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Suppression of inflammatory events associated to intestinal ischemia–reperfusion by 5-HT_{1A} blockade in mice

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ABSTRACT

Intestinal ischemia and reperfusion (I/R) is a potentially life-threatening disease, ensuing from various clinical conditions. Experimentally, either protective or detrimental roles have been attributed to 5-HT in the functional and morphological injury caused by mesenteric I/R. Recently, we proved the involvement of 5-HT_{2A} receptors in the intestinal dysmotility and leukocyte recruitment induced by 45 min occlusion of the superior mesenteric artery (SMA) followed by 24 h reperfusion in mice. Starting from these premises, the aim of our present work was to investigate the role played by endogenous 5-HT in the same experimental model where 45 min SMA clamping was followed by 5 h reflow. To this end, we first observed that ischemic preconditioning before I/R injury (IPC + I/R) reverted the increase in 5-HT tissue content and in inflammatory parameters induced by I/R in mice. Second, the effects produced by intravenous administration of 5-HT_{1A} ligands (partial agonist buspirone 10 mg kg^{-1}, antagonist WAY100135 0.5–5 mg kg^{-1}), 5-HT_{2A} antagonist sarpogrelate (10 mg kg^{-1}), 5-HT_3 antagonist alosetron (0.1 mg kg^{-1}), 5-HT_4 antagonist GR125487 (5 mg kg^{-1}) and 5-HT re-uptake inhibitor fluoxetine (10 mg kg^{-1}) on I/R-induced inflammatory response were investigated in I/R mice and compared to those obtained in sham-operated animals (S). Our results confirmed the significant role played by 5-HT_{2A} receptors not only in the late but also in the early I/R-induced microcirculatory dysfunction and showed that blockade of 5-HT_{1A} receptors protected against the intestinal leukocyte recruitment, plasma extravasation and reactive oxygen species formation triggered by SMA occlusion and reflow. The ability of α7 nicotinic receptor (α7nAchR) antagonist methyllycaconitine (5 mg kg^{-1}) to counteract the beneficial action provided by buspirone on I/R-induced neutrophil infiltration suggests that the anti-inflammatory effect produced by 5-HT_{1A} receptor antagonism could be partly ascribed to the indirect activation of α7nAch receptors.

1. Introduction

Intestinal ischemia is a clinical problem associated to different pathological conditions including neonatal necrotizing enterocolitis, mesenteric angina, intestinal infarction and intestinal transplantation [1]. Irritable bowel syndrome (IBS) is also one of the most frequently seen functional gastrointestinal disorders seriously impacting on patients' quality of life and it is associated with a considerable risk of ischemic colitis [2,3]. It is well known that ischemic injury rapidly damages intestinal tissues, but paradoxically the restoration of district blood supply exacerbates tissue dysfunctions, starting a series of deleterious events known as reperfusion injury: the intestine is probably one of the most sensitive organs to this patho-physiological process [4]. Among the variety of events affecting post-ischemic reperfused tissues, oxygen free radical formation, alteration of the microvasculature integrity, and neutrophil recruitment are pivotal: they sustain a local organ damage preceding the development of a systemic inflammatory response syndrome, followed, in its turn, by a serious multiple organ failure [5,6].

Exploiting an experimental model of intestinal ischemia/reperfusion (I/R) in rodents, our previous investigations demonstrated the participation of a number of neuronal, paracrine and plasmatic messengers in the functional and

Abbreviations: I/R, ischemia–reperfusion; 5-HT, 5-hydroxytryptamine; IPC, ischemic preconditioning; SERT, serotonin re-uptake transporter; nAchR, nicotinic receptor; MLA, methyllycaconitine; SMA, superior mesenteric artery; MPO, myeloperoxidase; MDA, malondialdehyde; ADP, adenosine diphosphate.

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morphological injury caused by mesenteric I/R [7–12]. More recently, we focused our attention on 5-HT, a known paracrine mediator and neurotransmitter involved in the physiological regulation of gut motility, perception and secretion [13]. Serotonergic signaling abnormalities have been implicated in the pathogenesis of several functional bowel diseases, like IBS: indeed, selective agonists and antagonists at 5-HT_3 and 5-HT_4 receptors, 5-HT receptor subtypes expressed at the intestinal level, or 5-HT selective reuptake inhibitors are now in use to combat these gut disorders [14,15].

Several findings suggest a critical involvement of 5-HT also in gut I/R conditions wherein 5-HT release, mainly from activated enterochromaffin cells, is initially augmented [16–18]. Controversial protective or detrimental roles have been ascribed to endogenous 5-HT in the sequels ensuing from gut hypoxia and re-oxygenation, depending on the organs affected and the experimental conditions adopted [19–21]. Indeed, pre-treatment with a 5-HT_{2A} antagonist was advantageous in dogs after small intestine I/R where liver injury was associated to 5-HT-induced platelet aggregation and vasoconstriction [19]. On the other hand, 5-HT plays a protective role in inflammation, since the amine, through 5-HT_{2A} receptors expressed on human peripheral blood mononuclear cells, counteracted the LPS-induced increase of the pro-inflammatory cytokine TNFα in vitro [22]. Clinical findings support this conflicting picture about serotonergic system also in intestinal disorders: 5-HT_3 antagonists alosetron and cilansetron, indicated for the treatment of diarrhea-predominant IBS, caused an enhancement of the prevalence of ischemic colitis [23]; the 5-HT_4 partial agonist tegaserod, approved for the treatment of IBS phenotype with constipation, was object of an FDA advisory because of post-marketing reports of cardiovascular ischemic events [24].

