Pathways involved in gut mucosal barrier dysfunction induced in adult rats by maternal deprivation: corticotrophin-releasing factor and nerve growth factor interplay

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Abstract
Neonatal maternal deprivation (NMD) increases gut paracellular permeability (GPP) through mast cells and nerve growth factor (NGF), and modifies corticotrophin-releasing factor (CRF) and corticosterone levels. CRF, corticosterone and mast cells are involved in stress-induced mucosal barrier impairment. Consequently, this study aimed to specify whether corticosteronaemia and colonic expression of both preproCRF and CRF are modified by NMD, and to determine if altered expression may participate in the elevated GPP in connection with NGF and mast cells. Male Wistar rat pups were either separated from postnatal days 2–14, or left undisturbed with their dam. At 12 weeks of age, adult rats were treated with mifepristone (an antagonist of corticoid receptors), a-helical CRF(9-41) (a non-specific CRF receptor antagonist), or SSR-125543 (CRF-R1 receptor antagonist). We also determined corticosteronaemia and both colonic preproCRF and CRF expression. Then, control rats were treated by CRF, doxantrazole (mast cell stabilizer), BRX-537A (a mast cell activator) and anti-NGF antibody. NMD did not modify colonic CRF level but increased colonic preproCRF expression and corticosteronaemia. Peripheral CRF, via CRF-R1 receptor, but not corticosterone, was involved in the elevated GPP observed in these rats, through a mast-cell-mediated mechanism, since the increase of GPP induced by exogenous CRF was abolished by doxantrazole. Anti-NGF antibody treatment also reduced the elevated GPP induced by CRF or BRX-537A. CRF acts through CRF-R1 receptors to stimulate NGF release from mast cells, which participates in the elevated GPP observed in NMD adult rats. This suggests that early traumatic experience induced neuro-endocrine dysfunction, involved in alterations of gut mucosal barrier.

There is evidence that early life trauma and ongoing psychological stress can affect the clinical course of intestinal disorders (Levenstein et al. 2000; Mayer et al. 2001) and also reactivate (Collins, 2001) or enhance (Gue et al. 1997) inflammation in experimental colitis. Thus, childhood adverse events, considered as potent stressors are often associated in humans with gastrointestinal diseases such as Crohn’s disease (Ringel & Drossman, 2001) or irritable
bowel syndrome (IBS) (Hislop, 1979; Lowman et al. 1987). In animal models, neonatal maternal deprivation (NMD) has been found to predispose to colonic barrier dysfunction (Barreau et al. 2004b, 2006) and to enhance mucosal response to a mild stress (Soderholm et al. 2002).

However, the mechanisms underlying the effects of neonatal stress on the induction or exacerbation of colonic mucosal barrier dysfunction remain largely unknown. In adult rats, acute or chronic stress induces short-term intestinal alterations, through mechanisms involving corticotrophin-releasing factor (CRF) (Santos et al. 1999; Saunders et al. 2002), glucocorticoids (Meddings & Swain, 2000) and mast cells (Santos et al. 2001). In agreement with the hypothesis of CRF involvement in stress-induced immediate gut permeability alterations, it has been reported that systemic administration of CRF increases gut permeability (Santos et al. 1999). Biological actions of CRF are exerted by interacting with two distinct CRF receptors subtypes, CRF-R1 and CRF-R2, both being expressed in the gastrointestinal tract (Chatzaki et al. 2004). These receptors belong to the family of G-protein-coupled receptors signalling through cAMP synthesis (Hauger et al. 2003). Although CRF is largely produced in the hypothalamus, a peripheral synthesis has been detected in colonic mucosal cells in the neighbourhood of the base of the crypts (Kawahito et al. 1994), and its local release may play a role in the modulation of the intestinal immune system and/or other gastrointestinal functions, either basally or under stressful conditions. However, the central or peripheral origin of CRF-mediated effects of stress on the mucosal barrier is still under debate.

