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Selective Serotonin-Receptor Antagonism and Microcirculatory Alterations During Experimental Endotoxemia

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Background. **Endothelial damage contributes greatly to the high mortality in septic shock. Recent experiments from our laboratory with the GP IIb/IIIa-antagonist abciximab describe a dominating role for platelets as mediators of endothelial dysfunction during endotoxemia. In many inflammatory conditions, serotonin is released by activated platelets into the circulation. Therefore, we investigated the effects of serotonin-receptor antagonism using methysergide and ketanserin (KET) on microcirculatory alterations during endotoxemia.**

Material and methods. **In male Wistar rats, venular wall shear rate, macromolecular efflux, and leukocyte-endothelial interaction were determined in mesenteric postcapillary venules using intravital microscopy at baseline 60 and 120 min after the start of the experiment. The experiments were separated into a pretreatment and a posttreatment part. In each part, rats were randomized into four groups. Animals in the treatment groups received either methysergide (MET) or KET. Endotoxin-challenged animals without MET or KET treatment served as a control group.**

Results. **Pretreatment as well as posttreatment with MET or KET is effective in maintaining venular wall shear rate, reducing leukocyte-endothelial interaction, and reducing macromolecular efflux during endotoxemia.**

Conclusions. **Serotonin-receptor antagonism with MET or KET represents a promising new therapy option to restore the microcirculation during endotoxemia.**

Key Words: **ketanserin; methysergide; endotoxin; macromolecular efflux; leukocyte-endothelial interaction; microcirculation.**

INTRODUCTION

Microcirculatory dysfunction as a marker for mortality plays a pivotal role in the development of the clinical manifestation of severe sepsis. In this process endothelial damage contributes greatly to the high mortality in septic shock [1]. Recent experiments from our laboratory with the GP IIb/IIIa-antagonist abciximab describe a dominating role for platelets as mediators of endothelial dysfunction during endotoxemia [2]. In various experimental settings, platelets emerge as key players in sepsis [3–6].

With inflammatory conditions such as thrombosis, ischemia, and endotoxemia, the activated platelets release serotonin [7, 8]. Serotonin [5-hydroxytryptamine (5-HT)] is synthesized and released in the circulation by enterochromaffin cells from gastric and intestinal mucosa. It is avidly taken up by endothelial cells and platelets, where it can be found at high concentrations. The released serotonin, interacting with its own receptors on the activated surfaces of platelets, is determinant for platelet aggregation [9]. Thereby, it enhances the extent of platelet aggregation and the release of intragranular platelet products and arachidonic acid metabolites in response to otherwise ineffective agonist concentrations. This propagates further platelet aggregation and serotonin release [10–12].

There are only few data available to determine the benefits and risks of modifying microcirculation during states of inflammation by serotonin. Therefore, we investigated the effects of serotonin-receptor antagonism using methysergide (MSG) and ketanserin (KET) on microcirculatory alterations during endotoxemia.

MATERIALS AND METHODS

Animal Preparation

All experimental procedures and protocols used in this investigation were reviewed and approved by the Governmental Animal Pro-

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tection Committee. Male Wistar rats weighing 250 to 350 g were used. Animals were kept in an animal facility at a 12-h light-dark cycle and housed in stainless-steel cages in a temperature- and humidity-controlled room. All animals received a diet of standard rat food and water *ad libitum*. Food was withheld from all animals for 4 h before the experiment; free access to water was maintained. Rats were anesthetized by intraperitoneal application of 60 mg/kg body weight (b.w.) sodium pentobarbital (Nembutal; Sanofi, Düsseldorf, Germany). The left carotid artery was cannulated with polyethylene tubing (outer diameter 0.9 mM, inner diameter 0.5 mM) for the measurement of mean arterial pressure via a pressure transducer connected to a monitoring system (Servomed, Hellige, Germany). For drug infusion, the right jugular vein was also cannulated with the same tubing. To maintain anesthesia, all animals received 10 mg/kg/h sodium pentobarbital intravenously. A tracheotomy was performed to facilitate spontaneous breathing. Rectal temperature was measured with a thermistor probe and maintained at 37°C using a heating lamp.

The abdomen was opened via a midline incision, and the ileal portion of the mesentery was carefully exteriorized over a plastic stage. At the beginning, the mesentery was continuously superfused with a thermostat-controlled (36.5°C), bicarbonate-buffered salt solution (132 mM sodium chloride, 4.7 mM potassium chloride, 2 mM calcium chloride, 1.2 mM magnesium chloride, and 18 mM sodium bicarbonate) equilibrated with 5% CO_2 in nitrogen to adjust the pH to 7.35.