A few years ago, our group proved the involvement of the 5-HT_{2A} receptor subtype in the intestinal dysmotility and leukocyte recruitment caused by reversible occlusion of superior mesenteric artery for 45 min followed by 24 h reperfusion in mice [25]. This background prompted us to get a deeper insight into the role played by 5-HT in the inflammation caused by 45 min of mesenteric ischemia followed by 5 h reperfusion: a condition characterized by a sustained inflammatory response as reported in different experimental models of I/R [26,27].

To this end, we first assessed the changes in 5-HT tissue content and in inflammatory parameters in I/R mice and in mice subjected to ischemic preconditioning before I/R injury (IPC + I/R). This phenomenon consists in multiple brief ischemic episodes that protect the tissue from a subsequent and more prolonged ischemic insult. IPC has been originally described in canine heart [28], then demonstrated in a number of animal and human tissues and also suggested as a preventive strategy against I/R injury of the intestine [4].

Second, we evaluated the contribution in I/R intestinal dysfunctions of selected 5-HT receptor subtypes (5-HT_{1A}, 5-HT_{2A}, 5-HT_3, 5-HT_4) widely distributed in the gastrointestinal tract, where they participate in the control of motor and secretory reflexes [13,15], as well as the role of 5-HT re-uptake transporter (SERT). Accordingly, we investigated the effects of treatments with selective 5-HT_{1A} ligands (buspirone, widely used in therapy as 5-HT_{1A} partial agonist, antagonist WAY100135), 5-HT_{2A} antagonist sarpogrelate, 5-HT_3 antagonist alosetron, 5-HT_4 antagonist GR125487 and 5-HT re-uptake inhibitor fluoxetine on early I/R damage in mice.

The inflammatory response was biochemically determined in intestinal tissues excised from I/R mice by measuring the indexes of leukocyte infiltration, lipid peroxidation, and capillary permeability. Intestinal tissues were histologically examined. Sham-operated animals (S mice) served as control. The results here obtained confirm a beneficial effect exerted by inhibition of 5-HT_{2A} receptors on neutrophil recruitment and platelets dysfunction and reveal that the I/R-induced increase of all the inflammatory parameters examined could be remarkably prevented by blockade of 5-HT_{1A} receptors. 5-HT_{1A} receptors are described as inhibitory presynaptic receptors primarily located on intrinsic cholinergic nerves controlling the release of acetylcholine [29]. Therefore, 5-HT_{1A} receptors antagonism could indirectly provide protection toward post-ischemic injury through an increased availability of defensive transmitters like acetylcholine. In order to substantiate this hypothesis, given the anti-inflammatory action recently described for α7 nicotinic receptors (α7nAchR) activation [30], we finally tested the effects produced by α7nAchR antagonist methyllycaconitine (MLA) in buspirone-treated I/R and S mice.

2. Materials and methods

2.1. Experimental procedures

This investigation conforms to the rule for the care and use of laboratory animals of the European Community and is in accordance with Italian Law (DL 116/92). Experiments were performed on female adult Swiss mice (20–25 g; Charles River, Italy) that were housed under standard conditions and fasted 12 h before the experiment with free access to water.

2.2. Ischemia/reperfusion

Mice were anaesthetized with pentobarbital (50 mg kg^{-1} i.p.) and after laparotomy the small bowel was retracted to the left and the superior mesenteric artery (SMA) was temporarily occluded using a microvascular clip [25]. After 45 min, the reperfusion was allowed by gently removing the clip. At the end of the period of reperfusion, the animals were euthanized with CO_2 inhalation. Intestinal tissues were then excised and processed for biochemical assays.

In the first part of the study, animals were randomly assigned to the following three experimental groups ($n = 10$ for each group): mice subjected to ischemia (45 min) followed by 5 h reperfusion (I/R); mice subjected to one transient ischemic insult for 10 min and 10 min of reperfusion (ischemic preconditioning or IPC), followed by I/R (IPC + I/R); sham operated mice that underwent the same surgical manipulations except for SMA occlusion (S).

In the second part of the investigation, the following treatments were intravenously administered to 10–12 mice of I/R and S groups: vehicle (0.9% NaCl, w v^{-1}, 10 ml kg^{-1}), buspirone (10 mg kg^{-1}), WAY100135 (0.5, 1.5 and 5 mg kg^{-1}), sarpogrelate (10 mg kg^{-1}), alosetron (0.1 mg kg^{-1}), GR125487 (5 mg kg^{-1}) and fluoxetine (10 mg kg^{-1}). The drugs were administered immediately before the beginning of ischemia and were used at doses that have been reported as effective but not toxic [31–37].

In a separate group of experiments, α7nAchR antagonist MLA (5 mg kg^{-1}) was subcutaneously injected 30 min before ischemia alone or in association with buspirone (10 mg kg^{-1} i.v. administered immediately before ischemia).

2.3. 5-HT tissue levels

5-HT levels were measured in intestinal tissues excised from S, I/R and IPC + I/R mice. Briefly, ileal segments were collected and homogenized through sonication: the samples were quickly frozen and stored at −20 °C until assayed for 5-HT using a commercially available enzyme linked immunosorbent assay (ELISA kit) (Immunotech SAS, Marseille France). Data were expressed as the output of 5-HT in ng per g of dry weight tissue.