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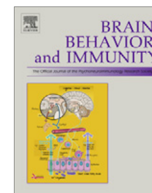

Chronological assessment of mast cell-mediated gut dysfunction and mucosal inflammation in a rat model of chronic psychosocial stress

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ABSTRACT

Life stress and mucosal inflammation may influence symptom onset and severity in certain gastrointestinal disorders, particularly irritable bowel syndrome (IBS), in connection with dysregulated intestinal barrier. However, the mechanism responsible remains unknown. Crowding is a validated animal model reproducing naturalistic psychosocial stress, whose consequences on gut physiology remain unexplored. Our aims were to prove that crowding stress induces mucosal inflammation and intestinal dysfunction, to characterize dynamics in time, and to evaluate the implication of stress-induced mast cell activation on intestinal dysfunction. Wistar-Kyoto rats were submitted to 15 days of crowding stress (8 rats/cage) or sham-crowding (2 rats/cage). We measured spontaneous and corticotropin-releasing factor-mediated release of plasma corticosterone. Stress-induced intestinal chrono-pathobiology was determined by measuring intestinal inflammation, epithelial damage, mast cell activation and infiltration, and intestinal barrier function. Corticosterone release was higher in crowded rats throughout day 15. Stress-induced mild inflammation, manifested earlier in the ileum and the colon than in the jejunum. While mast cell counts remained mostly unchanged, piecemeal degranulation increased along time, as the mucosal content and luminal release of rat mast cell protease-II. Stress-induced mitochondrial injury and increased jejunal permeability, both events strongly correlated with mast cell activation at day 15. Taken together, we have provided evidences that long-term exposure to psychosocial stress promotes mucosal inflammation and mast cell-mediated barrier dysfunction in the rat bowel. The notable resemblance of these findings with those in some IBS patients, support the potential interest and translational validity of this experimental model for the research of stress-sensitive intestinal disorders, particularly IBS.

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1. Introduction

The increasing incidence of stress-related morbidity in modern societies is in close dependence with raised people expectations, seemingly endless, as a mandatory toll to overcome everyday life confrontation against ubiquitous environmental, psychosocial, and economic determinants (Chrousos, 2009). Relevant to digestive disorders, there is now compelling evidence for the modulatory role of physical and psychological stresses, whether acute or chronic, in shaping the clinical course of a number of functional and inflammatory conditions of the gastrointestinal tract (Maunder and Levenstein, 2008). In particular, epidemiological, empirical and clinical observations provide valuable support for life stress as a common co-morbid event in the irritable bowel syndrome (IBS), which may strongly influence symptom onset, severity, and persistence in certain IBS subtypes (Bennett et al., 1998; Faresjo et al., 2007; Nicholl et al., 2008; Palsson and Drossman, 2005). Furthermore, intestinal dismotility, visceral hyperalgesia, reactivation of mucosal inflammation, and epithelial dysfunction have been all related to stress episodes in post-infectious and diarrhea-predominant IBS (Fukudo et al., 1998; Mönnikes et al., 2001; Murray et al., 2004). However, the mechanisms by which stress impacts on IBS pathophysiology remain to be fully established. In this regard, the development of animal models reproducing stress-related gastrointestinal dysfunction has lately evolved as a helpful approach to improve our understanding on IBS pathophysiology (Kiank et al., 2010). Relevant experimental models include studies

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in naturally or genetically modified stress-sensitive rodent species and the use of paradigms such as neonatal maternal deprivation (Levine, 1967; Söderholm et al., 2002b), water avoidance stress (Santos et al., 2000), and intestinal infection (McLean et al., 1997) or irritation (Al-Chaer et al., 2000; Collins et al., 1996), in both early life and adulthood. From these models we have learned that single acute or repetitive exposure to homotypic stresses of different nature leads to intestinal pathobiology revealed as increased ion secretion, macromolecular permeability, microscopic inflammation, visceral hypersensitivity, dysmotility, and even bacterial penetration (Collins and Bercik, 2009; Santos et al., 2008b; Söderholm et al., 2002a). In addition, these responses involve dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and are mediated by stress-related neuropeptides such as corticotropin-releasing factor (CRF), neural mechanisms and mast cells (Santos et al., 2008a; Keita et al., 2010), a profile which has been also described in IBS patients (Guilarte et al., 2007).

Despite much information has been gathered we still lack detailed information on the time course of specific stress-related mucosal alterations in the small and the large intestine of these animals. Furthermore, the vast majority of laboratory stress protocols applied for the study of intestinal disorders have been criticized for the use of repetitive homotypic stimuli. The predictive validity of those models remains disputed because in socially organized mammals, dominant stressors in natural conditions are frequently heterotypic and complex and best represented by social experiences (Tamashiro et al., 2005). Crowding stress (CS) is a well-known validated rat model of chronic social stress that might adjust to the type and intensity of stressors experienced by these animals in their natural environment on a daily basis (Armario et al., 1984) and may also reflect well life stress for humans. Several metabolic, endocrine, and HPA axis disturbances including changes in body temperature, food intake and body weight gain, have been reported in response to CS (Bhatnagar et al., 2006). However, the effect of CS on gastrointestinal function remains largely unexplored. Therefore, the goals of the present study were to determine the ability of long-term CS to provoke functional and structural abnormalities in the rat intestine, to characterize the dynamics of these responses, and to evaluate its potential as a model for the study of IBS pathophysiology.

