Psychological stress induces eosinophils to produce corticotrophin releasing hormone in the intestine

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► A supplementary file containing methods, a table and four figures is published online only at http://gut.bmj.com/content/vol58/issue11

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ABSTRACT

Background and aims: Psychological stress plays an important role in an array of intestinal disorders. Corticotrophin releasing hormone (CRH) is involved in the pathogenic process induced by psychological stress. The peripheral sources of CRH remain to be further understood. This paper aims to identify the sources of CRH in the intestine.

Methods and results: Mice were treated with chronic restraint stress. A double-labelling approach was taken to localise CRH expression in immune cells (including dendritic cells, mast cells, lymphocytes, enterochromaffin cells and eosinophils) in the intestine by confocal microscopy and flow cytometry. As CRH was identified in eosinophils, a cell line of eosinophil, EoL-1 cells were treated with an array of putative stress mediators. The results showed that substance P (SP) induced the expression/release of CRH in eosinophils via neurokinin receptor 1 and 2. Co-culturing SP-primed eosinophils with the mast cell line, HMC-1 cells, we found that HMC-1 cells were activated by eosinophil-derived CRH that further induced T84 monolayer barrier dysfunction, which was further confirmed by a mouse model study.

Conclusion: Eosinophils express CRH in the jejunum in response to psychological stress. SP and its receptors mediate the effect of stress in the CRH expression in eosinophils. Eosinophil-derived CRH activates mast cells to induce the jejunum epithelial barrier dysfunction.

We and others have revealed that psychological stress has a strong impact on the induction of intestinal epithelial barrier dysfunction in which the corticotrophin releasing hormone (CRH) plays a critical role.[1 2] Using CRH receptor antagonists efficiently abolishes the stress-induced epithelial barrier dysfunction,[2 3] which implicates a therapeutic potential for those stress-related disorders. Although the known source of CRH is mainly localised in the nervous system, CRH can also be detected in serum;[4] the fact implicates that peripheral sources of CRH exist. It is reported that the intestinal nervous system can be a source of CRH.[5 6] However, in a preliminary study, we treated mouse intestinal tissue with nerve blocker, tetrodotoxin, in Ussing chambers, the intestinal tissue still responded to substance P (SP) to release CRH (measured with protein precipitation and western blotting approaches (see supplementary fig S1) as well as increased the ion secretion and permeability to macromolecular protein tracer (supplementary fig S1). The fact indicates that there are other CRH sources in the intestine in addition to the nervous system. Based on that the blockade of CRH or CRH receptors abolished stress-induced intestinal epithelial barrier dysfunction, skewed immune responses in the intestine,[2 3] additional studies showing expression of CRH in ileum lamina propria cells,[7] and the tetrodotoxin blocking experiment as shown in supplementary fig S1, we hypothesised that CRH could be produced by immune cells in the intestine in response to stress.

Substance P is capable of modulating the immune activities in the body via activating its receptors neurokinin receptor (NK)1, or NK2 or NK3. It is also involved in stress-induced immune disorders in the body, such as exacerbation of inflammatory bowel disease.[8 9] In preliminary studies, we observed a significant increase in SP in jejunal tissue of mice after treatment with restraint stress (supplementary fig S2). The facts that stress induces release of SP, pretreatment of experimental animals with CRH antagonist, α-helical, abolishes stress-induced disorders in the body[1 2 9] implicate that SP is involved in the CRH-related mucosal disorders. However, the underlying mechanisms are to be further elucidated.

Eosinophils are residential cells in the mucosa such as in the airway and intestine. The activation of eosinophils is involved in initiation and propagation of diverse inflammatory responses.[10] One key issue remains to be addressed is how eosinophils are activated in non-IgE mediated inflammation. Since eosinophils express the receptors of SP,[10] and stress drives the nerve endings to release SP,[11] it is likely, but not clear, that stress may activate eosinophils; the latter release mediators to participate initiation and propagation of immune inflammation in the body.

In this study, we aimed to investigate the possibility that (1) psychological stress increases the expression of CRH in immune cells in the intestine; (2) stress activates the CRH-secreting cells to release CRH; and (3) immune cell-derived CRH impairs intestinal epithelial barrier function with a cell culture model and a murine stress model. Indeed, the results show that stress induces eosinophils to express CRH via activities of SP. Eosinophil-derived CRH plays a role in the induction of epithelial barrier dysfunction.

