Morphological and Functional Changes of Coronary Vasculature Caused by Transcellular Biosynthesis of Sulfidopeptide Leukotrienes in Isolated Heart of Rabbit

By Angelo Sala, Gjumrakch M. Aliev, Giuseppe Rossoni, Ferruccio Berti, Carola Buccellati, Geoffrey Burnstock, Giancarlo Folco, and Jacques Maclouf

Morphological and functional modifications occurring in Langendorff rabbit heart preparations perfused with purified human leukocytes (PMNL), as an organ model of sulfidopeptide-leukotrienes (sLT) transcellular biosynthesis, were studied. Coronary perfusion pressure (CPP), monitored as an index of coronary vasospasm, increased by 295% after challenge with the Ca²⁺-ionophore A-23187 (0.5 μM) for 30 s, accompanied by a significant formation of sLT. Increase in CPP was prevented by PMNL pretreatment with the 5-lipoxygenase inhibitor MK-886 (1 μM) or by heart pretreatment with the LTD₄-receptor antagonist SKF 104353, indicating a pivotal role of PMNL-derived 5-lipoxygenase (5-LO) products in the observed functional modifications. Similar effects were obtained using granulocyte macrophage-colony stimulating factor-primed PMNL challenged with the tripeptide n-formyl-methionyl-leucyl-phenyalanine. Scanning electron microscopy (SEM) of coronary arteries showed craters on the vessel luminal surface, PMNL adhering to endothelial cells (EC), increased number of microvilli on EC, decreased number of patent microvessels. These morphological alterations were significantly blunted by MK-886 or SKF 104353. These data provide evidence of close interaction between PMNL and myocardial EC, resulting in enhanced sLT formation via transcellular biosynthesis, originating from transfer of PMNL-derived LTA₄ to EC. These potent proinflammatory autacoids are responsible for coronary vasospasm and the morphological alterations observed.

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ADHERENCE OF polymorphonuclear leukocytes (PMNL) to the vascular endothelial cells (EC) appears to represent one of the earliest steps in the pathogenesis of ischemia/reperfusion tissue injury. PMNL adherence to endothelial cells involves several adhesion molecules on the surface of both cell types. Of particular interest is the upregulation and enhanced expression of cell surface adhesion glycoproteins, which mediate cell-cell interactions and orchestrate a complex series of events occurring between the EC and PMNL. The activation of adhered leukocytes in response to various noxious stimuli characterizes the first step in the initiation of an acute inflammatory response, involving diapedesis, release of proinflammatory lipid mediators and alterations in vasotonic and vascular leakage. Numerous, structurally diverse lipid molecules derived from arachidonic acid are synthesized during inflammatory reactions “in vivo”; among them leukotrienes (LT) have attracted considerable interest. Leukotriene C₄ and D₄ (LTC₄, LTD₄) are potent vasoactive mediators that can constrict small and large vessels, modify cardiac and coronary functions, the microcirculation and the manifestations of ischemia-reperfusion injury. Additionally, they are known to have vasoregulatory properties that might have relevance for the extravasation of leukocytes from the vessel lumen to the tissue. The existence of PMNL-platelet interactions via the lipoxygenase pathway, which may be important in hemostasis and inflammation has been first documented by Marcus et al. More recently cooperation of donor PMNL with acceptor EC in processing the reactive intermediate LTA₄ into biologically active LTC₄ has been shown; this process has been termed “transcellular biosynthesis” and suggests that the cellular environment (ie, cell-cell interactions) is an important control in the production of eicosanoids. Moreover, the challenge of PMNL present within the coronary vasculature has been shown to cause a sulfidopeptide leukotirole-dependent coronary vasoconstriction.

The cooperation and synergy between PMNL and EC seem to have a profound impact in the mediation of inflammatory disorders and associated alterations of vascular function. In the present report we provide functional, biochemical, and morphological evidence of the pathological events that occur at the level of coronary vascular bed ultrastructure in a model of acute, PMNL- and sLT-dependent cardiac ischemia; histological evidences of conditions characterized by close contact between LTA₄-donor and -acceptor cells, such as adhesion between blood cells and vessel walls, are presented.