Among immune cells, mast cells play a critical role in the regulation of epithelial transport both in human (Crowe et al. 1997; Santos et al. 1998) and rodent intestine (Perdue et al. 1991; Berin et al. 1998), and it is widely accepted that nerve–mast cell interactions are involved in intestinal epithelial dysfunction (Argenzio, 1997; Coelho et al. 2000). Nerves and mast cells participate in the development of stress-induced increase of colonic paracellular permeability (Castagliuolo et al. 1998; Santos et al. 1999). Indeed, a mast cell stabilizer, doxantrazole, abolishes acute stress-induced ion secretion and permeability increase in the rat colon, and these effects are not observed in mast cell-deficient mice (Castagliuolo et al. 1998). However, it remains to be confirmed that mast cells are involved in the increased gut paracellular permeability (GPP) induced by CRF.

Concerning the effects of neonatal stress on mast cells, some investigations have reported that NMD increases the number of colonic mucosal mast cells (Barreau et al. 2004b), and that daily handling of new-born rats enhances the number of mast cells within thalamus nuclei in adult rats (Lafreniere et al. 2001). Moreover, we have previously shown that mast cell stabiliser doxantrazole and anti-NGF (nerve growth factor) antibody (Ab) treatment in adult rats abolished the increase in GPP induced by NMD (Barreau et al. 2004a). Thus, both mast cells and NGF play together a pivotal role in the elevated GPP observed in NMD adult rats. However, although NGF may stimulate mast cell proliferation and degranulation, the pathway involving NGF and mast cells in the regulation of GPP is unknown. Neonatal stress also affects the hypothalamic-pituitary-adrenal (HPA) axis, modifying the adrenocortical response to novelty in adult rats (Biagini et al. 1998). NMD increases supraspinal (brain) CRF expression (Husum & Mathe, 2002; Vazquez et al. 2003) associated with an altered expression of glucocorticoid receptors (Ogawa et al. 1994) in the central nervous system.

Taken together, these data suggest that even though NGF is involved, during the neonatal period, in the genesis of long-term effect of NMD, corticosterone, CRF, mast cells and NGF also play a pivotal role in the gut barrier alterations induced by NMD in adult rats. Consequently this study aimed (1) to assess in adult rats whether corticosterone and CRF are involved in the permanent alterations of GPP induced by NMD; (2) to determine the type of...
CRF receptor involved and; (3) to investigate whether CRF and mast cells are involved in cascade with NGF.

Methods
Animals
Primiparous pregnant female Wistar rats were individually housed in standard polypropylene cages containing 2.5 cm of wood chip bedding material. They were kept at a constant temperature (23±1°C) and maintained on a 12:12 h light:dark cycle (lights on at 7 am). Food (UAR pellets, Epinay, France) and water were available ad libitum. Mothers and their pups, and then the young rats after weaning on day 22, were kept in the same conditions.

Nenonatal maternal deprivation
NMD was performed according to a previously validated method (Rosztoczy et al. 2003; Barreau et al. 2004b). Briefly, the litters were culled to 10 pups after delivery (day 1). NMD was performed daily for three consecutive hours (from 0900 to 1200 h), during which pups were removed from the home cage and kept in temperature-controlled cages at 28±1°C, where bedding was changed every day. This procedure was applied between postnatal days 2 and 14. Control pups were left with their dam. From days 15–22, all control and maternally deprived pups were maintained with their dam. Weaning was performed on day 22, siblings were sex-matched, and males were selected.

Gut paracellular permeability
Assessment of GPP was performed using 51Cr-ethylenediaminetetraacetic acetic (EDTA) as a selective marker of paracellular permeation of tight junctions. Thus, 0.7 µCi of 51Cr-EDTA (Perkin Elmer Life Science, Paris, France) was diluted in 500 µl water and administered by oral route. Rats were then placed in metabolic cages, and faeces and urine were separately collected during 24 h. The radioactivity found in urine was measured by a gamma-counter (Cobra II; Packard, Meriden, CT, USA). Permeability to 51Cr-EDTA was expressed as a percentage of the total administered radioactivity.

