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Acute stress modulates the histamine content of mast cells in the gastrointestinal tract through interleukin-1 and corticotropin-releasing factor release in rats

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Stress results in activation of the hypothalamic pituitary adrenal axis and affects illnesses such as neuroinflammatory syndrome. *In vivo* acute stress (restraint stress) induces gastrointestinal function disturbances through colonic mast cell activation. This study investigated the effect of acute stress in histamine content of colonic mast cells, and the central role of interleukin-1 (IL-1) and corticotropin-releasing factor (CRF) in this effect. After a restraint stress session colonic segments were isolated and submitted to three protocols: (i) determination of histamine levels by radioimmunoassay (RIA) after incubation with 48/80 compound, (ii) evaluation by histology of mucosal mast cell (MMC) number and (iii) determination of histamine immunoreactivity of MMC. These procedures were conducted (1) in sham or stressed rats, (2) in stressed rats previously treated with intracerebroventricular (I.C.V.) IL-1ra or α-helical CRF9–41, (3) in naive rats pretreated with I.C.V. rhIL-1β or CRF and (4) in rats treated with central IL-1β and CRF plus α-helical CRF and IL-1ra, respectively (cross-antagonism reaction). Acute stress increases histamine content in colonic mast cells, without degranulation. I.C.V. pretreatment with IL-1ra or α-helical CRF9–41 blocked stress-induced mast cell histamine content increase. Both I.C.V. rhIL-1β and CRF injections reproduced the stress-linked changes. I.C.V. treatment with CRF antagonist blocked I.C.V. rhIL-1β-induced mast cell histamine content increase, whereas central IL-1ra did not affect stress events induced by I.C.V. CRF administration. These results suggest that in rats acute stress increases colonic mast cell histamine content. This effect is mediated by the release in cascade in the brain first of IL-1 and secondly of CRF.

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Stress results in activation of the hypothalamic pituitary adrenal axis and can affect illnesses such as autoimmune and neuroinflammatory syndrome (Stenberg & Wilder, 1993). Stress participates in or worsens certain neuro-inflammatory disorders, such as neurogenic pruritis and intestitial cystitis (Sant & Theoharides, 1994), two diseases associated with mast cell activation. Moreover, stress can reactivate or enhance experimental colitis in animals (Collins *et al.* 1996; Gue *et al.* 1997*a*). In addition, stress stimulates colonic motility through the central release of corticotropin-releasing factor (CRF) (Williams *et al.* 1988; Erkenbrecht, 1989). Recently it has been reported that colonic responses to immobilization stress such as mucines and PGE2 release are linked to mucosal mast cell degranulation (Castagliuolo *et al.* 1996). In a previous study, we demonstrated that stress-induced rectal hyper-algesia is suppressed by a mast cell stabilizer, suggesting that it depends upon mast cell degranulation (Gue *et al.* 1997*b*). Moreover, it has been shown that psychological factors such as anxiety and stress are important in the symptomatology of irritable bowel syndrome (IBS) patients (Gwee *et al.* 1996). Intestinal degranulation of mast cells can result in the release of mediators such as histamine, serotonin and cytokines, known to facilitate the response of afferent fibres inducing a range of systemic symptoms like visceral pain, the major symptom described in IBS patients (Collins, 1991; Miner, 1991). Controversial results were described concerning the presence of a greater number of mucosal mast cells detected in the gastro-intestinal tract of IBS (Miner, 1991; Weston *et al.* 1993; Gwee *et al.* 1996). In addition, in stress-induced visceral hypersensitivity, we also illustrated the involvement of CRF receptors (Gue *et al.* 1997*b*). These receptors are known to be found not only on neurons but also in different immune cells including macrophages, lymphocytes and mast cells (Singh, 1989; Webster *et al.* 1990), which when activated can release pro-nociceptive mediators. CRF can also trigger lymphocyte proliferation, as well as interleukine-1 (IL-1) secretion from monocytes (Woloski *et al.* 1985). Recently, Nguyen *et al.* (1998) showed a brain IL-1 protein expression in rats after exposure to acute stress session. In addition, infectious agents initiate

behavioural reactions similar to stress and central administration of IL-1 mimicks neurochemical and behavioural reactions identical to that induced by environmental stressors (Shintani *et al.* 1995*a*; Maier & Watkins, 1998). Moreover, in rats IL-1 receptor antagonist (IL-1ra) has been reported to block hypothalamic monoamines and pituitary–adrenal response to immobilization stress (Shintani *et al.* 1995*b*).