MATERIALS AND METHODS

Reagents

Anti-CRH antibody (clone 2B11), antalarmine, antisauvagine-30, cortisol, ACTH, methacholine, GR-159897, SB-222200, substance P, noradrenaline, SAR-MET-substance P, horseradish peroxidase, tetrodotoxin were obtained from Sigma-Aldrich (Oakville, Ontario, Canada). CP-96345 and

CP-99994 were from Pfizer (Kirkland, Quebec, Canada). enhanced chemiluminescence (ECL) reagents were from GE Healthcare Life Science (Baie d'Urfe, Quebec, Canada); nitrocellulose membranes were Hy-bond C Super (Amersham, Baie d'Urfe, Quebec, Canada); the RNeasy Mini kit was from Qiagen (Mississauga, Ontario, Canada); the iScriptTMcDNA Synthesis Kit was from Bio-Rad (Mississauga, Ontario, Canada); and the SuperScript III Platinum SYBR Green Two-Step qPCR Kit was from Invitrogen, Burlington, Ontario, Canada). Antibodies against CD11c, major basic protein, 5-HT was from Abcam (Cambridge, Massachusetts, USA); and antibodies against mouse mast cell protease 1, CD3 and CD68 were from Santa Cruz Biotechnology, Santa Cruz, California, USA). Cell culture materials were purchased from Invitrogen (Burlington, Ontario, Canada); and the CRH ELISA kit from Cosmo Bio (Tokyo, Japan). The sensitivity of the ELISA was 0.313 ng/ml. Immunogens of purchased antibodies are listed in supplementary table S1.

Mice

Balb/c mice (6–8 weeks old) were purchased from Charles River Canada (St. Constant, Quebec, Canada). *W/W*^{v} mice were purchased from Jackson Laboratory (Bar Harbor, Maine, USA). Mice were maintained in a pathogen-free environment.

Restraint stress

The strainer using in restraint stress was a 50 ml conic plastic tube with 10 holes (0.8 cm in diameter) on the walls and both ends. The stress was 1 h per session and carried out at 10–11 a.m. for 10 consecutive days. Mice were killed immediately after the last stress session by decapitation.

Immunohistochemistry

Jejunal segments were excised immediately after the mice had been killed. Cryosections were fixed with cold acetone for 20 min. Incubation with the primary and fluorescence-labelled secondary antibodies was for 1 h each. The nuclei were stained with propidium iodide (10 μg/ml) for 15 min for morphological viewing. Sections were observed under a confocal microscope.

Western blotting

Jejunal protein extracts were separated on pre-cast SDS–PAGE and transferred to nitrocellulose membranes. Membranes were incubated overnight with the primary biotin labelled antibodies (1 μg/ml) at 4°C. Peroxidase–avidin was added at a concentration of 1 μg/ml to the membranes for 1 h at room temperature. The membranes were then developed by ECL reagents and the signal was recorded by *x* ray film.

Real time quantitative reverse transcription polymerase chain reaction

The procedures were the same as our previous report[12] and are also detailed in the supplementary methods.

Flow cytometry

Cells were fixed with 1% paraformaldehyde on ice for 30 min and then treated with permeable kit. After washing with phosphate-buffered saline (PBS), cells were stained with fluorescence-labelled antibodies (1 μg/ml) for 30 min on ice. The stained cells were analysed with a FACSarray (BD Bioscience, San Jose, California, USA). The data were analysed by FlowJo.

Culture of cell lines

The eosinophil cell line, EoL-1 cells, generated from a patient with acute myeloid leukaemia following hypereosinophilic syndrome, was purchased from the RIKEN Cell Bank (Tsukuba, Japan). The human mast cell line, HMC-1 cells, was a gift from Dr JH Butterfield (Mayo Clinic, Rochester, Minnesota, USA). EoL-1 and HMC-1 cells were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum and antibiotics at 37°C and 5% CO_2.

Assessment of T84 monolayer barrier function

Transepithelial electric resistance (TER) of T84 monolayers was determined using the Millicell ERS apparatus (Millipore, Bedford, Massachusetts, USA). Horseradish peroxidase (HRP) flux was carried out to the indicator of T84 monolayer permeability.[13]

Ussing chamber studies

Procedures of Ussing chamber studies were the same as our previous report[1] and are also described in the supplementary methods.

Enzyme-linked immunosorbent assay

CRH levels were determined with an ELISA kit. The analytical procedures were carried out according to the manufacturer's instructions. The detectable concentration range for CRH is $\leqslant$5 pg/ml.