PreproCRF immunohistochemistry
Under anaesthesia (i.p. administration of 0.6 mg kg-1 acepromazine (Vetoquinol, Lure, France) and 120 mg kg-1 ketamine (Rhone-Méérieux, Lyon, France)), rats were exsanguinated by beheading, and a 2 cm-long portion of the colon was excised and washed in sterile saline. The collected fragments were fixed in Duboscq-Brazil solution for 24 h, dehydrated in ethanol solution, embedded in paraffin blocks, and cut into 5 µm sections. Paraffin sections were rehydrated and submerged in antigen retrieval solution (citrate buffer, 10mm, pH6, 95°C, 3 min). After inhibition of endogenous peroxidases with 0.6% H2O2 in 100% methanol for 30 min, and incubation in blocking solution (phosphate-buffered saline containing 1% bovine serum albumin and 2% normal donkey serum), sections were incubated with goat anti-preproCRF antibody (Santa Cruz, Le Perray en Yvelines, France) (1/100; overnight, +4°C) followed by a biotinylated donkey anti-goat IgG immune serum (Interchim, Montluçon, France) 1/1000; 30 min, RT. Slides were subsequently incubated with ABC complexes coupled to peroxidase (Abcys, Paris, France). 3-3′diaminobenzidine (ICN Pharmaceuticals, Costa Mesa, CA, USA) was used as chromogen. Immunoreactivity was graded from 0 to 3 indicating the degree of preproCRF expression (0, none; 1, weak; 2, intermediate; 3, strong). Grading was done in a blind fashion.

Corticosterone assay
Plasma samples were stored at-20°C until assayed. Plasma corticosterone concentrations were determined using an adapted high-performance liquid chromatography (HPLC) after a solid/liquid extraction on C8 cartridge and in the presence of internal standard (flumethasone 2.5 µg/ml). An Inertsil ODS 33 µm (150×4.0 mm) column was eluted by H2O/methanol
(50/50; v/v) mixing, and a 0.5 ml min-1 rate of flow was used to separate cortisol and corticosterone. The corticosterone detection was performed at 254 nm and the quantification limit of the method was 25 ng ml-1.

Quantification of NGF release under CRF stimulation
Under anaesthesia (i.p. administration of 0.6 mg kg-1 acepromazine (Vetoquinol, Lure, France) and 120 mg kg-1 ketamine (Rhone-M’erieux, Lyon, France)), rats were exsanguinated by beheading and four segments (0.5 cm) of distal colon were immediately taken from each animal. Two colonic pieces were used to investigate the CRF-induced NGF release, and two others for basal NGF release. Each segment was incubated for 15 min at 37°C, in a Tyrode solution (composition, (mm): NaCl 136.9, KCl 2.68, CaCl2 1.8, MgCl2 1.05, NaHCO3 1.19, NaH2PO4 0.42 and glucose 0.55). Then Tyrode solution containing CRF (10 µg ml-1) or CRF-vehicle was added, and NGF released was determined for 1 h. NGF levels in the supernatant were measured by ELISA. Briefly, anti-NGF polyclonal Ab, which binds soluble NGF, was used at 100 µl well-1. The sample incubation was 6 h at room temperature. The captured NGF is bound by a second specific monoclonal antibody (mAb). After washing, the amount of specifically bound mAb is detected using an anti-rat IgG conjugated to HRP. After, incubation with a chromogenic substrate, the colour change is measured at 450 nm after reaction was stopped. All these products were obtained from Promega (Lyon, France). NGF levels were expressed in pmol (ml supernatant)-1.

Quantification of colonic mucosal CRF levels
Under anaesthesia (i.p. administration of 0.6 mg kg-1 acepromazine (Vetoquinol, Lure, France) and 120 mg kg-1 ketamine (Rhone-M’erieux, Lyon, France)), rats were exsanguinated by beheading, and segments (4 cm) of distal colon were immediately taken from each animal. Mucosa was recovered and homogenized in PBS buffer supplemented with anti-protease cocktail (Roche, Meylan, France). Sample protein content was determined by a modified Lowry-based assay (Bio-Rad, Marnes-La-Coquette, France). CRF levels were determined by ELISA (CosmoBio, Tokyo, Japan) according to the manufacturer’s instructions. CRF was quantified against a CRF standard curve and expressed as ng (mg protein)-1.