Consequently, on the basis of these observations, this study was designed first to evaluate, in rats, if an acute restraint stress is able to produce changes in the number and/or histamine content of mast cells within the gut. This work also aimed to evaluate the involvement of brain IL-1 and CRF in stress-induced colonic mast cell histamine level changes.

METHODS

Animal preparation

Thirteen groups of ten male Wistar rats weighing 250–300 g were used. The animals were housed in polypropylene cages and kept in a temperature-controlled room (21 ± 1 °C). They were allowed free access to water and fed laboratory pellets (U.A.R., Epinay, France).

Eleven groups of animals were fitted with a small polyethylene catheter (ID, 0.3 mm; OD, 0.7 mm) inserted into a lateral ventricle of the brain using the following co-ordinates from the bregma: anteroposterior −1.3 mm; lateral 1.8 mm; central 3.5 mm. Two screws were implanted in the bone surface and the catheter was secured with dental cement. The position of the catheter was checked on completion of the experiment. All surgery for intracerebroventricular (I.C.V.) cannula implantations was performed under a deep anaesthesia consisting of an intraperitoneal injection of acepromazine (0.6 mg kg^{-1}; Calmivet, Vetiquinol, Lure, France) as a sedative, and ketamine (120 mg kg^{-1}; Imalgene 1000, Rhone Merieux, Lyon, France) as anaesthetic. Recovery from surgery was visually monitored for 4 h in specific individual cages. All rats presented normal behaviour and food intake during the following 24 h. This experimental protocol was approved by the local Institut National de la Recherche Agronomique (INRA) Care and Use Committee (agreement no. 97024A).

Stress procedure

Acute partial restraint stress, a relatively mild, non-ulcerogenic model of restraint was used in all stress sessions. Partial restraint stress, which was reported to increase plasma levels of adrenocorticotropic hormone (ACTH) and cortisone (Williams *et al.* 1988), was used in all stress sessions. Briefly, the animals were lightly anaesthetized with ethyl ether, and their foreshoulders, upper forelimbs and thoracic trunk were wrapped in a confining harness of paper tape to restrict, but not to prevent body movement for 2 h. Control sham stressed animals were anaesthetized but were not wrapped and replaced in their home cages. Partial restraint stress was always performed between 10.00 and 12.00 h.

Mast cell counting and histamine release

Twenty minutes after the stress session, the animals were killed by decapitation according to European Commision guidelines. Two segments (0.5 cm) of proximal colon (taken from 2 cm from the caecocolonic junction, proximal segment), and two distal segments (taken at 8 cm from the caecocolonic junction, distal segment) were excised for histological mucosal mast cell (MMC) number determination, and local *in vitro* histamine release determination. For histological studies, colonic segments were fixed in Carnoy's solution, cleared in xylene, and embedded in paraffin blocks. Transverse sections (5 μm) were cut and stained with Alcian Blue-Safranin in order to identify the MMC population. Indeed MMCs predominate in the rat gut (96 % of gut MMC are present in the large intestine). Alcian Blue-Safranin staining was performed at low pH (Alcian Blue: pH 3 and Safranin: pH 3). This staining permitted the identification of resident mucosal mast cells, which appeared in blue. Then only blue mast cells in the mucosa and submucosa (where mucosal mast cells have been clearly described) were counted and expressed per high-powered field (HPF, using a ×20 objective) by an observer blinded for each treatment (Bradesi *et al.* 2002).