Statistics

The values were expressed as the means with the SD of at least three independent experiments. The data were analysed using the two-tailed unpaired Student t test when data were consisted of two groups or by ANOVA when three or more groups were compared. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Chronic stress induces expression of CRH in eosinophils of the intestine

CRH is an important mediator in the induction of psychological stress-related disorders.[1 2 14 15] Since treating intestinal epithelial tissue with CRH antagonists blocked stress induced epithelial barrier dysfunction,[1 2 14] we inferred that intestinal immune cells were one of the sources of CRH in the intestine during stress treatment. Thus, Balb/c mice were treated with restraint stress daily for 10 days. The mice were killed immediately after the last stress session. Jejunal cryosections or lamina propria mononuclear cells (LPMCs; by density centrifugation) were prepared and stained with antibodies against CRH and markers of a batch of immune cells that included CD11c (a marker of dendritic cells), mast cell protease 1 (a marker of mast cells), CD3 (a marker of T cells), major basic protein (a marker of eosinophils). As shown by confocal microscopy (fig 1) and flow cytometry (supplementary fig S3), abundant CRH^{+} eosinophils were localised in the intestinal lamina propria or in LPMC of mice treated with chronic restraint stress; only a few CRH^{+} eosinophils were found in naïve mice. The protein of CRH in intestinal tissue extracts was detected by western blotting. The number of CRH^{+} eosinophils and CRH protein in the intestine were increased 1 day after the stress session on day 1, reached the peak value on day 6 and slightly fell down in day 8 and day 10, but did not disappear immediately. After treatment with restraint stress ceased, the CRH expression in intestinal

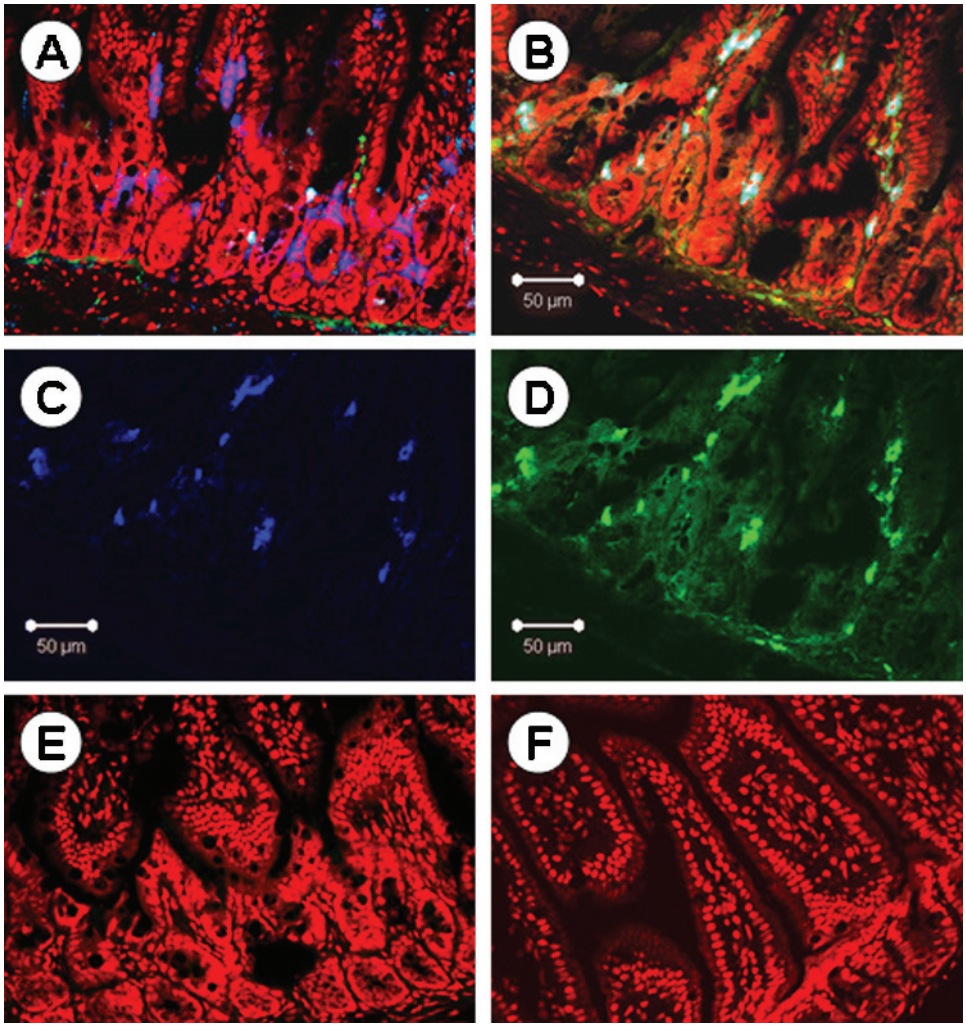

Figure 1 Eosinophils express corticotrophin releasing hormone (CRH) in the intestine. Intestinal cryosections were stained with antibodies against the major basic protein (MBP) and CRH. Confocal images show the co-localisation of major basic protein (in blue) and CRH (in green) The intestine of naive mice (A) or mice treated with chronic restraint stress (B–D). The light-blue colour is merged by blue (C; MBP) and green (CRH). (E) Negative staining control. Intestinal tissue was collected from stressed mice. The primary antibody was replaced by an isotype IgG. (F) Stained with specific antigen (major basic antigen and CRH) absorbed antibodies (the same as those used in B–D).

