulation. (C) A mast cell (MC) in the mucosa of a stressed +/+ rat showing enlarged granules with solubilisation of intragranular contents, fusion of intergranular membranes (arrows), and absence of extruded material, indicative of a mixed anaphylactic-piecemeal degranulation pattern. Magnification: A–C, $\times 3000$.

anaphylactic-type degranulation or mixed patterns were occasionally observed (fig 3A–C). Frequently, mast cells showing piecemeal-type degranulation were in close proximity to nerves (fig 3B).

EFFECTS OF STRESS ON FAECAL OUTPUT

Defecation was increased during exposure to stress, as shown by the higher number of faecal pellets expelled by stressed versus sham stressed rats. This effect was significant for both +/+ (10.6 (0.9) pellets/h) and Ws/Ws (12.4 (0.9)) stressed rats compared with their controls (+/+ 1.0 (0.1) pellets/h, $F_{1,36}=95$; Ws/Ws: 1.0 (0.2), $F_{1,36}=146$; $p<0.001$ for both).

DURATION OF STRESS EFFECTS ON EPITHELIAL FUNCTION

Stress induced enhancement of HRP flux in +/+ rats continued to be significantly ($p<0.05$) elevated on days 1 (16.2 (1.1) pmol/h/cm², $n=4$) and 3 (10.6 (1.2) pmol/h/cm², $n=4$) after cessation of stress compared with tissues from sham treated rats on days 1 (6.4 (1.1) pmol/h/cm², $n=4$) and 3 (6.6 (0.5) pmol/h/cm², $n=4$), respectively. However, by day 7 after stress, HRP fluxes in the colon were similar in both groups (7.1 (0.5) and 6.4 (0.8) pmol/h/cm² in stressed and sham rats, respectively, $n=4$ in each group).

Discussion

We have shown that repetitive exposure to water avoidance stress, a psychological stressor, induced sustained abnormalities in colonic epithelial barrier function, and that mast cells played an important role in this response. Participation of mast cells in intestinal epithelial dysfunction has been documented in several rodent models, such as immediate hypersensitivity,^{8,9} acute stress,¹² *Clostridium difficile* infection,²² as well as in inflamed human colon.⁶ We have previously shown that in the rat colon, a single exposure to acute stress stimulates epithelial ion secretion and increases transepithelial transport of macromolecules.¹² However, a model of more chronic stress, rather than acute stress, may better represent ongoing exposure to stressful life events in humans. Information regarding the effects of chronic stress on gut epithelial function and the role of mast cells is scarce, particularly in the colon. A recent study documented that the epithelial secretory response to 5-hydroxytryptamine in the distal colon was altered after three days of immobilisation for two hours/day,²³ and chronic stress was also shown to stimulate colonic epithelial cell proliferation.²⁴ In the present study, we report for the first time that chronic stress markedly increased HRP flux indicating enhanced epithelial permeability to

macromolecules in the rat distal colon. Moreover, enhanced HRP flux involved both transcellular and paracellular routes across the epithelium. Baseline *I_{sc}*, indicating net ion secretion, was also increased by chronic stress. A key finding was that these epithelial abnormalities were manifested only in *+/+* rats with normal mucosal mast cell numbers, suggesting that mast cells are required for expression of stress induced epithelial transport abnormalities in the rat colon.

In parallel with abnormalities of epithelial function, we found ultrastructural evidence of mitochondrial changes in colonocytes of stressed *+/+* rats whereas *Ws/Ws* mast cell deficient rats did not develop any changes in epithelial morphology. In the gastrointestinal tract, to our knowledge, mitochondrial abnormalities have only been reported in enterocytes of rats after 24 hours of restraint stress.²⁵ It is known that certain severe stressors such as activity stress can induce overt damage involving bleeding and thinning of the colonic mucosa.²⁶ However, our finding that a mild chronic stressor provoked ultrastructural changes of the epithelium, but only in tissues with mast cells, is a novel observation. Similar mitochondrial changes in intestinal epithelia have been induced by uncoupling of oxidative phosphorylation and associated with an increased epithelial permeability,^{27, 28} involving rearrangement of F-actin and tight junction proteins.²⁹ Swelling of mitochondria may lead to rupture of the outer mitochondrial membrane and release of cytochrome *c*, a strong activator of the caspases which trigger the cell to undergo apoptosis.³⁰ However, depending on the cellular concentration of ATP and oxygen radicals, cell necrosis could also follow. Epithelial events following mitochondrial damage found in our model of chronic stress need further investigation, and such studies are underway in our laboratory.