In vitro, levels of histamine released by proximal and distal colonic segments were evaluated as previously described (Eutamene *et al.* 1998). The colonic segments were incubated (60 min) in a Ringer solution containing the 48/80 compound (1.5 mg ml^{-1}), a potent rat mast cell degranulating agent (Riley & West, 1995). Antiproteases (0.2 M of AEBSF, 10 μM of leupeptine and 1 μM of pepstatine) were added to the medium. After homogenization, the total histamine concentration in the supernatant was measured by a radioimmunoassay (RIA) kit using polyclonal histamine antibodies (Immunotech, Marseille, France). Total tissue protein concentrations were determined according to the Bradford (1976) method. In the tissues, the histamine level was expressed in nanomoles per gram of total protein.

Histamine immunoreactivity of colonic mucosal mast cells

In both stressed and sham stressed animals, mucosal mast cell histamine content was determined in two additional proximal and distal colonic segments by immunohistochemistry. Colonic segments were paraplast embedded and transverse sections (3 μm) were cut for peroxidase immunochemistry. Sections were pretreated with 100 % methanol plus H_2O_2 at room temperature for 30 min. Rabbit anti-histamine (Chemicon International Inc.), was diluted 1/50 in 10 % normal rabbit serum in phosphate-buffered saline (PBS; pH 7.6). The anti-rabbit peroxidase affinity secondary antibodies were diluted 1/500 in 10 % normal rabbit serum, in PBS (pH 7.6). Peroxidase activity was revealed using diaminobenzidine (DAB) as chromogene and hydrogen peroxidase. Sections were then mounted on gelatin-coated slides, dehydrated, cleared in toluene and coverslipped. The presence of MMC histamine immunoreactivity was detected as a dark brown reaction product in cell cytoplasm under a light microscope. All individual cytoplasm staining cells were estimated using an image grabbing program (Neotech, Paris, France) and an Optilab image analysis software package (Graftek, Paris, France) running on an Apple Macintosh IICI computer. Two average density levels of histamine-positive cytoplasm cell were determined using a grey histogram level pallet (HLP). The first HLP (105–116 grey level) was determined in order to discriminate target cells from background, and only MMCs that had significant levels of DAB reaction product in their cytoplasm were counted (high intensity: +). The second HLP (105–147 grey level) was determined in order to identify all the MMCs coloured with DAB reaction product (low intensity). Then a selective target cell size was determined (20–300 pixels). Both low MMC intensity and high MMC intensity (+) were counted per site on three sections of both proximal and distal colon. The number of MMC with a significant

DAB colouration (intensity +) were expressed as a percentage of total MMC coloured with DAB.

Experimental design

In the first series of experiments two groups of animals were submitted to partial restraint stress or a sham stress for 2 h. Twenty minutes later, the animals were killed and the three following parameters were evaluated: number of MMC, colonic mast cell histamine level released *in vitro* after 48/80 compound stimulation, and MMC histamine immunoreactivity.

In a second series of experiments, pharmacological blockade of stress effects were investigated. Twenty minutes before the immobilization stress session, three groups of animals were injected I.C.V. with either 5 μl of sterilized saline (control) or α-helical CRF9–41 (a CRF receptor antagonist at 20 μg kg^{-1}) or IL-1ra (a IL-1 receptor antagonist at 40 μg kg^{-1}) 30 min before the stress session. The sham stressed group was treated with I.C.V. saline. Twenty minutes after the stress session the animals were killed and the same parameters except MMC histamine immunoreactivity were evaluated.

In a third series of experiments, the effect of central (I.C.V.) IL-1 and CRF administration were evaluated. Vehicle (sterilized saline 0.9 %) or rh-IL-1β (80 ng kg^{-1}) or CRF (20 μg kg^{-1}) were I.C.V. administered to three groups of conscious freely moving rats. 170 minutes after administration of these compounds, the animals were killed and the protocols previously described were performed.

In a fourth series of experiments using two distinct groups of rats, we determined the cascade of activation of IL-1 and CRF receptors in the brain. Twenty minutes before the stress session, one of them was I.C.V. pretreated with α-helical CRF9–41 (20 μg kg^{-1}) and 15 min later received central rhIL-1 (80 ng kg^{-1}). In contrast, the second group received first central IL-1ra (40 μg kg^{-1}) followed (15 min) by I.C.V. CRF administration (20 μg kg^{-1}).