eosinophils declined gradually and was positively correlated with the time of restraint stress (fig 2). In addition, we also observed CRH expression in colonic immune cells with flow cytometry. Most CRH^+ cells were eosinophils; only a few other immune cells were also CRH^+ (supplementary fig S3). The SP content in jejunal tissue increased after the treatment of 10 day stress, which returned to baseline 1 day after the stress (supplementary fig S2).

Substance P is required for the expression of CRH in eosinophils

We next sought to identify the factors induced CRH expression in eosinophils. An eosinophil cell line, EoL-1 cells, was employed as a study platform, which were derived from a patient with acute myeloid leukaemia following hypereosinophilic syndrome and have the features of human eosinophils as shown the expression of markers for mature eosinophils such as major basic protein and eosinophil peroxidase.[16] EoL-1 cells were exposed to the following putative stress mediators in the intestine including cortisol, adrenocorticotropic hormone (ACTH), SP, methacholine, noradrenaline and an isotype IgG in culture for 24 h. As shown by quantitative real time RT-PCR (qPCR) (fig 3A) and western blotting (fig 3B), the expression of CRH was detected in EoL-1 cells in response to SP, but not in the remaining five molecules. To further confirm the results, EoL-1 cells were exposed to the NK1 receptor agonist SAR-MET-SP in culture for 8 h. As expected, the expression of CRH in EoL-1 cells increased in a dose-dependent manner (fig 3C). To ensure this result, we examined naïve EoL-1 cells with flow cytometry; over 90% EoL-1 cells express both NK1 and NK2 receptors (data not shown).

We then examined the receptor types on EoL-1 cells by which SP induced the expression of CRH. EoL-1 cells were treated with NK1 receptor antagonist CP-96345 (2.5 μg/ml), or CP-99994 (5 μg/ml), or NK2 receptor antagonist GR-159897 (0.12 μg/ml), or NK3 receptor antagonist SB-222200 (2 μg/ml) in culture 30 min prior to exposure to SP. As shown by western blotting, the expression of CRH in EoL-1 cells induced by SP was inhibited by NK1 antagonist, but not by NK2 or NK3 antagonists (fig 3D). Collectively, the results indicate that SP binds NK1 receptor on eosinophils to induce expression of CRH in eosinophils.

Substance P triggers CRH release from eosinophils

To elucidate the mechanism of CRH release from eosinophils, EoL-1 cells were cultured in the presence of SP at graded concentrations. As shown by an immunoblotting assay, CRH in culture media of those EoL-1 cells without SP stimulation was under the detectable levels, while the presence of SP induced CRH release from EoL-1 cells in a SP dose-dependent manner. Pretreatment of EoL-1 cells with NK2 (but not NK1 or NK3) antagonists resulted in abrogation of the SP-induced CRH release by EoL-1 cells (fig 3E). The results indicate that SP