MATERIALS AND METHODS

Isolated perfused heart preparation. Albino rabbits weighing between 2.5 and 3.0 kg were used. Hearts were isolated and perfused retrogradely at 37°C through the aorta as previously described. The rate of perfusion was maintained with a roller pump (Gilson Minipulse 2, BioLabs, Milano, Italy). A latex balloon was inserted into the left ventricular cavity for measurement of left ventricular pressure (LVP) and dP/dt, recorded with a Hewlett Packard carrier amplifier (mod. 8805B) and recorder (mod 7754A; Hewlett Packard Italia, Cernusco, Milano, Italy). All hearts were equilibrated for 30 minutes at a flow rate of 20 mL min⁻¹ to allow extensive rinsing of the
vascular bed; the hearts were then perfused in a recirculating system at the same flow of 20 mL min⁻¹ with a total volume of 50 mL. Coronary perfusion pressure (CPP), heart rate (HR), LVP and LV end-diastolic pressure (EDP) were monitored continuously. Synthetic LTC₄ (2 µg) (Cayman Chem, Ann Arbor, MI), was administered by bolus injection in the recirculating medium.

Cell perfused hearts. Recirculating rabbit hearts were perfused with PMNL to study interactions between blood cells and the heart. These human cells were obtained from blood (40 mL) withdrawn from healthy donors that had not taken medications for at least one week and purified by a discontinuous Percoll density gradient (42% and 51%, vol/vol, in platelet-poor plasma [PPP]). After centrifugation for 10 minutes at 180 g, the lower PMNL containing band was transferred; cells were washed twice with 10 mL of a 1:1 solution of PPP in saline. Cells were finally resuspended to appropriate volume in Tyrode’s solution without Ca²⁺ and Mg²⁺. This preparation contains more than 95% PMNL, as checked on cytocentrifugates fixed in methanol and stained with Giemsa-losung (Merck-Kohlschutter, Germany).

When cells had to be pretreated with the leukotriene biosynthesis inhibitor, compound MK-886, (1S,4S)-1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl)-2,2-dimethylpropanoic acid, before heart perfusion, they were supplied with Ca²⁺ (2 mM) and Mg²⁺ (0.5 mM). Preincubated at 37°C for 2 minutes and then exposed to MK-886 (1 µM) for 5 minutes; cells were subsequently washed once, resuspended in Tyrode’s solution without Ca²⁺ and Mg²⁺ (Tyrode =) and counted.

Sulfidopeptide-leukotriene (sLT) receptor antagonist, compound SKF 104353 (2S)-hydroxy-3(R)-(2-carboxyethylthio)-3-(2(8-phenylocty1)phenyl)-propanoic acid, was added (10 µM) to the recirculating medium 15 minutes before the addition of blood cells.

Pretreatment of PMNL was carried on using granulocyte macrophage-colony stimulating factor (GM-CSF) 1 nmol/L (Amersham International, Little Chalfont, UK), for 30 minutes in Tyrode’s solution.

PMNL (5 x 10⁷ cells for A-23187 experiments and 10 x 10⁶ cells for FMLP experiments) were supplied with Ca²⁺ (2 mM/L) and Mg²⁺ (0.5 mM/L) before infusion into the recirculating medium of isolated rabbit hearts, at a flow rate of 0.6 mL/min in a volume representing 2% to 6% (1 to 3 mL) of the total perfusate, to avoid mechanical obstruction of coronary vasculature.

Challenge was performed 10 minutes after addition of cells to the recirculating reservoir, with either the calcium ionophore A-23187 0.5 µmol/L (Calbiochem, San Diego, CA) or n-formyl-methionyl-leucyl-phenylalanine (fMLP) 1 µmol/L (Sigma Chemical Co, St Louis, MO). Isolated hearts were observed up to 30 minutes after A-23187 (0.5 µmol/L) was added (up to 60 minutes after fMLP challenge).

Analysis of heart perfusates. The entire heart reservoir (~45 mL) was collected into 1 vol ice-cold methanol, spiked with 25,000 dpm ¹³C-LTC₄ and stored at ~20°C overnight. Incubates were then centrifuged for 15 minutes at 3,500 g, the supernatant diluted to 15 mL with H₂O, and extracted using a solid-phase cartridge (Supelclean LC18; Supelco). Ninety percent aqueous methanol eluates were taken to dryness using a SpeedVac evaporating centrifuge (Savant Instruments, Farmingdale, NY), reconstituted and analyzed as described.

Scanning and transmission electron microscopy studies (SEM, TEM). The morphological examinations were performed on the following experimental groups: (a) isolated, perfused hearts of rabbit, challenged for 30 minutes with the Calcium ionophore A-23187 (0.5 µmol/L); (b) hearts perfused with unstimulated PMNL (10⁶ cells/mL); (c) hearts perfused with PMNL and challenged with A-23187; (d') hearts that had been pretreated with the sLT-receptor antagonist SKF 104353 (10 µmol/L); perfused with PMNL and challenged with A-23187; (d') hearts that had been perfused with MK-886-treated PMNL (MK-886 1 µmol/L) and challenged with A-23187.