Experimental protocol
All experiments were performed in 12-week-old male rats. In a first set of experiments, four groups of eight control and eight NMD rats were used. In a first group of rats, colonic preproCRF immunoreactivity, mucosal colonic CRF level (distal segment) and corticosterone plasma level were determined. Rats (control and NMD) were both anaesthetized (10 AM) by i.p. administration of 0.6 mg kg-1 acepromazine (Vetoquinol, Lure, France) and 120 mg kg-1 ketamine (Rhone-M’erieux, Lyon, France), and both aortic blood and colonic samples were collected. Then rats were killed by cervical dislocation.

A fourth group of rats received oral SSR-125543 (a selective CRF-R1 antagonist (Griebel et al. 2002); 10 mg kg-1 in 0.5 ml) 6 h and 1 h before assessing GPP. Another group of rats was used to evaluate the release of NGF from colonic strips in basal condition and under CRF stimulation.

In a second set of experiments, six groups of eight control rats were used.
Group 1 received two i.p. administrations of CRF 1 h before and 6 h after starting the permeability measurement.

Group 2 was treated by two i.p. administrations of doxantrazole (mast cell stabilizer; 5 mg kg\(^{-1}\) in 0.2 ml; 6 h and 1 h before permeability measurement) and CRF (50 µg kg\(^{-1}\) in 0.2 ml; 1 h before and 6 h after starting the permeability measurement).

Group 3 received two administrations of doxantrazole (5 mg kg\(^{-1}\) in 0.2 ml; 6 h and 1 h before permeability measurement).

Group 4 was treated by two i.p. administrations of anti-NGF antibodies (15 µg kg\(^{-1}\) in 0.2 ml; 6 h and 1 h before permeability measurement) and CRF (50 µg kg\(^{-1}\) in 0.2 ml; 1 h before and 6 h after starting the permeability measurement).

Group 5 received two i.p. administrations of BRX-537A (2 mg kg\(^{-1}\) in 0.2 ml; 1 h before and 6 h after starting the permeability measurement).

Group 6 was treated by two i.p. administrations of anti-NGF antibodies (15 µg kg\(^{-1}\) in 0.2 ml; 6 h and 1 h before permeability measurement) and BRX-537A (2 mg kg\(^{-1}\) in 0.2 ml; 1 h before and 6 h after starting the permeability measurement).

All experimental protocols described in this study were approved by the local Institutional Animal Care and Use Committee.

**Drugs**

Doxantrazole was kindly supplied by Wellcome Research laboratories (Beckenham, UK), and was dissolved in 5% sodium bicarbonate. Mifepristone was a gift from Roussel-Uclaf (Paris, France), and was dissolved in olive oil. \(\alpha\)-helical CRF(9-41) was purchased from Fisher (Illkirch, France), and was diluted in sterile saline solution. CRF was purchased from Calbiochem (VWR, Fontenay-sous-bois, France), and was diluted in saline solution. SSR-125543 was kindly supplied by Sanofi-Aventis, and was dissolved in DMSO 5% and cremophor-EL 5%. Anti-NGF Ab was purchased from R&D Systems (R&D, Lille, France), and was diluted in sterile saline solution. BrX-537A (bromolasalocid ethanolate) was kindly supplied by Roche Laboratories (London, UK) and was dissolved in DMSO.

**Statistical analysis**

Results are expressed as means±s.e.m. Multiple groups were compared by Dunnett’s test after one-way ANOVA. Single comparisons were performed by non-parametric test (unpaired Student’s t test) for statistical analysis of CRF immunoreactivity and CRF effects on gut paracellular permeability. Differences were considered significant for P <0.05.