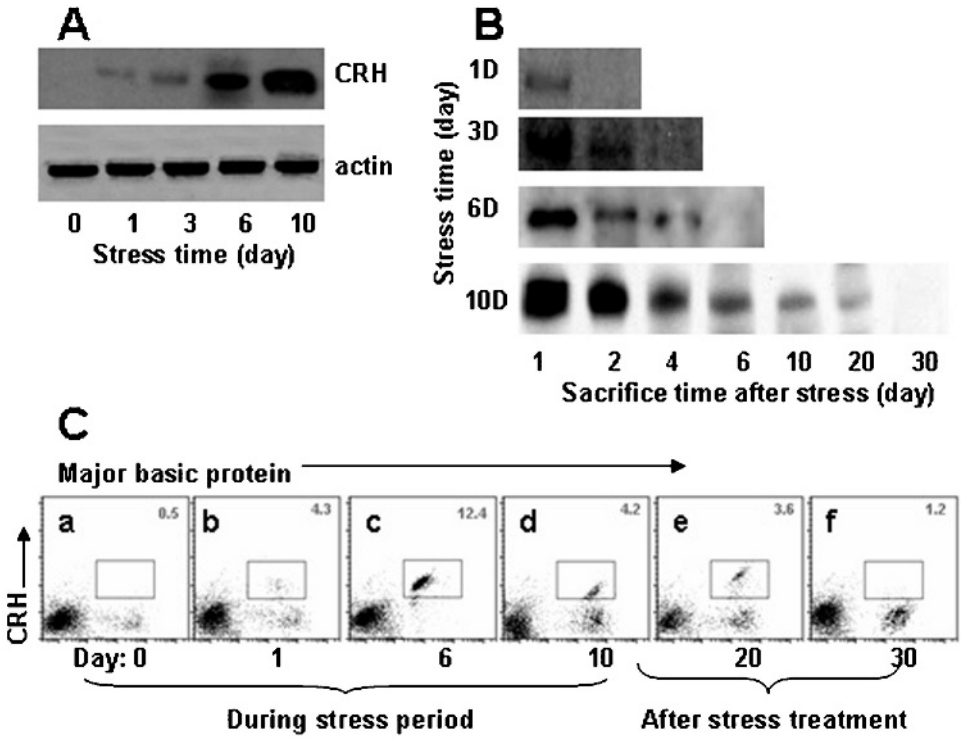

Figure 2 Stress increases the expression of corticotrophin releaseing hormone (CRH) in the intestine. Grouped mice were treated with restraint stress for 0–10 days. Mice were killed immediately after the last stress session (A) or killed at various time points after stress (B). The western blotting gels show CRH immune blots from intestinal protein extracts. (C) Flow cytometry plots show cells stained positively for both CRH and major basic protein (the gated cells; the numbers in upper right corners indicate the ratio of positive cells in isolated LPMCs from naïve mice (a) or mice treated with chronic restraint stress (b–f) from day 1 to day 30 (f). Each group consisted of six mice.

increases the release of CRH via activating NK2 receptors. We next observed the time course of CRH release from EoL-1 cells. As shown in fig 3F, CRH was detected at half an hour after the addition of SP and reached a peak value at 2 h, then declined and returned to baseline 48 h afterwards (fig 3F).

Eosinophil-derived CRH induces intestinal epithelial barrier dysfunction via activation of mast cells

Mast cell activation plays a critical role in psychological stress-induced epithelial barrier dysfunction.[1 2 14 17] CRH is able to activate mast cells.[18 19] We speculated that under a psychological

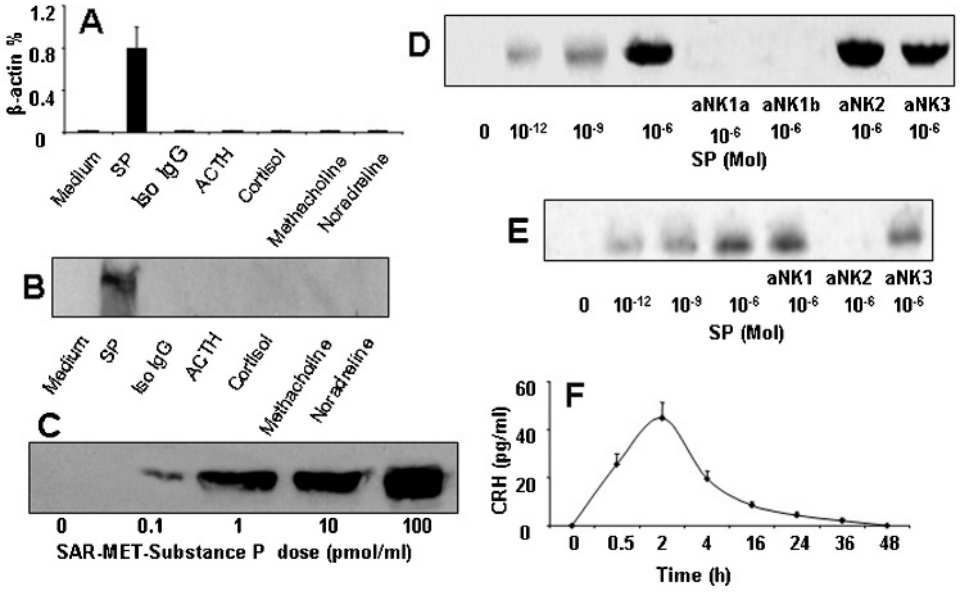

Figure 3 Expression of corticotrophin releasing hormone (CRH) by eosinophils. EoL cells (eosinophil cell line) were cultured in the presence of putative simulators. CRH levels were assessed at the levels of mRNA and protein in EoL-cell extracts (A–D) and culture media (E,F). (A) Bars indicate CRH mRNA levels in EoL-1 cells (means with the SD) that were determined by real-time RT-PCR. (B) Western blotting gel show CRH protein in EoL-1 cells after treatment with putative stimulators. (C) Western blotting gel shows CRH protein in EoL cells after treatment by NK1 agonist (SAR-MET-substance P). (D) Western blotting gel indicates the expression of CRH in EoL cell extracts in the presence of SP and NK1 antagonist. a: antagonist. aNK1a: CP96345. aNK1b: CP99994. (E) Western blots show CRH in EoL-1 cell culture medium in the presence of SP and NK2 antagonist. (F) Time course of CRH release in culture media by EoL cells in response to SP (10^{-6} mol/l).