Ws/Ws rats have a 12 base deletion in the tyrosine kinase domain of the *c-kit* gene that results in lack or deficiency of melanocytes, erythrocytes, interstitial cells of Cajal in the myenteric plexus, and mast cells.^{17, 31} Mast cells have been reconstituted in mast cell deficient mice but due to the heterogeneous genetic background of the *Ws/Ws* and *+/+* rats (*F₂* generation of two inbred rat strains), reconstitution is not feasible in *Ws* rats because of cell rejection. Therefore, we cannot rule out other phenotypic abnormalities as contributors to the observed responses in *Ws/Ws* rats. However, the fact that *Ws/Ws* rats show normal intestinal epithelial responses to carbachol and forskolin in the presence of neural blockade (Berin and Perdue, unpublished results), together with pharmacological evidence of mast cell involvement in stress responses in the colon,^{12, 13} highlight the role of mast cells in the colonic response to stress and make unlikely a large contribution of other phenotypic abnormalities.

Our study showed that stress induced mast cell hyperplasia in the colonic mucosa of *+/+* rats. An increase in mast cell numbers has previously been shown in the rodent brain after

chronic psychological stress.³² The presence of increased numbers of mast cells may indicate either local hyperplasia of resident mast cells or influx of newly immigrated mast cells from bone marrow or the submucosa or, most probably, a combination of both processes. Although our study did not explore the mechanism accounting for mast cell hyperplasia, several molecules such as interleukin 3, interleukin 4, nerve growth factor, or stem cell factor promote mast cell proliferation. Of these, nerve growth factor has been shown to be released during stress,³³ making it a possible candidate for mast cell hyperplasia in our system. In addition, activated mast cells are known to release a host of cytokines/chemokines and in fact to stimulate recruitment of additional mast cells into the tissue.³⁴ Whether mast cell hyperplasia represents a direct response to stress mediators or a secondary phenomenon to stress induced epithelial damage remains to be elucidated.

We and others have shown that acute stress results in mast cell activation, assessed by either release of specific mediators or by histological criteria in the human jejunum⁷ and rat brain.³⁵ Here, we demonstrated that chronic stress increased the number of mucosal mast cells fivefold, as well as the degree of activation, as indicated by ultrastructural changes. In some mast cells we observed a mixed pattern of piecemeal-type and anaphylactic-type degranulation. However, the majority of activated mast cells showed the pattern of piecemeal-type degranulation, as indicated by loss of granular density without fusion of intergranular membranes and no signs of compound exocytosis.¹⁹ This latter pattern has been reported in the brain of rats submitted to stress³⁵ as well as in the gastrointestinal tract of IBD patients.³⁶ Although the significance of these morphological changes is unclear, they have been noted primarily, as in our study, in mast cells which are in close proximity to nerve fibres,^{20, 35} and may represent either an event preceding exocytosis or nerve mediated activation.³⁷ Release of histamine without apparent signs of exocytosis in rat intestinal mast cells has previously been described after electrical stimulation of the vagus nerve.³⁸

Increased colonic motor activity is a well known effect of different types of acute stress.³⁹ In acute restraint stress, changes in colonic smooth muscle motility to facilitate colonic transit and evacuation have been found to be correlated with faecal pellet output.²¹ In contrast with changes in epithelial function, we found no difference between *+/+* and *Ws/Ws* rats in the stress induced increase in faecal pellet expulsion. This is in agreement with a previous study of immobilisation stress in mast cell deficient mice,¹³ and suggests that motor disturbances induced by stress involve pathways less dependent on mast cells than those inducing epithelial dysfunction.

Finally, as in the jejunum,¹⁵ enhanced colonic permeability to macromolecules lasted for three days after stress exposure. Persistent abnormalities in barrier function could have implications for the development of intestinal

inflammation. Patients with Crohn's disease have increased intestinal permeability.⁴⁰ This barrier dysfunction appears to precede mucosal inflammation,⁴¹ and recurrence of inflammation can be avoided by diversion of the faecal stream.⁴² In gene engineered models of IBD, germ free rodents do not develop intestinal inflammation.⁴³ Increased uptake of luminal microbial antigens may be required to activate mucosal immune cells leading to an inflammatory response involved in the pathogenesis and/or reactivation of IBD. Whether increased colonic permeability and epithelial damage induced by stress, as in this study, eventually leads to intestinal inflammation remains to be elucidated.

In summary, in the present study we have shown that chronic stress caused prominent and lasting alterations in epithelial function in the rat colon and that the stress induced enhanced epithelial antigen uptake was mast cell mediated. The study provides further support for the role of mast cells, not only as key cells in the regulation of gut epithelial physiology, but also as effectors in stress induced colonic permeability. Stress has been linked to the course of colonic inflammation,¹⁻⁵ and mast cells, which play a key role in colonic epithelial responses to stress, are also implicated in the pathogenesis of intestinal inflammation.²² Therefore, mast cells may represent a therapeutic target in the management of stress related mucosal pathophysiology in the gastrointestinal tract.