**Results**

**Corticosterone and maternal deprivation**

In control rats, the corticosterone plasma concentration was 90±9 ng ml\(^{-1}\). In NMD rats, this value was significantly increased to 138±17 ng ml\(^{-1}\) (P <0.05) (Fig. 1A). In NMD rats, GPP was significantly increased in comparison with control rats (4.1±0.2% versus 2.7±0.3%; P <0.05). However, mifepristone (4mg kg\(^{-1}\); s.c.) did not modify GPP in either control or NMD rats (P >0.05) (Fig. 2).

**PreproCRF, CRF and maternal deprivation**

An intense preproCRF labelling of enterocytes was observed in both control and NMD rats (Fig. 1B), while labelling of colonic bottom crypts and submucosa was weaker. In NMD rats, preproCRF immunoreactivity was significantly increased (P <0.05) in colonic mucosa, lamina propria layers and colonic bottom crypts, in comparison with control rats (Table 1 and Fig. 1B). Surprisingly, as presented in Fig. 1C, no difference of CRF colonic mucosal levels was
observed between control and NMD rats (3.9±0.4 ng (mg protein)-1 versus 4.2±0.2 ng (mg protein)-1; P >0.05).

In NMD rats, an increased GPP was observed (3.9±0.3% versus 2.4±0.2%; P <0.05), both a-helical CRF(9-41) and SSR-125543 suppressed the increase in GPP induced by maternal deprivation (P <0.05) (Fig. 3A and B). In control rats, GPP was not modified by either a-helical CRF(9-41) or SS-125543 treatment (P >0.05).

**CRF and mast cells**

In control rats, CRF (50 µg kg-1) increased GPP (3.9±0.3% versus 2.1±0.2%; P<0.05). Doxantrazole treatment abolished the increase of GPP induced by CRF (2.6±0.4% versus 3.9±0.3%; P <0.05), but had no effect under basal conditions (1.9±0.2% versus 2.1±0.2%; P >0.05) (Fig. 4).

![Figure 1](image)

**NGF, CRF and mast cells**

In control rats, CRF (50 µg kg-1) increased GPP (3.9±0.3% versus 1.6±0.2%; P<0.05) (Fig. 5). Anti-NGF Ab treatment reduced significantly the increase of GPP induced by CRF administration (2.8±0.3% versus 3.9±0.3%; P <0.05). However, the GPP observed after CRF plus anti-NGF Ab treatments, remained elevated compared with control rats (2.8±0.3% versus 1.6±0.3%; P <0.05) (Fig. 5). Moreover, anti-NGF-neutralizing antibody treatment did not modify the GPP in adult control rat (data not shown; P >0.05).

Similarly, mast cell stimulation by BRX-537A increased GPP (4.1±0.4% versus 1.8±0.2%; P <0.05) (Fig. 6). Anti-NGF Ab treatment reduced the increase of GPP induced by BRX-537A (3.1±0.4% versus 4.8±0.4%; P <0.05). However, the GPP still remained higher than that observed in control rat (3.1±0.4% versus 1.8±0.2%; P <0.05) (Fig. 6).
In basal condition, the spontaneous release of NGF from colonic strips of NMD and control rats was measured in the cleared supernatant obtained after 1 h of incubation (Fig. 7). The NGF amount released by colon sample from NMD rats was significantly higher (1073.2±48.4 ng ml-1 versus 514.3±20.6 ng ml-1; P < 0.01) than that of control rats. On colonic strips, CRF stimulated NGF release with a greater response in NMD than in control rats (1413.2±26.1 ng ml-1 versus 843.3±113.7 ng g-1; P < 0.001) (Fig. 7).

Figure 2. Effect of mifepristone on the increased GPP induced by NMD in adult rats
Control (left) and NMD rats (right) were treated by vehicle (open bars) or mifepristone (filled bars). Values are means ± S.E.M.; n = 8/group. *P < 0.05 versus vehicle controls.

