Inhalation of methane preserves the epithelial barrier during ischemia and reperfusion in the rat small intestine

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Background. *Methane is part of the gaseous environment of the intestinal lumen. The purpose of this study was to elucidate the bioactivity of exogenous methane on the intestinal barrier function in an antigen-independent model of acute inflammation.*

Methods. *Anesthetized rats underwent sham operation or 45-min occlusion of the superior mesenteric artery. A normoxic methane (2.2%)-air mixture was inhaled for 15 min at the end of ischemia and at the beginning of a 60-min or 180-min reperfusion. The integrity of the epithelial barrier of the ileum was assessed by determining the lumen-to-blood clearance of fluorescent dextran, while microvascular permeability changes were detected by the Evans blue technique. Tissue levels of superoxide, nitrotyrosine, myeloperoxidase, and endothelin-1 were measured, the superficial mucosal damage was visualized and quantified, and the serosal microcirculation and mesenteric flow was recorded. Erythrocyte deformability and aggregation were tested in vitro.*

Results. *Reperfusion significantly increased epithelial permeability, worsened macro- and microcirculation, increased the production of proinflammatory mediators, and resulted in a rapid loss of the epithelium. Exogenous normoxic methane inhalation maintained the superficial mucosal structure, decreased epithelial permeability, and improved local microcirculation, with a decrease in reactive oxygen and nitrogen species generation. Both the deformability and aggregation of erythrocytes improved with incubation of methane.*

Conclusion. *Normoxic methane decreases the signs of oxidative and nitrosative stress, improves tissue microcirculation, and thus appears to modulate the ischemia-reperfusion–induced epithelial permeability changes. These findings suggest that the administration of exogenous methane may be a useful strategy for maintaining the integrity of the mucosa sustaining an oxido-reductive attack. (Surgery 2017;■:■-■.)*

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Acute mesenteric ischemia progresses rapidly and leads to irreversible damage of the mucosa, but reperfusion can cause injury in excess of that induced by ischemia alone.[1] It is commonly accepted that re-established circulation is associated with the production of reactive oxygen and nitrogen species (ROS and RNS, respectively), which leads subsequently to membrane breakdown and loss of cellular integrity. In this way, events during intestinal ischemia-reperfusion (IR) increase quickly the mucosal permeability which leads to excessive fluid losses and an influx of luminal foreign material into the lamina propria.[2]

It is also recognized that the gastrointestinal (GI) lumen contains a range of potentially bioactive gas metabolites, such as carbon dioxide,[3] hydrogen,[4] ammonia,[5] and hydrogen sulfide.[6] Methane (CH_4) is also present in the intestinal atmosphere and is measurable in the exhaled breath of approximately one-third of humans.[7] The functional consequences of CH_4 production are subject to debate; nevertheless, the formation of CH_4 in

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mammals is regarded as a specific indicator of carbohydrate fermentation by the anaerobe intestinal flora. This latter route dependent on anaerobic flora is probably not exclusive; various in vitro and in vivo experiments have demonstrated alternative routes for the nonbacterial generation of CH_4 in conditions of oxido-reductive stress.[8,9]

Although some data suggest a role in the regulation of GI motility,[10] the in vivo biologic effects of biotic or abiotic CH_4 formation in the gut are still not completely understood. A normoxic CH_4-air mixture decreases the biochemical signs of inflammation after an IR challenge,[11] and a number of other observations confirm the anti-inflammatory properties of CH_4, demonstrated by decreases in levels of inflammatory cytokine and markers of oxidative stress.[12-16] In addition, many published articles suggest that CH_4-based treatments have antiapoptotic effects in model experiments.[17-23]

These converging research findings suggest that CH_4 can influence the permeability status of the mucosa, a most critical factor in GI injuries in clinical settings. Consequently, we devised experiments to investigate the kinetics of CH_4 distribution in the blood and small intestine and the effects of exogenous CH_4 which leads to an approximately twofold increase in the intraluminal CH_4 concentration over background levels; we evaluated these effects on the epithelial and endothelial permeability and secondary inflammatory reactions in a standardized rat model of mesenteric IR.

Because the mucosal response may take the form of either the rapid exacerbation of an injury after the ischemic episode or of a slowly developing alteration,[24] another goal of the study was to characterize the consequences of normoxic CH_4 inhalation separately in an early and a late period of an IR-induced, antigen-independent inflammatory challenge. Finally, additional in vitro model experiments were performed to test the influence of CH_4 on erythrocyte membrane rigidity to investigate possible mechanisms of changes in the microcirculation of the small intestine.

MATERIALS AND METHODS

The experiments were carried out on 76, male, Sprague-Dawley rats (280–320 g body weight [bw]) in accordance with the National Institutes of Health guidelines on the handling and care of experimental animals and the EU Directive 2010/63 for the protection of animals used for scientific purposes; the study was approved by the National Scientific Ethical Committee on Animal Experimentation (National Competent Authority), with license number V/148/2013. The animals were housed in plastic cages in a 12/12-h day/night cycle under standard air temperature and humidity conditions. All chemicals were obtained from Sigma-Aldrich Inc (Budapest, Hungary) unless stated otherwise.

Operative procedure. Rats fed on a normal laboratory diet with tap water ad libitum were allocated randomly into one or the other of the experimental groups. After overnight fasting, the animals were anesthetized with sodium pentobarbital (50 mg/kg bw intraperitoneal) and placed in a supine position on a heating pad. The trachea was dissected free and cannulated with a silicone tube; then the right jugular vein was cannulated with PE50 tubing for fluid administration and Ringer's lactate infusion (10 mL/kg/h) during the experiments.

Experimental protocol. The experiments were performed in 2 series (Fig 1). In study 1 (the "early reperfusion" study), the animals were killed 60 min after the re-establishment of the mesenteric blood flow; in the second set (the "late reperfusion" study), the reperfusion period and the corresponding control phase in the sham-operated animals lasted for 180 min.

After a midline laparotomy, the superior mesenteric artery (SMA) was dissected free. Group 1 ($n = 6$) served as a sham-operated control, while in Group 2 (IR, $n = 6$), the SMA was occluded using an atraumatic vascular clip for 45 min. In the CH_4-treated Group 3 (IR + CH_4, $n = 6$) an artificial gas mixture containing 2.2% CH_4, 21% O_2, and 76.8% N_2 (Linde Gas, Budapest, Hungary) was administered for 5 min before the end of the 45-min ischemia and for 10 min at the beginning of the reperfusion (Fig 1). In study 2, the protocol followed was identical, but the durations of the observation and the reperfusion phases were different.

Epithelial permeability. Epithelial permeability was determined with the 4 kDa fluorescein isothiocyanate-dextran (FD4) method, as described previously.[25] In short, a 5-cm-long segment of the terminal ileum supplied by 3 blood vessel arcades was isolated at a distance of 10 cm from the ileocecal valve. Silicone cannulas were placed and fixed into the oral and aboral ends of the segment, and the lumen was gently flushed with 5 mL of 37°C 154 mM NaCl and 5 mL air; then the distal end was closed.

Before performing measurements, the renal pedicles were ligated. Exactly at the moment of reperfusion (the "early reperfusion" study) or