Drugs

rhIL-1β was kindly provided by Dr Widner (Immunex, Seattle, WA, USA). IL-1ra was a gift from Synergen (Boulder, CO, USA). CRF and α-helical CRF9–41 were purchased from Sigma (USA). The doses were chosen according to the literature cited in this manuscript.

Statistics

Values of mast cell number, histamine concentration and intensity of DAB colourations obtained after each treatment were compared using a Kruskall-Wallis test. The criteria for statistical significance was $P < 0.05$. Data are expressed as means ± S.E.M. for each group of rats.

RESULTS

MMC number and histamine content after acute stress

The histological analysis of both proximal and distal colonic segments showed no significant difference ($P < 0.05$) in the number of mucosal mast cells identified in the lamina propria of stressed animals compared with sham stress (Table 1).

Levels of histamine released *in vitro* after 48/80 compound stimulation from both proximal and distal colonic segments provided from stressed animals were 6- and 9-fold higher than those obtained for control (sham stress, 0.34 ± 0.11 and 0.11 ± 0.05 nmol (g total proteins)$^{-1}$) in proximal and distal colonic segment, respectively (Fig. 1*A*).

Comparison of histamine immunoreactivity labelling in proximal and distal colonic segments of both stressed and sham rats showed a significant increase in histamine content in mast cells of stressed animals compared with sham rats (2- and 4-fold higher, respectively; Figs 1*B* and 5).

CRF and IL-1 antagonism on stress-induced MMC activation

Both α-helical CRF9–41 receptor antagonist (20 μg kg^{-1}) and IL-1ra (40 μg kg^{-1}) administered I.C.V. 30 min before the stress session, abolished stress-induced enhancement of colonic mast cell histamine content, since in the proximal and distal colon, values obtained were significantly ($P < 0.05$) lower when compared with vehicle stressed animals (0.39 ± 0.17 and 0.15 ± 0.09 nmol (g total proteins)$^{-1}$) and similar to that observed in sham

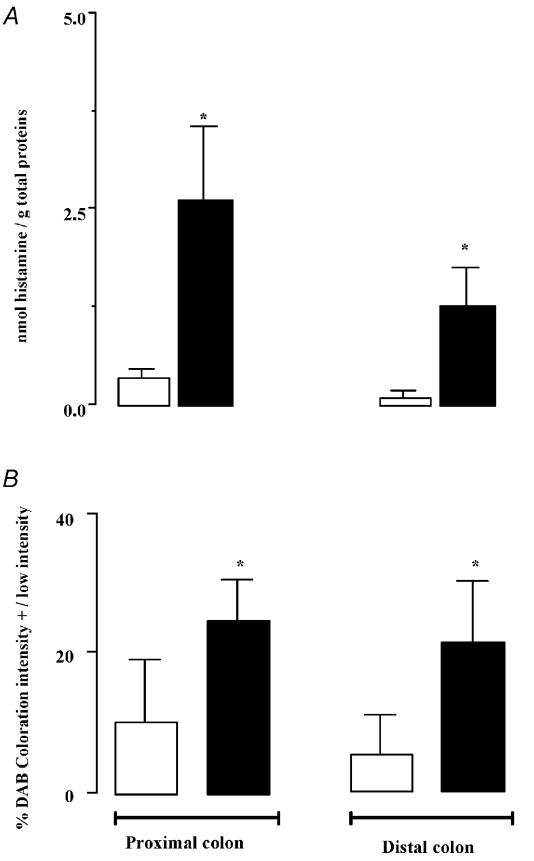

Figure 1

A, influence of stress on histamine mast cell release after 48/80 compound stimulation. Note that stress increases histamine mast cell content. *B*, MMC histamine immunohistochemical labelling in stressed (■) *vs.* control (□) rats. The number of MMC with a high DAB colouration: intensity +, was expressed as a percentage of total MMC coloured with DAB (low intensity). A significant increase of MMC histamine level label in stressed rats was observed compared with the label control. Results are expressed as means ± S.E.M., $^{*}P < 0.05$ from control values.

