Endothelin-1 induces mucosal mast cell degranulation and tissue injury via ET\textsubscript{A} receptors

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ABSTRACT

The effects of endothelin-1 (ET-1) on mucosal mast cells are of special interest, since they may be an important component of the tissue response that occurs during ischaemic preconditioning or ischaemia/re-oxygenation injuries. Increasing doses of ET-1 were administered intravenously to anaesthetized rats. In a second series of experiments, animals were pretreated with the ET\textsubscript{A} receptor antagonists BQ-610 or ETR-P1/fl peptide, or with the ET\textsubscript{B} receptor antagonist IRL-1038. Intestinal perfusion changes were recorded, and the proportion of degranulated mast cells and the degree of mucosal damage were determined in ileal biopsies. ET-1 induced dose-dependent alterations in the haemodynamic and morphological parameters, and caused significant mast cell degranulation. These changes were inhibited significantly by pretreatment with the ET\textsubscript{A} receptor antagonists, but not with the ET\textsubscript{B} receptor antagonist. We conclude that a cross-talk exists between endothelial cell-derived humoral mediators and the intestinal mast cell system.

INTRODUCTION

The mucosal mast cells (MCs) of the gastrointestinal tract are a unique cellular source of both preformed and \textit{de novo} synthesized mediators. They are located mainly around postcapillary venules, from where they can influence local tissue reactions [1]. Intestinal MCs have both ET\textsubscript{A} and ET\textsubscript{B} receptors on their membrane surface, and this suggests a possible cross-talk between endothelial cell-derived humoral mediators and the MC system [2,3].

One of the early events in low-flow conditions is the production of activators of secondary circulatory responses. There is a growing body of evidence that, in addition to the role of endothelin-1 (ET-1) as a dominant vasoconstrictor [4], this peptide may also influence the biological activity of other cell types in the cardiovascular system, including polymorphonuclear leucocytes [5]. The effect of ET-1 on MCs is of special interest, since it may also be an important component of the tissue response that occurs in the mucosa during inflammation or ischaemia/reperfusion injuries.

Our studies were directed towards an examination of whether and how intestinal MCs respond to increasing doses of exogenously administered ET-1. To this end, ET-1-induced mucosal morphological changes were correlated with the degree of MC degranulation. Secondly, we used ET\textsubscript{A}- and ET\textsubscript{B}-receptor-selective antagonists to investigate the roles of these receptor subtypes in mediating ET-1-induced intestinal MC activation.

MATERIALS AND METHODS

Animals
The experiments were performed in accordance with U.S. National Institutes of Health guidelines on the use...
of experimental animals. A total of 42 male Sprague-Dawley rats (body weight 200 ± 20 g) were deprived of food, but not water, for 12 h prior to the experiments. The animals were anaesthetised with sodium pentobarbital (60 mg/kg, intraperitoneal). The left carotid artery and jugular vein were cannulated for the recording of mean arterial pressure and the injection of test compounds respectively. Throughout the experiment, the animals received an infusion of Ringer’s lactate at a rate of 40 ml · kg⁻¹ · h⁻¹. After a transverse laparotomy, a segment of the terminal ileum perfused by a single artery was selected. The marginal vessels were divided and ligated, and the intestinal segment with intact neu-ovascular connections was covered by plastic sheets.

**Experimental protocol**

In the first series of experiments, dose responses to ET-1 (Alexis Corp., Läufelfingen, Switzerland) were obtained. The animals were randomly allotted into the following groups: group 1, sham-operated (n = 6); group 2, 0.1 nmol/kg ET-1 (n = 5); group 3, 1 nmol/kg ET-1 (n = 5); group 4, 3 nmol/kg ET-1 (n = 6). At 30 min after the end of baseline measurements, a solution of 0.1 ml of ET-1 or vehicle was infused intravenously into the systemic circulation over 15 min. In an additional group of animals (n = 5) segmental intestinal ischaemia was induced by a 15-min occlusion of the ileal artery.

In the second series of experiments, an ET₄ receptor antagonist (ETR-P1/ll peptide (Kurabo Ltd, Osaka, Japan) or BQ-610 [homopiperidinyl-carbonyl-Leu-D-Trp(CHO)-D-Trp-OH, Alexis Corp.]), or the ET₄ receptor antagonist IRL-1038 ([Cys¹¹,Cys¹⁸]endothelin-1(11–21)), was infused intravenously (0.3 μmol/kg) for 30 min, followed by a 15-min infusion of ET-1 into the systemic circulation after the end of BQ-610, ETR-P1/ll peptide or IRL-1038 pretreatment. The circulatory changes were observed for 60 min, and at the end of the observation period a tissue sample was taken from the intestinal segment.

**Histology**

Intestinal biopsy samples were placed into Carnoy’s fixative and trimmed along the longitudinal axis. The samples were embedded in paraffin, sectioned (6 μm) and stained with haematoxylin/eosin and Alcian Blue/safranin O (pH 0.4). An image analysis system (IVM; Pictron Kft., Budapest, Hungary) was used to digitize the x and y coordinates of the sections. Three non-overlapping fields were processed in each section, and the average height of a single villus was measured from its origin to the villus tip. Mucosal damage was assessed according to the standard scale of Chiu et al. [5a]. The grading was performed with the following criteria: grade 0, normal mucosa; grade 1, development of subepithelial space at the tip of the villus; grade 2, extension of the space with epithelial lifting; grade 3, massive epithelial lifting; grade 4, denuded villi; grade 5, disintegration of the lamina propria. Positively stained MCs were quantified in the villi of an average of 20 villus-crypt units. Counting was performed in coded sections at ×400 optical magnification by one investigator. Loss of intracellular granules, with stained material dispersed diffusely within the lamina propria, was taken as evidence of MC degranulation.