2. Material and methods

2.1. Animals

Male Wistar-Kyoto rats (175–200 g on arrival, Iffa Credo Laboratories, L'Arbresle, Lyon, France) were maintained on a normal 12:12 h dark:light cycle (0700/1900 h) and provided with food and water *ad libitum*. Rats were allowed for acclimation for 2 weeks before the experimental procedures. Procedures were approved by the Animal Care Committee at Institut de Recerca Vall d'Hebron, and performed in accordance with the European Union Directive for the Protection of Vertebrate Animals used for experimental and other scientific purposes (86/609/EEC).

2.2. Stress protocol

Rats were randomly assigned to one of the following experimental protocols and kept in separate housing areas: CS, in which groups of eight animals were housed together in standard cages ($48 \times 23 \times 14$ cm) half-filled with bedding for up to 15 consecutive days to increase their social stress; sham-crowding (SC), in which control rats were housed in pairs for equal time in cages containing hollow plastic cylinders to minimize environmental stress. Experiments were performed between 9 and 12 AM to control the effect of circadian rhythm.

2.3. HPA axis activity

The efficacy of CS and SC to switch on the stress HPA axis circuitry and adaptational changes were assessed by measuring the spontaneous release of plasma corticosterone at days 1, 3, 7, 12 and 15. The residual capacity of the HPA axis to respond to additional stimuli was determined by measuring corticosterone release at day 15, 30 minutes after intraperitoneal (i.p.) administration of vehicle (0.9% NaCl) or CRF (10 μg/kg, Sigma, Barcelona, Spain). Rats were euthanized by decapitation and truncal blood was collected into chilled tubes containing EDTA (1.5 mg/mL, Sarsted, Barcelona, Spain). Plasma corticosterone concentration was measured with an immunoassay kit (IDS Ltd., Boldon, UK).

2.4. Assessment of stress responses in the rat intestine

Rats were euthanized at day 1, 3, 7, 12, or 15, and the entire intestine excised. Thereafter, the small intestine and the colon were half-divided into proximal and distal segments, and coded for blind evaluation. Stress-induced intestinal chrono-pathobiology was determined using selected histophysiological indicators.

2.4.1. Myeloperoxidase (MPO) activity

We measured MPO activity, an index of neutrophil infiltration, in mucosal scrapings as previously described (Vicario et al., 2005). MPO is expressed as U/g wet tissue.

2.4.2. Transmission (TEM) and scanning (SEM) electron microscopy

Transmural intestinal fragments (jejunum, ileum, proximal colon and distal colon) were fixed for 4 h in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), postfixed for 2 h in 1% (w/v) osmium tetroxide containing 0.8% (w/v) of potassium hexacyanoferrate, and dehydrated through a graded acetone series. TEM samples were infiltrated in Epon's resin, polymerized, contrasted, and examined using a Hitachi H-7000 microscope at 75 kV equipped with a MegaView III camera (Soft Imaging System). After critical point drying with CO_2, SEM samples were mounted on metallic tubs and coated with gold, examined under a SEM Hitachi S-570 (15 kV) device, and images were captured with the Quartz PCI v.5.5. Chemicals (electron microscopy grade) were obtained from Sigma. Images were used to evaluate epithelial damage, with special focus on mitochondrial abnormalities including enlargement, swelling, loss of cristae, and vacuolisation, under a fixed size observation window of 300 μm^2. Intact and damaged mitochondria were quantified and results expressed as the percentage of intact mitochondria per area in each segment. In addition, we evaluated qualitative differences and changes in cellular density on the luminal surface of intestinal tissues along time.

2.4.3. Flow cytometry

We next examined the cellular content in the lumen of the small intestine of SC and CS rats at day 15. After an overnight fast, the entire small intestine (containing the jejunum and the ileum) was excised and gently flushed with 50 mL of RPMI (Gibco, Barcelona, Spain). Luminal content was centrifuged (200g, 5 min, 4 °C) to eliminate pelleted debris. Cells were purified by gradient centrifugation with Percoll (Amersham Biosciences, Uppsala, Sweden). Cell suspension was washed, resuspended in PBS (pH 7.4) containing 2% (v/v) fetal calf serum and 0.5 g/L sodium azide, and cell counting and viability (>90% in all cases) were determined by trypan blue exclusion (Sigma). Double-color immunofluorescence was performed by incubating with monoclonal mouse anti-rat antibodies CD45-PE-conjugated and CD3-FITC-conjugated (Becton Dickinson,

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