Table 1. Mucosal mast cell number determination after specific colouration by Alcian Blue-Safranin

Mast cell number/HPF	Proximal colon	Distal colon
Sham stress	16.2 ± 2.2	8.1 ± 1.1
St essed	18.0 ± 1.9	8.9 ± 1.7
Stress + α-helical (20 μg kg^{-1} I.C.V.)	15.9 ± 2.8	7.7 ± 1.9
Stress + IL-1 a (40 μg kg^{-1} I.C.V.)	17.1 ± 1.6	8.0 ± 1.0
CRF (20 μg kg^{-1} I.C.V.)	18.5 ± 3.1	7.9 ± 2.1
rhIL-1β (80 ng kg^{-1} I.C.V.)	16.3 ± 2.4	8.4 ± 2.0
α-helical (20 μg kg^{-1} I.C.V.) + rhIL-1β (80 ng kg^{-1} I.C.V.)	15.7 ± 2.9	7.4 ± 1.9
IL-1 ra (40 μg kg^{-1} I.C.V.) + CRF (20 μg kg^{-1} I.C.V.)	16.8 ± 2.1	8.0 ± 1.8

(n=10)

stressed rats (Fig. 2). Futhermore, neither central α-helical CRF9–41 nor IL-1ra administration before the stress session changed the MMC number at both proximal and distal colonic segments when compared with sham stress (Table 1).

Peripheral CRF and brain rhIL-1 and CRF administration on MMC activation

rhIL-1β and CRF administered I.C.V. at 80 ng kg^{-1} and 20 μg kg^{-1}, respectively, significantly enhanced histamine levels of colonic mast cells when compared with control rats in both proximal and distal segments (0.43 ± 0.09 and 0.05 ± 0.14 nmol (g total proteins)$^{-1}$, respectively) and increased the *in vitro* mast cell histamine release at a level similar to that observed after a stress session (Fig. 3). At the doses used central rhIL-1β or CRF administrations were unable to modify the number of MMC detected in the mucosa of both proximal and distal colon when compared with sham stressed animals (Table 1).

Relationships between activation of central rhIL-1 and CRF receptors

α-Helical CRF9–41 (20 μg kg^{-1}) injected I.C.V., 15 min before rhIL-1 (80 ng kg^{-1} I.C.V.) antagonized rhIL-1β-induced increase of histamine mast cell content as well as histamine released *in vitro* after 48/80 compound in stressed animals (Fig. 4).

In contrast, in animals pretreated with IL-1ra (80 μg kg^{-1} I.C.V.), CRF (20 μg per rat I.C.V.) did not affect CRF-induced histamine content enhancement in mast cells in both proximal and distal colon; values obtained were significantly different ($P < 0.05$) from histamine levels observed in controls (0.43 ± 0.12 and 0.10 ± 0.03 nmol (g total proteins)$^{-1}$, respectively) (Fig. 4).

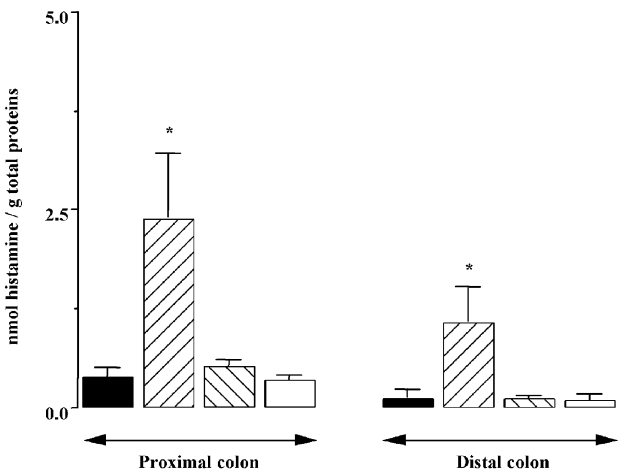

Figure 2. Effect of α-helical CRF and IL-1ra on stress-induced histamine content modulation in mast cells

Note the antagonistic effect of both α-helical CRF and IL-1ra in stress-induced enhancement of histamine synthesis in proximal and distal colonic mast cells. Results are expressed as means ± S.E.M., *$P < 0.05$ from control values. ■, control; ▨, stressed + saline I.C.V.; ▧, stressed + α-helical CRF (20 μg kg^{-1} I.C.V.); □, stressed + IL-1ra (40 μg kg^{-1} I.C.V.).