Supported by grants from the Medical Research Council of Canada, the Acadèmia de Ciències Mèdiques de Catalunya i Balears, and the Societat Catalana de Digestologia (1997/99). J Santos was a recipient of a Medical Research Council/Pharmaceutical Manufacturers' Association of Canada (Astra Research Initiative Fellowship). JD Söderholm was supported by the Swedish Institute and the Swedish Society of Medicine. We acknowledge the expert statistical assistance of Mr Todd Prior.

- 1 Drossman D. Presidential address: Gastrointestinal illness and the biopsychosocial model. *Psychosom Med* 1998;**60**: 258-67.
- 2 Söderholm JD, Perdue MH. Stress and intestinal barrier function. *Am J Physiol* 2001;**280**:G7-13.
- 3 Collins SM, McHugh K, Jacobson K, et al. Previous inflammation alters the response of the rat colon to stress. *Gastroenterology* 1996;**111**:1509-15.
- 4 Qiu BS, Vallance BA, Blennerhasset PA, et al. The role of CD4+ lymphocytes in the susceptibility of mice to stress-induced reactivation of experimental colitis. *Nat Med* 1999;**5**:1178-82.
- 5 Million M, Taché Y, Anton P. Susceptibility of Lewis and Fischer rats to stress-induced worsening of TNB-colitis: protective role of brain CRF. *Am J Physiol* 1999;**276**: G1027-36.
- 6 Crowe SE, Luthra GK, Perdue MH. Mast cell mediated ion transport in intestine from patients with and without inflammatory bowel disease. *Gut* 1997;**41**:785-92.
- 7 Santos J, Saperas E, Nogueiras C, et al. Release of mast cell mediators into the jejunum by cold pain stress in humans. *Gastroenterology* 1998;**114**:640-8.
- 8 Berin MC, Kiliaan AJ, Yang PC, et al. The influence of mast cells on pathways of transepithelial antigen transport in the rat intestine. *J Immunol* 1998; **161**:2561-6.
- 9 Perdue MH, Masson S, Wershil BK, et al. Role of mast cells in ion transport abnormalities associated with intestinal anaphylaxis. Correction of the diminished secretory response in genetically mast cell-deficient W/W^v mice by bone marrow transplantation. *J Clin Invest* 1991;**87**:687-93.
- 10 Coelho AM, Fioramonti J, Bueno L. Systemic lipopolysaccharide influences rectal sensitivity in rats: role of mast cells, cytokines, and vagus nerve. *Am J Physiol* 2000;**279**: G781-90.
- 11 Argenzio RA. Neuro-immune pathobiology of infectious enteric disease. *Adv Exp Med Biol* 1997;**412**:21-9.
- 12 Santos J, Saunders PR, Yang P-C, et al. Corticotropin-releasing hormone mimics stress-induced colonic epithelial pathophysiology in the rat. *Am J Physiol* 1999;**277**:G391-9.
- 13 Castagliuolo I, Wershil BK, Karalis K, et al. Colonic mucin release in response to immobilization stress is mast cell dependent. *Am J Physiol* 1998;**274**:G1094-1100.
- 14 Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology* 1997;**134**:319-29.
- 15 Santos J, Benjamin M, Yang P-C, et al. Chronic stress impairs rat growth and jejunal epithelial barrier function: role of mast cells. *Am J Physiol* 2000;**278**:847-54.
- 16 Levenstein S, Prantera C, Varvo V, et al. Stress and exacerbation in ulcerative colitis: a prospective study of patients enrolled in remission. *Am J Gastroenterol* 2000;**95**:1213-20.
- 17 Niwa Y, Kasugai T, Ohno K, et al. Anemia and mast cell depletion in mutant rats that are homozygous at "white spotting (Ws)" locus. *Blood* 1991;**78**:1936-41.
- 18 Kiliaan AJ, Saunders PR, Bijlsma PB, et al. Stress stimulates transepithelial macromolecular uptake in rat jejunum. *Am J Physiol* 1998;**275**:G1037-44.
- 19 Dvorak AM, Tepper RI, Weller PF, et al. Piecemeal degranulation of mast cells in the inflammatory eyelid lesions of IL-4 transgenic mice. Evidence of mast cell histamine release in vivo by diamino oxidase-gold enzyme-affinity ultrastructural cytochemistry. *Blood* 1994;**83**:3600-12.
- 20 Dvorak AM, Mihm MC Jr, Dvorak HF. Degranulation of basophilic leukocytes in allergic contact dermatitis reactions in man. *J Immunol* 1976;**116**:687-95.