Table 1. Effect of neonatal maternal deprivation on colonic preproCRF immunohistochemistry in adult rats

<table>
<thead>
<tr>
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<th>Control</th>
<th>NMD</th>
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<tr>
<td>Submucosa</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.2</td>
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<tr>
<td>Lamina propria</td>
<td>0.2 ± 0.2</td>
<td>1.1 ± 0.3*</td>
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<tr>
<td>Enteroendocrine</td>
<td>1.1 ± 0.3</td>
<td>2.4 ± 0.4*</td>
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<tr>
<td>Colonic bottom crypts</td>
<td>0.6 ± 0.4</td>
<td>1.5 ± 0.2*</td>
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<tr>
<td>Total CRF</td>
<td>2.5 ± 0.5</td>
<td>5.9 ± 0.7*</td>
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Results were obtained from at least six different rats from control and NMD groups. Data (mean ± S.E.M.) are expressed as the total scoring, graded from 0 to 3 (0, no immunoreactivity; 1, weak immunoreactivity; 2, intermediate immunoreactivity; 3, strong immunoreactivity). *P < 0.05 from control (Student’s t test).

Discussion
This report demonstrates that NMD both increases corticosteronaemia and preproCRF immunoreactivity in the different colonic mucosa areas, but does not modify colonic mucosal levels of CRF in adult NMD rats.
Moreover, CRF but not corticosterone plays a major role in long-term alterations of the gut epithelial barrier triggered by NMD. This is supported by our data showing that the effects of NMD are suppressed by treatment of adult rats with α-helical CRF(9-41) or a CRF-R1 receptor antagonist, but not by the glucocorticoid/progesterone receptor antagonist mifepristone. We demonstrate for the first time that CRF favors the release of NGF from colonic mast cells, which in turn is responsible for the increase of GPP. Indeed, doxantrazole treatment abolishes the increased GPP induced by CRF, and anti-NGF Ab reduces the CRF or BRX-537A-induced increase of GPP. This result is confirmed by the observed greater release of NGF in response to CRF in adult NMD rats.

The neonatal period, roughly extending in rats from birth to day 14, is often referred to as a stress hyporesponsive period characterized by a diminished ACTH (adrenocorticotrophic hormone) and corticosterone response to most stressors (Rosenfeld et al. 1991).
Although NMD is known to affect the HPA axis function (Levine, 2001), the effects of NMD on plasma corticoid levels are in opposition. These differences may be explained by the rodent strain (Ellenbroek & Cools, 2000), sex (Wigger & Neumann, 1999; Barna et al. 2003), or the period where NMD (Van Oers et al. 1998) is applied (chronic or acute). In this study we have shown that NMD increased plasma corticosterone in adult rats. Several hypotheses may be considered. Firstly, NMD induced NGF overexpression in colonic tissue of adult rats (Cirulli, 2001; Barreau et al. 2004a). Since NGF promotes adrenal gland hypertrophy (Bigi et al. 1992; Alleva & Santucci, 2001) and glucocorticoid secretion (Otten et al. 1979, 1981), NGF overexpression may also stimulate corticosterone synthesis and accumulation in the blood. Secondly, NMD rats exhibited hyperresponsiveness to psychological stressors as compared to controls (Caldji et al. 2000; Ladd et al. 2000). This hyperresponsiveness results from a resistance to glucocorticoid-mediated negative feedback (Caldji et al. 2000; Ladd et al. 2000), which may participate in the elevated corticosteronemia. Thirdly, since NMD triggers bacterial translocation (Barreau et al. 2004b), leading to a downstream activation of the HPA axis (Ando et al. 2000), bacterial translocation induced by NMD may participate to increase corticosteronaemia in NMD rats. Recently, it was shown that acute stress increases plasma corticosterone, which in turn increases GPP (Meddings & Swain, 2000). However, in NMD rats, glucocorticoid receptor antagonist treatment failed to affect GPP. Consequently, a direct involvement of corticoids in the increased GPP can be excluded.
By contrast with glucocorticoids, the effects of NMD on CRF release and CRF receptor expression were extensively investigated in the central nervous system. Maternal deprivation induces a significant increase of CRF, CRF-R1 and CRF-R2 mRNA expression in the cortex, and a decrease of CRF-R1 and CRF-R2 mRNA expression in the amygdala (Vazquez et al. 2003). Although CRF secretion is mainly localized in the central nervous system, a colonic CRF production has been detected in the mucosal cells in the neighbourhood of the base of the colonic crypts (Kawahito et al. 1994), and an elevated colonic CRF expression has been observed in biopsies of patients with ulcerative colitis (Kawahito et al. 1995). In agreement with these previous studies, in the present study we have reported that both preproCRF and mature CRF are present within the colonic mucosa. We have also shown for the first time that NMD increases preproCRF expression in several colonic mucosal areas, while it does not modify colonic mucosal CRF content in adult rats. This differential effect of NMD on colonic mucosal preproCRF and CRF levels may be explained by the fact that NMD may only alter preproCRF synthesis without modifying the rate of maturation of preproCRF into CRF. In addition, colonic content of CRF may result from both central and peripheral production of CRF. Consequently, this can explain the lack of relationship between preproCRF expression and tissue CRF concentration.