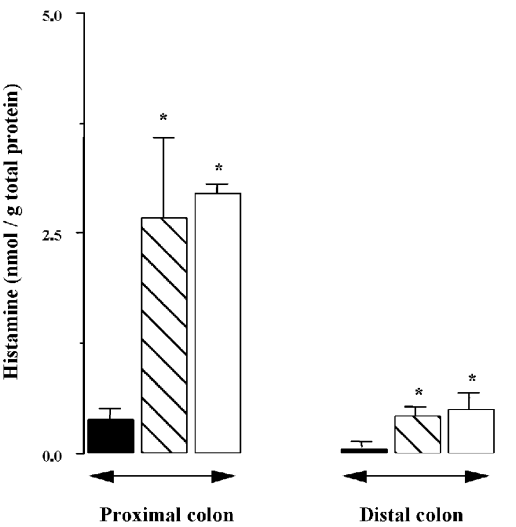

Figure 3. Influence of central rhIL-1β and CRF administration on histamine content enhancement in mast cells

Note that CRF administered I.C.V. enhanced histamine levels in mast cells. A similar effect was observed in animals treated by an I.C.V. rhIL-1β administration. Results are expressed as means ± S.E.M., *$P < 0.05$ from control values. ■, control; ▧, rhIL-1β (80 ng kg^{-1} I.C.V.); □, CRF (20 μg kg^{-1} I.C.V.).

DISCUSSION

This study shows in the experimental protocol design used, a modulatory role of stress on colonic mast cell mediators, reflected by intracellular enhanced levels of histamine. We also demonstrate that this effect is linked to the activation in cascade of brain receptors of IL-1 and CRF.

These results do not fully agree with previous data showing that immobilization stress promotes mast cell degranulation or at least increases rat mast cell protease II (RMCPII) activity (Castagliuolo *et al.* 1996). Such a difference can be explained by the experimental protocols performed, since patterns of neuronal activation differ between stressors applied (Li *et al.* 1996). Indeed, Castagliuolo *et al.* (1996) evaluated mucosal mast cell activity by RMCPII level release determination in the serum and in colonic explants cultured *in vitro*, for 30 min and 2.5 h after acute stress, respectively. In the present study, histamine mast cell measurement was performed *in vitro* in colonic segments after 48/80 compound stimulation, 20 min after the stress session. Compound 48/80 such as substance P are members of a family of polybasic mast cell secretagogues that are known to activate trimeric G proteins, primarily those of the G_i and G_o categories. It is now well established that RBL-2H3, a mucosal-like mast cell line, insensible to 48/80, can be made to respond to compound 48/80 by prior exposure to quercetin (a kinase inhibitor), which increases the expression of G_{i2} and G_{i3} proteins (Senyshyn *et al.* 1998; Chahdi *et al.* 2000; Trnovsky *et al.* 1993). The increased expression of these G_i proteins accounts for the acquired sensitivity to compound 48/80 inducing mast cell secretion. Recently Grammatopoulos *et al.* (2001) have shown that a peptide largely released in stress events, corticotropin-releasing hormone (CRH), activates five different G proteins (G_s, G_i, G_o and G_z) in rat cerebral cortical membrane suspensions.