stress environment, eosinophil-derived CRH activated mast cells to impair intestinal epithelial barrier function. An ex vivo experiment was carried out to test the hypothesis. EoL-1 cells were exposed to SP (10^{-6} mol/l) for 24 h and washed with fresh culture media. The EoL-1 cells were then co-cultured with human mast cell line HMC-1 cells in the basal chambers of trans-well system where confluent T84 cells were cultured in inserts.[13] The co-culture significantly reduced the transepithelial electrical resistance and increased the permeability of T84 monolayers in an EoL-1 cell number-dependent manner indicating the epithelial barrier dysfunction. The induced epithelial barrier dysfunction could be inhibited by pretreating HMC-1 cells with α-helical CRH, which indicates that EoL-1 cells release CRH after being primed by SP. CRH activates HMC-1 cells via binding CRH receptors and induces the epithelial barrier dysfunction (fig 4).

To gain further insight into the role of eosinophil-derived CRH on the regulation of the intestinal epithelial barrier function, we treated mice with restraint stress daily for 10 days. The intestinal epithelial barrier function was assessed by using an Ussing chamber approach. The short circuit current (Isc) and HRP flux were employed as markers of epithelial barrier functional status. In the first attempt, we tested if SP impairs intestinal barrier function at given concentrations. At the concentrations (10^{-12} to 10^{-6} mol/l) tested, we observed that SP did not directly affect the epithelial barrier function. As compared with the naïve group, stress-treated mice showed significantly higher Isc and HRP flux in response to stimulation of SP in Ussing chambers. To clarify if mast cells play any roles in the epithelial barrier dysfunction caused by SP, we treated a group of mast cell deficient mice, WBB6F1/J-*Kit*^{W}/*Kit*$^{W-v}$ (*W*/*W*^{v}) mice, with restraint stress daily for 10 days; the intestinal epithelial barrier function was then examined. Data from Ussing chamber experiments showed that Isc and HRP flux were significantly lower in stressed *W*/*W*^{v} mice than that from stressed littermates, the +/+ mice in response to stimulation of SP (fig 5). The results indicate that mast cells play a critical role in SP-induced intestinal epithelial barrier dysfunction in stressed individuals.

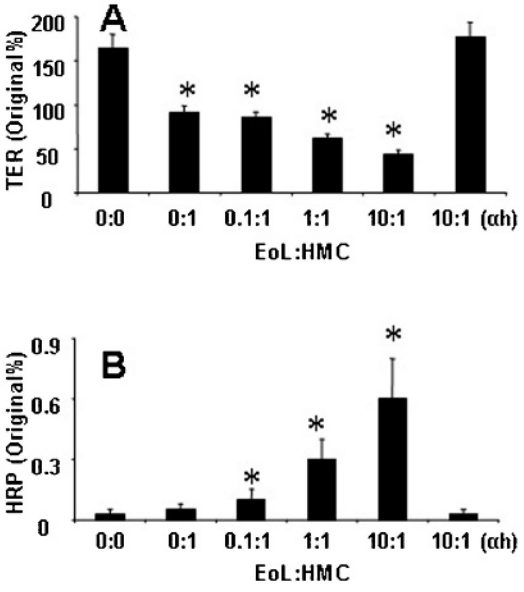

Figure 4 In vitro study. Eosinophil-derived corticotrophin releasing hormone (CRH) induced T84 monolayer barrier dysfunction. Substance P-primed EoL-1 cells were co-cultured with HMC-1 cells at the basal chambers of trans-well where confluent T84 monolayers were cultured in apical chambers. The transepithelial resistance (TER) was recorded before and 24 h after the addition of EoL-1 and HMC-1 cells. Horseradish peroxidase (HRP) flux was carried out during 24–26 h after the addition of EoL-1 and HMC-1 cells. Data were normalised by baseline records and presented as the percentage of baseline TER (A) or percentage of HRP added to apical chambers (B).