CRF acts within the brain and pituitary to coordinate the overall endocrine and behavioural stress response. CRF is known to be involved in various stress-induced abnormalities in the gastrointestinal tract (Castagliuolo et al. 1996; Santos et al. 1999; Tache et al. 2001). A peripheral injection of CRF mimics stress-induced changes in colonic function regarding mucin release (Castagliuolo et al. 1996), ion secretion and permeability (Santos et al. 1999). Moreover, physical and psychological stress in rats enhanced colonic epithelial permeability via peripheral CRF (Saunders et al. 2002). In this context, in this study we have shown that CRF plays an important role in the elevated GPP induced by NMD through peripheral and/or central CRF-R1 receptor. Intraperitoneal a-helical CRF-(9-41) or SSR-125543 administration suppress the stress-induced increase of GPP. Furthermore, the suppressive effect of doxantrazole treatment on the increased GPP induced by CRF points out the involvement of mast cells. Because we have previously shown that colonic mucosa from adult NMD animals exhibit an elevated number of mast cells (Barreau et al. 2004b), we can also hypothesize that for a similar CRF colonic content, more mast cells may be activated and, consequently, larger amounts of mediators participating in the elevated GPP induced by NMD can be released.

As mast cells synthesize and release NGF (Leon et al. 1994), and NGF is known to be involved in the elevated GPP induced by NMD (Barreau et al. 2004a), we investigated CRF, NGF and mast cell interplay in the regulation of GPP. Firstly, we have shown that NGF...
release from mast cells under CRF and BRX-537A (mast cell activator) stimulation participates in the increased GPP in control rats. Indeed, anti-NGF Ab treatment significantly reduces the increase of GPP induced by CRF and BRX-537A. Secondly, in this study we have shown that CRF stimulation triggers a greater NGF release from colon biopsies of NMD rats than in control animals. Consequently, these data also support that NGF release from mast cells under CRF stimulation participates in the increased GPP in rats. However, the NGF receptor subtype (TrKa and P-75) (Vega et al. 2003) and cell type (nerve ending, epithelial cell, lymphocyte . . .) (Vega et al. 2003) involved in NGF-mediated increase of GPP remain unclear. Indeed, NGF released from mast cell may directly interfere with receptors located on epithelial cells or on nerve endings, stimulating the release of mediators or stimulating immune cells (lymphocytes) (Ferrier et al. 2003), which in turn increase GPP. Nevertheless, since anti-NGF Ab only partially lower the increase of GPP induced by CRF or BRX-537A, it can be suggested that other mediators such as rat mast cell protease II, cytokines (IL-4, IFNγ ) or neuromediators (substance P) are also involved.

In summary, this study shows that NMD promotes long-term alterations of colonic preproCRF expression and corticosteronaemia level in adult NMD rats. CRF may interact with the CRF-R1 receptor to stimulate mast cell release of NGF, which in turn participates in the increased GPP in adult NMD rats. These results also provide evidence that adverse experiences in early life can induce neuroendocrine changes (HPA axis, NGF) associated with alterations of gut mucosa physiology in adult rats, and could have implications in the development of intestinal disorders such as IBS.

References


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