- 21 Barone FC, Deegan JF, Proce WJ, et al. Cold-restraint stress increases rat fecal pellet output and colonic transit. *Am J Physiol* 1990;**258**:G329-37.
- 22 Wershil B, Castagliuolo I, Pothoulakis C. Direct evidence of mast cell involvement in Clostridium difficile toxin A-induced enteritis in mice. *Gastroenterology* 1998;**114**: 956-64.
- 23 Goldhill J, Porquet M-F, Angel I. The response of rat colonic mucosa to 5-hydroxytryptamine in health and following restraint stress. *Eur J Pharmacol* 1988;**353**:289-96.
- 24 Hori T, Wanibuchi H, Yano Y, et al. Epithelial cell proliferation in the digestive tract induced by space restriction and water-immersion stress. *Cancer Lett* 1998;**125**:141-8.
- 25 Perov I, Evdokimov VV. Ultrastructure of the duodenal mucosa in rats under stress. *Bull Exp Biol Med (translated)* 1976;**81**:625-8.
- 26 Andrianopoulos G, Bombeck T, Nyhus LM. Influence of maturation on activity-stress related pathology in the rat colon. *Physiol Behav* 1986;**36**:1105-10.
- 27 Madara JL. Intestinal absorptive cell tight junctions are linked to the cytoskeleton. *Am J Physiol* 1987;**253**:C171-5.
- 28 Somasundaram S, Rafi S, Hayllar J, et al. Mitochondrial damage: a possible mechanism of the "topical" phase of NSAID induced injury to the rat intestine. *Gut* 1997;**41**: 344-53.
- 29 Tsukamoto T, Nigam SK. Tight junction proteins form large complexes and associate with the cytoskeleton in an ATP depletion model for reversible junction assembly. *J Biol Chem* 1997;**272**:16133-9.
- 30 Green DR, Reed JC. Mitochondria and apoptosis. *Science* 1998;**281**:1309-12.
- 31 Horiguchi K, Komuro T. Ultrastructural characterization of interstitial cells of Cajal in the rat small intestine using control and Ws/Ws mutant rats. *Cell Tissue Res* 1998;**293**:277-84.
- 32 Cirulli F, Pistillo L, de Acetis L, et al. Increased number of mast cells in the central nervous system of adult mice following chronic subordination stress. *Brain Behav Immun* 1998;**12**:123-33.
- 33 Aloe L, Bracci-Laudiero L, Alleve E, et al. Emotional stress induced by parachute jumping enhances blood nerve growth factor levels and the distribution of nerve growth factor receptors in lymphocytes. *Proc Natl Acad Sci USA* 1994;**91**:10440-4.
- 34 Marshall JS. Repeated antigen challenge in rats induces a mucosal mast cell hyperplasia. *Gastroenterology* 1993;**105**: 391-8.
- 35 Theoharides TC, Spanos C, Pang X, et al. Stress-induced intracranial mast cell degranulation: a corticotropin-releasing hormone-mediated effect. *Endocrinology* 1995; **136**:5745-50.
- 36 Dvorak AM, McLeod RS, Onderdonk A, et al. Ultrastructural evidence for piecemeal and anaphylactic degranulation of human gut mucosal mast cells in vivo. *Int Arch Allergy Immunol* 1992;**99**:74-83.
- 37 Theoharides CT, Bondy PK, Tsakalos ND, et al. Differential release of serotonin and histamine from mast cells. *Nature* 1982;**297**:229-31.
- 38 Williams RH, Berthoud HR, Stead RH. Vagal afferent fibres contact mast cells in rat small intestinal mucosa. *Neuroimmunomodulation* 1997;**4**:266-70.
- 39 Taché Y, Martinez V, Mulugeta M, et al. Corticotropin-releasing factor and the brain-gut motor response to stress. *Can J Gastroenterol* 1999;**13**(suppl A):18-25A.
- 40 Meddings JB. Intestinal permeability in Crohn's disease. *Aliment Pharmacol Ther* 1997;**11**(suppl 3):47-56.
- 41 Söderholm JD, Holmgren-Peterson K, Olaison G, et al. Epithelial permeability to proteins in the non-inflamed ileum of Crohn's disease? *Gastroenterology* 1999;**117**:65-72.
- 42 Rutgeerts P, Geboes K, Peeters M, et al. Effect of the faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. *Lancet* 1991;**338**:771-4.
- 43 Taurog JD, Richardson JA, Croft JT, et al. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J Exp Med* 1994;**180**: 2359-64.