**Statistics**

The Friedman test followed by Dunnett’s method was applied for multiple comparisons with a control. Differences between groups were analysed by Kruskal-Wallis one-way ANOVA on ranks. P values of < 0.05 were considered significant. Mean values ± S.D. are given.

**RESULTS**

The resting haemodynamic parameters were similar in each of the groups studied (results not shown). In the sham-operated group, the villus MC count was unchanged, and no significant increase in degranulation was observed in biopsies taken at the end of the observation period. A significant, dose-dependent diminution of villus height was induced by ET-1 infusion as compared
Endothelin-1 and mucosal mast cell degranulation

**Figure 2** ET-1-induced mucosal damage
Upper panel: grading of mucosal damage after infusion of 0.1, 1 or 3 nmol/kg ET-1, or after 15 min of ischaemia/60 min of reperfusion (I-R). Significance of differences: *P < 0.05 compared with sham-operated group. Lower panel: mucosal damage in rats that received pretreatment with an ET receptor antagonist.

with the control group. The shortening of the villi was statistically significant after the administration of 1 or 3 nmol/kg ET-1, and there was a significant difference in this parameter between the 0.1 and 3 nmol/kg ET-1 groups (Figure 1, upper panel). The MC degranulation ratio exhibited a significant increase after ET-1 treatment. The ET-1 infusions elevated the proportion of degranulated MCs almost 2-fold in each of the ET-1-treated groups (Figure 1, lower panel). Simultaneously, mucosal alterations, as assessed on the Chiu scale, were statistically different from the control in the 1 and 3 nmol/kg ET-1 groups (Figure 2).

The ET_A receptor antagonists ETR-P1/fl peptide and BQ-610 attenuated the ET-induced villus shortening and mucosal damage (Figure 3, upper panel). Similarly, MC degranulation was significantly inhibited by pretreatment with the ET_A receptor antagonists (Figure 3, lower panel). Administration of the ET_B receptor antagonist IRL-1038 did not influence the MC degranulation and morphological alterations induced by 3 nmol/kg ET-1.

**DISCUSSION**

Previous studies have revealed that even a short period of intestinal arterial occlusion leads to structural damage to the mucosal layer and triggers the discharge of a variety of MC-derived inflammatory mediators into the mesenteric circulation [1]. The major finding of the present study is that the vasoconstrictor mediator ET-1 induces intestinal mucosal damage, and concomitantly exerts significant effects on MC degranulation via ET_A receptors. Pretreatment with ET_A receptor antagonists was effective in reducing the morphological signs of ET-1-induced structural damage.

Depending on the localization of the ET_A receptors, at least three possible mechanisms may be hypothesized to account for the observed results. (1) One possibility is a direct effect of ET-1 on MCs. This notion is supported by data demonstrating ET_A receptor expression on the surface of MCs in the rat [2,3]. A direct interaction between MC-degranulating peptide and G-proteins in MCs has also been reported [6]. Given the very close structural similarities between ET-1 and MC-degranulating peptide, a similar interaction between ET-1 and G-proteins in MCs might be suggested. (2) The profound ET_A-receptor-mediated microvascular vasoconstriction and the ensuing ischaemic injury could be another plausible explanation for the observed MC degranulation. In this case, structural injury to the small intestinal mucosa may be connected directly to the
haemodynamic consequences of ET administration. The mucosal lesions observed in the present study were similar to those described in animal models of intestinal ischaemia/reperfusion or after administration of nanomolar doses of exogenous ET-1 [7]. In this case, tissue hypoxia, or oxygen-derived free radicals generated during local ischaemia/reperfusion injury, could also be MC-degranulating factors. (3) Another explanation may be provided by the alteration to the osmolarity of the intestinal mucosa that occurs as a result of localized perivascular oedema. The fragility of the MC membranes under conditions of osmotic stress has been demonstrated. Indeed, Filep et al. [8] have shown that ET-1 causes dose-dependent increases in vascular permeability through the activation of ET\textsubscript{A} receptors as a consequence of the disruption of the endothelial barrier. Following the decrease in arterial inflow, the declining energy supply for active membrane transport processes and the lack of removal of metabolites may be accompanied by rapid fluid movement from the vascular lumen to the lamina propria. An acute circulatory breakdown may therefore rapidly cause perivascular oedema, leading to MC degranulation.

In conclusion, infusion of exogenous ET-1 significantly enhanced degranulation of intestinal MCs by an ET\textsubscript{A}-receptor-dependent mechanism. Previous data indicate that MCs and ET-1 may both be involved in the mechanisms of endothelial-cell–leucocyte interactions and the sequestration of polymorphonuclear leucocytes after ischaemia [5,7,9]. Our results demonstrate that ET\textsubscript{A} receptor antagonism may have additional beneficial activity through the inhibition of MC reactions during intestinal pathologies. Similarly, these data suggest that an important interaction exists between endothelial cell-derived humoral mediators and the perivascular MC system. If ET-1 acts as an amplifier of the process of leucocyte activation, any alteration in this mechanism could have important consequences for local tissue responses.

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**REFERENCES**