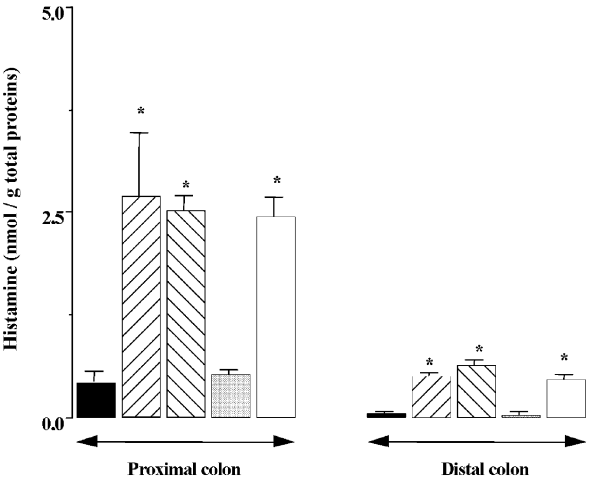

Figure 4. Relationship between activation of central rhIL-1β and CRF receptors

Note that α-helical CRF suppresses rhIL-1β-induced enhancement of histamine synthesis, whereas IL-1ra failed to reverse the I.C.V. CRF-induced histamine synthesis enhancement in mast cells. Results are expressed as means ± S.E.M., *: $P < 0.05$ from control values. ■, control; ▨, rhIL-1β (80 ng kg^{-1} I.C.V.); ▧, CRF (20 μg kg^{-1} I.C.V.); ▩, α-helical CRF (20 μg kg^{-1} I.C.V.) + rhIL-1β (80 ng kg^{-1} I.C.V.); □, IL-1ra (40 μg kg^{-1} I.C.V.) + CRF (20 μg kg^{-1} I.C.V.).

Since in our study we have shown a total mast cell degranulation induced by 48/80 in colonic segments, these different data permit us to speculate that a 2 h restraint

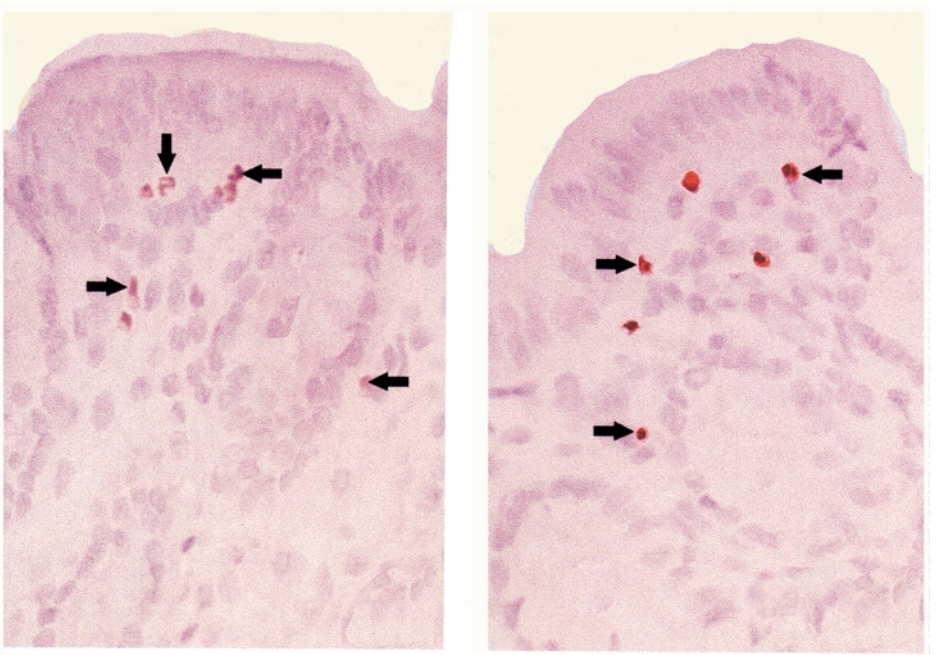

Figure 5. Histamine immunoreactivity labelling in a distal colonic segment in both naive and stressed rats

Note a higher intensity of DAB colouration in stressed animals (right-hand panel) compared with controls (left-hand panel).

stress session permits the release of a range of mediators such as CRH, which are able to increase the expression of G_i proteins in mucosal mast cells. These G_i proteins seem to play a key role in acquired sensitivity to compound 48/80, permitting mucosal mast cell secretion.

MMCs, like other immune cells, reveal an intimate relationship with nerve fibres and their activity can be regulated by neuronal input (Stead *et al.* 1987). In agreement with this argument, Gottwald (1995) reported that cervical vagi electrical stimulation enhances histamine MMC content in the gastrointestinal tract without apparent degranulation (Gottwald *et al.* 1995). In 1989, MacQueen *et al.* illustrated that a psychological conditioning stress induces the sensitization of intestinal mucosal mast cells (MacQueen *et al.* 1989). Moreover, neurotransmitters such as substance P, present in primary afferent neurons activate MMC in a dose-dependent manner by changing their sensitivity and/or potentiating their ability to degranulate (Janiszewski *et al.* 1994). In agreement with these data, Rogers (1998) has shown that acute social stress in adult female monkeys causes a significant decrease in NK cell toxicity. This reduction in NK cell lytic function is correlated with an increase in granule content.