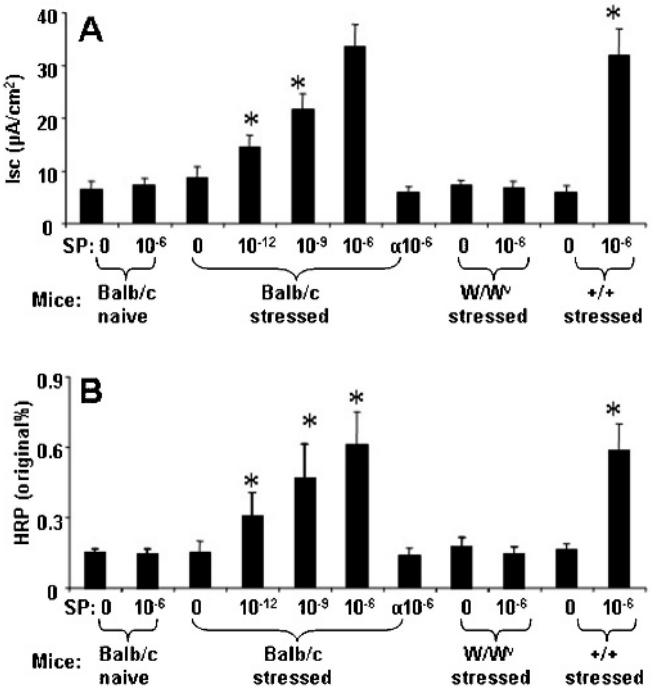

Figure 5 In vivo study. Eosinophil-derived corticotrophin releasing hormone (CRH) impairs epithelial barrier function. Grouped mice (six mice per group) were treated with restraint stress for 10 days. Intestinal epithelial short circuit current (A) and horseradish peroxidase (HRP) flux (B) were assessed by the Ussing chamber technique. Unit of SP: mol/l. $\alpha 10^{-6}$: Intestinal epithelial layers were treated with α-helical CRH for 10 min prior to the addition of SP at a concentration of 10^{-6} mol/l.

We next sought to determine whether SP activates mast cells directly to impair the intestinal epithelial barrier function, or drives eosinophils to release CRH; the latter activates mast cells to affect epithelial barrier function. A group of Balb/c mice was treated with the CRH antagonist, α-helical CRH (20 μg/mouse), prior to each stress session. As expected, data of Isc and HRP flux from these mice were similar to that from naïve controls (fig 5). The results indicate that SP did not activate mast cells directly, but induces CRH release from eosinophils first, the released CRH then binds CRH receptors on mast cells and induces mast cell activation.[20] The results are also in line with other's report that SP does not activate mast cells in the intestine.[21]

We further observed the roles of CRH receptor subtypes, CRH-R1 and CRH-R2, in SP-mediated intestinal epithelial barrier dysfunction. With the same procedures in fig 5, mice were pretreated with CRH-R1 antagonist, antalarmin (30 μg/mouse), or CRH-R2 antagonist, antisauvagine-30 (50 μg/mouse) prior to each stress session. The data from the Ussing chamber study showed that both CRH-R1 and CRH-R2 were involved in SP-mediated intestinal barrier dysfunction (supplementary fig S4). Blocking both CRH-R1 and CRH-R2 with α-helical CRH abolished the effect of SP-mediated intestinal epithelial barrier dysfunction (fig 5).

DISCUSSION

Apart from playing critical roles in the initiation of allergic reactions such as asthma, eosinophils are also effector cells in the induction of non-IgE mediated inflammation such as eosinophilic pneumonia, some subtypes of eosinophilic oesophagitis and eosinophilic gastroenteritis. The mechanism of activation of eosinophils in non-IgE mediated disorders remains largely unknown. The present study provides novel evidence that eosinophils produce CRH upon stimulation of psychological stress. SP plays a critical role in the expression of CRH in eosinophils via activation of its receptor NK1. Furthermore, SP also activates NK2 on eosinophils to evoke the release of CRH. On the other hand, eosinophil-derived CRH has the ability to activate mast cells and further induced intestinal epithelial barrier dysfunction.

Although CRH has been found in the serum, its peripheral cellular sources remain to be further understood.[4 22] Using cellular and molecular approaches, the present study provides evidence that intestinal immune cells, including eosinophils, macrophages, T cells and enterochromaffin cells, express CRH. The results are in line with previous studies showing intestinal macrophages are CRH^{+}[23] and enterochromaffin cells express CRH.[24] Notably, a novel finding of the present study shows that most CRH^{+} cells are eosinophils in the intestine. Although the expression of CRH was not observed in naïve status of eosinophil cells, upon psychological stress, marked increase in the amount of CRH in eosinophils was noted.

That the increase in CRH expression in eosinophils is in parallel with stress time is in accordance with previous reports, the longer time of stress, the more severe symptoms in stress-related clinical phenomena.[25] On the other hand, the data show that the amounts of CRH in the intestine decline automatically and gradually after remove of stress. The longer time in the treatment with stress, the longer time required to recover or stop the expression of CRH in the intestine. This is in line with our previous studies on recovery time of intestinal epithelial barrier dysfunction induced by psychological stress; it recovers quickly after acute stress (1 h only), but requires much longer time (3–4 weeks) to recover after chronic stress (a 10 day water avoidance stress protocol) (Yang, *et al*, unpublished data). The findings implicate that to spend some "no-stress" time favours the recovery of stress-induced disturbance of the homeostasis in the body.