Intravital Microscopy

The mesenteric microcirculation was observed using a microscope (Orthoplan; Leica, Wetzlar, Germany) equipped with a 40-fold objective (Achroplan 40/0.75W; Zeiss, Jena, Germany). The exteriorized mesentery was visualized either by transillumination (150-W cold light fountain; KL 1500 electronic, Schott, Wiesbaden, Germany) or by epi-illumination using an epifluorescence illuminator (Ploemopak; Leica) that consisted of a 100-W short-arc mercury lamp (Osram, Munich, Germany) and a filter system for the fluorescence excitation (green light excitation: N 2.1; blue light excitation: I 3; Leica). To protect the preparation from heat, a heat protection filter (KG1; Leica) was located in the body of the microscope. Microscopic images were transferred to a monitor (PVM 1444QM; Sony Corp., Tokyo, Japan) by a low-light camera (Kappa CF 8/1; Kappa Messtechnik, Gleichen, Germany) and recorded on videotape using a video recorder (Panasonic S-VHS AG-7350-E; Matsushita, Japan).

Measurement of Erythrocyte Velocity

Mean red blood cell (RBC) velocities (V_{RBC}) in single unbranched postcapillary venules (25–35 μm diameter) were analyzed offline at 0, 60, and 120 min after administration of endotoxin, using a computer-assisted microcirculation analysis system (Cap image; Zeintl, Heidelberg, Germany). Therefore, fluorescent-labeled erythrocytes from donor rats were injected before microscopy (0.5 mL/kg b.w.; hematocrit 50%). These erythrocytes were labeled with a red fluorescent cell linker kit (PKH26-GL; Sigma Chemical, Deisenhofen, Germany). For velocity measurement, the distance through which a labeled erythrocyte traveled within two subsequent video frames was divided by the known video frame time interval of 20 ms. Mean blood cell velocity in a vessel was calculated by averaging the velocities of 20 to 30 individual erythrocytes. To calculate venular wall shear rate, the vessel diameters (D_v) of the same venules were measured (Cap image; Zeintl). Venular wall shear rate (γ) was calculated based on the Newtonian definition: $\gamma = 8\ (V_{RBC}/D_v)$.

Leukocyte-Endothelial Interactions

The behavior of leukocytes was visualized using transillumination microscopy at 0, 60, and 120 min. Rolling leukocytes were assayed offline during playback of the videotaped images and subsequent quantitative analysis. Leukocyte-endothelial interaction was expressed as the number of adherent leukocytes per square millimeter.

Quantitation of Macromolecular Leakage

To quantify albumin leakage across mesenteric venules, 50 mg/kg b.w. of fluorescein isothiocyanate–labeled bovine albumin (FITC-albumin; Sigma Chemicals, Deisenhofen, Germany) was injected intravenously before baseline measurements. The recorded fluorescent images were digitized, and the gray levels reflecting the fluorescence intensity [gray levels ranging from 0 (black) to 255 (white)] were measured within three segments of the venule under study (I_v) and in three continuous areas of the perivenular interstitium (I_i). Macromolecular leakage was determined as the ratio I_i/I_v at 0, 60, and 120 min.

Experimental Protocol

The experiments were separated into a pretreatment and a posttreatment part. In each part, rats were randomized into four groups of 10 animals each in the pretreatment part, respectively, into eight animals each in the posttreatment part. Animal preparation and mesenteric exteriorization were followed by a 30-min stabilization period on the stage of the intravital microscope. Exactly five minutes later, all animals received PKH26-GL–labeled erythrocytes and FITC-labeled albumin. After this procedure, baseline measurements were determined.

Pretreatment

Animals in the lipopolysaccharide (LPS) group received a continuous intravenous infusion of 2 mg/kg/h endotoxin (LPS *Escherichia coli* 026:B6; Sigma Chemicals) diluted in saline 0.9% after baseline measurement, to induce normotensive endotoxemia with a model lethality of less than 5 percent. In the MSG pre/LPS group, animals were given 1 mg/kg b.w. MSG (Novartis Pharma, Wehr, Germany) before midline laparotomy, in addition to the endotoxin infusion. The KET pre/LPS group received 1 mg/kg b.w. KET (Biotrend Chemikalien, Köln, Germany) before midline laparotomy, in addition to the infusion of endotoxins. Animals in the saline group received a volume-equivalent intravenous infusion of saline 0.9% instead of endotoxin. The LPS group served as the control group, whereas the saline group was used as the unaffected reference group.

Posttreatment

Again, animals in the LPS group received a continuous intravenous infusion of 2 mg/kg/h endotoxin (LPS *E. coli* 026:B6; Sigma Chemicals) diluted in saline 0.9% after baseline measurement. In the LPS/MSG post group, animals were given 1 mg/kg b.w. MSG (Novartis Pharma) 30 min after start of the continuous intravenous infusion of endotoxin (LPS *E. coli* 026:B6; Sigma Chemicals). The LPS/KET post group received 1 mg/kg b.w. KET (Biotrend Chemikalien) 30 min after start of the continuous intravenous infusion of endotoxin, in addition to the endotoxin infusion described earlier. Animals in the saline group received a volume-equivalent intravenous infusion of saline 0.9% instead of endotoxin. Again, the LPS group served as the control group, whereas the saline group was used as the unaffected reference group.

Statistical Analysis

All data are presented as mean ± SEM. For statistical analysis, two-way repeated measures analysis of variance followed by the Scheffé test were used. Differences were considered significant at $P < 0.05$.