Taken together, these observations suggest that the activation of MMC observed after acute stress herein, depends on the nervous system, and is affected by neuromediators released in a sufficient concentration to sensitize mast cells in terms of an increase of histamine and probably of other mediator levels without subsequent degranulation.

Central administration of either IL-1 or CRF receptor antagonists suppresses stress-induced increase of histamine content at both proximal and distal colonic levels. These results show that the release of IL-1 and CRF is involved, at the brain level, in the effect of stress on colonic mast cell changes. This is also supported by the fact that I.C.V. administration of rhIL-1β or CRF mimics colonic mast cell activation induced by an acute stress session. CRF mRNA and CRF itself are expressed and released a few minutes after the stress session in the paraventricular nucleus of the hypothalamus, the area that contains the largest concentration of CRF neurons (Lightman & Youg, 1989). Specific subclasses of CRF receptors are distributed in both central and peripheral autonomic nervous systems (Potter *et al.* 1994; Perrin *et al.* 1995). Moreover, CRF excites myenteric neurons in guinea-pig small intestine (Hanani & Wood, 1992). These data suggest that CRF can stimulate the autonomic nervous system by acting on brain specific nuclei or directly on peripheral nerves, and in turn modifying intestinal responses.

In addition, the present study suggests an activation in cascade of IL-1 and CRF receptors since the effect of central rhIL-1 is blocked by central CRF antagonists, whereas the IL-1 receptor antagonist does not reduce the effect of I.C.V. administration of CRF. Acute stress induces IL-1β release in specific brain areas in rats (Nguyen *et al.* 1998). Further, Minami *et al.* (1991) have also described that immobilization stress increases IL-1β mRNA particularly in the hypothalamus, suggesting a neuronal origin of IL-1 in this model. Activation of the HPA axis by IL-1 is mediated through parvocellular neurosecretory neurons that express CRF and import central drive to pituitary adrenal output (Berkenbosch *et al.*1987; Sapolsky *et al.* 1987). However, only a few studies have evaluated the neuronal pathways involved in central IL-1 synthesis or release induced by stress. Furthermore, IL-1 is also released by glial cells in the brain (Fontana *et al.* 1992), and indirectly in a paracrine fashion, through different mediators such as prostaglandins, and can act on the neurons in order to change the neuronal activity.

Finally, all these observations highlight the idea that a long reflex pathway is required to enhance histamine MMC content in the gut without degranulation, and this pathway involves at least brain IL-1 and CRF in cascade.

The pathophysiological meaning of these results concerns the understanding of pathologies with unclear aetiology such as irritable bowel syndrome (IBS). Indeed, it has been shown that psychological factors are important in triggering IBS symptoms (Collins, 1991). Experimental stress induces a hypersensitivity of the colon in IBS patients, which is not observed in healthy volunteers (Metivier *et al.* 1996). Moreover, a previous study in rats showed that a 2 h restraint stress session triggers visceral hypersensitivity in response to rectal distension, which was suppressed by doxantrazole, a mast cell stabilizer, thus suggesting the involvement of mast cell degranulation in this nociceptive response (Gue *et al.* 1997).

These different observations are the basis of a mechanism we propose herein for events linked to stress-induced mast cell activation. First, stress-induced mast cell sensitization acts as a primer, changing mast cell mediator contents. Secondly, when a baric stimulus (such as rectal distension) is added, mast cell degranulation occurs with the release of cytogenic substances such as serotonin and SP, which sensitize the terminals of primary afferents.

Consequently, our findings indicate that an increase of histamine content in MMC without hypermastocytosis or degranulation may be an important parameter in neuro-immune communication for understanding and effectively treating the IBS pathology in which stress events exacerbate gastrointestinal clinical symptoms.

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