SP and its NK1 receptor play an important role in the modulation of stress responses in the central nervous system[26] as well as in stress-induced intestinal immune inflammation.[8] Our data also show that SP plays a role in mediating effect of stress to the expression of CRH in intestinal eosinophils. SP is found in picomolar amounts in colonic tissues and in the vagal, pelvic, splanchnic and lumbar colonic nerves.[27] Based on these findings, we may envisage a scenario that restraint stress evokes central nervous system activity; the latter sends instructive signals via these neural pathways to arrive the intestine where the nervous endings release SP to induce the downstream physiological or pathophysiological responses in the intestine.

The released SP may act on nervous endings, blood vessels or immune cells to induce diverse responses in the intestine. Eosinophils express the receptors of SP[10] and have the potential to be activated by stress-induced SP. Indeed, the present data show that SP activates EoL-1 cells via NK1 receptor to express CRH. The finding is in line with previous reports that CRH is localised in the intestine,[23 24 28] which can be upregulated upon the stimulation of psychological stress.

As a residential component in the intestine, eosinophils are involved in an array of physiological responses and inflammatory reactions. Apart from being involved in inflammation related to food allergy, eosinophils also play a critical role in eosinophilic oesophagitis and gastroenteritis. Although the association between eosinophils and pathogenesis of these disorders is quite clear, the triggers of activation of eosinophils remain obscure. Based on the present data, it indicates that psychological stress induces the release of SP in the intestine. SP further induces eosinophils to produce CRH, and then, CRH can be involved in the induction of these inflammatory disorders. The results are in line with previous studies that SP and CRH are also involved in microbial product-related intestinal inflammation.[29]

Mast cells are another residential cell population in the intestine which is involved in both allergic and non-allergic inflammation as effector cells. Recent advances in mast cell studies indicate that mast cells express CRH receptors[18] and can be activated by CRH to impair the intestinal epithelial barrier function[14] and initiate inflammation in the intestine.[1 2] The present study has extended existing knowledge by revealing that psychological stress-derived SP induces the expression of CRH in eosinophils in the jejunum that further activate mast cells.

The released CRH activates mast cells which may, in turn, activate the sensory neurons in the intestine to release SP.[30] SP released by these neurons may further amplify the peripheral responses via the mechanisms described above. This scenario may explain the delay in enhanced CRH expression by eosinophils (see fig 2) under stressful environment, especially in chronic stress.

Intact epithelial barrier function is essential to maintaining the homeostasis in the body. Intestinal epithelial barrier dysfunction is involved in a broad array of intestinal disorders including inflammatory bowel disease,[31] food antigen-related adverse responses,[12] and other types of intestinal inflammation.[32 33] However, the causative mechanisms in intestinal epithelial barrier dysfunction are not completely understood. The present data provide novel evidence that eosinophil-derived CRH plays a role in the induction of intestinal epithelial barrier dysfunction. We therefore summarise the evidence into such a pathway, stress → SP release from the nervous endings in the intestine (eg, in the jejunum) → induces eosinophil to produce CRH → CRH activates mast cells to induce intestinal epithelial barrier dysfunction. Therefore, under psychological stress, intestinal epithelial barrier may be impaired via this pathway resulting in absorption of macromolecular antigens, microbial products or even bacteria into intestinal tissue to induce inappropriate immune responses and inflammation in the intestine.

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Competing interests: None.

Ethics approval: The procedures of experiments carried out on the mice in this study were approved by the Animal Care Committee at McMaster University.

Provenance and peer review: Not commissioned; externally peer reviewed.

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Sir Francis Avery Jones BSG Research Award 2010

Applications are invited by the Education Committee of the British Society of Gastroenterology, who will recommend to Council the recipient of the 2010 Award. The recipient will be required to deliver a 30 minute lecture at the annual meeting of the Society in Liverpool in March 2010. Applications should comprise:

- A manuscript (two A4 pages ONLY) describing the work conducted.
- A bibliography of relevant personal publications.
- An outline of the proposed content of the lecture, including title.
- A written statement confirming that all or a substantial part of the work has been personally conducted in the UK or Eire.

Entrants must be $\leqslant$40 years on 31 December 2009 but need not be a member of the Society. Applications should be composed electronically in Word or Rich Text format and emailed as an attachment to the BSG Office (A.Orgusaar@bsg.org.uk) by 31 October 2009.