At the end of the observation period, hearts were perfused for 5 minutes via the aortic cannula at constant flow (20 mL/min) with Karnovsky’s fixative solution (1.25% [wt/vol] glutaraldehyde and 1.25% [wt/vol] paraformaldehyde in 0.1 mol/L phosphate buffer, Sigma) pH 7.33.

Samples from each heart were obtained from the left-descending coronary artery (LDCA) and from the heart muscle (myocardium), stored for 24 hours at +4°C in Karnovsky’s fixative solution and treated for SEM as described previously. Tissue specimens were finally examined with a Hitachi S-405 scanning electron microscope (Hitachi Scientific Instruments, Wokingham, Berks, UK), operating at 80 kV.

Morphometric measurements. Morphometric analysis was performed in a double blind fashion by measuring: (1) the number of microvilli per square micrometer present on the luminal surface of the endothelium of the coronary artery and related microvessels; (2) the number of adhered leukocytes per square millimeter of coronary artery endothelium and related myocardial microvessels; (3) the number of patent capillary vessels per square millimeter of myocardial muscle cross-section. Results for each sample represent the mean of at least 50 observations.

The distinction between a patent and nonpatent capillary vessel was made by observing a cross-section of a frozen-fracture muscle sample at low magnification. A patency in the same section of myocardial muscle higher than 80% of that observed in sham-isolated hearts was considered sufficient to define a capillary vessel as patent.

Statistical analysis. All values in the text and tables are expressed as mean ± SE of n observations. A two-way analysis of variance was employed to analyze results from repeated measurements. Endpoint experiments were analyzed by one-way Analysis of Variance (ANOVA), followed by a least significant difference procedure to determine the nature of response (SPSS Inc, Chicago, IL). Probability values less than 0.05 were considered statistically significant.

RESULTS

Functional studies. When isolated rabbit hearts were perfused under recirculating conditions at a constant flow of 2 mL ice cold methanol, containing 30 ng of PGB₂, and the samples stored at ~20°C overnight. Incubates were then centrifuged for 15 minutes at 3,500 g, the supernatant diluted to 15 mL with H₂O, and extracted using a solid-phase cartridge (Supelclean LC18; Supelco). Ninety percent aqueous methanol eluates were taken to dryness using a SpeedVac evaporating centrifuge (Savant Instruments, Farmingdale, NY), reconstituted and analyzed as described.
20 mL min⁻¹, CPP averaged 46.25 ± 1.41 mm Hg (n = 16) and remained constant for the observation period that extended up to 30 minutes. Similarly HR (beats per minute 163 ± 12, n = 16), LVP (86 ± 2 mm Hg, n = 16) and LVEDP (8.62 ± 0.40 mm Hg, n = 16) did not vary throughout the experimental period. Administration of synthetic LTC₄ (2 µg) by bolus injection in the recirculating medium, resulted in a modest but significant increase in CPP (+70.8% ± 6.4% over basal).

Challenge of the rabbit heart with A-23187, 0.5 µmol/L (group a), per se, did not alter myocardial contractility nor CPP. When purified PMNL (5 × 10⁶ cells) were perfused through the rabbit heart, no changes in the functional parameters were detected (group b). However, challenge with A-23187 (0.5 µmol/L) in presence of purified PMNL (group c) induced a severe increase in CPP; at 30 minutes the resistance to perfusion pressure had increased by 294.6% (P < .025 v group a), which was significantly ablated by pretreatment of the heart with SKF 104353 (group d') (89.9% inhibition of rise in CPP, P < .03 v group c), or by pretreatment of PMNL only with MK-886 (group d") (78.9% inhibition of rise in CPP, P < .05 v group c).

HPLC analysis of the total volume of the circulating perfusate (44 to 47 mL) at the end of the experiment, allowed positive identification of LTs by on-line UV-spectrum analysis (Fig 1). A significant increase in sulfidopeptide leukotrienes was observed in the perfusate of PMNL-perfused hearts of rabbit (Fig 2A). Pretreatment of PMNL only with MK-886 (1 µmol/L) (group d") resulted in a marked suppression of the rise in LTs (Fig 2A), proving the pivotal role of an intact PMNL 5-LO in the observed production of sulfidopeptide leukotrienes.

Challenge of PMNL preparations in suspension, showed a substantial release of LTE₄ as indicated by presence of its nonenzymatic degradation products (Δ⁵-trans-LTB₄, 12-epi,Δ⁵-trans-LTB₄, 5(S),6(S)-diHETE and 5(S),6(R)-diHETE), whereas the amount of LTC₄ observed could not account for the increase in sLT observed in PMNL-perfused rabbit hearts with respect to A-23187 challenged heart alone (Fig 2B).

The challenge with the chemotactic peptide (fMLP, 1 µmol/L) of GM-CSF–primed PMNL-perfused rabbit heart, resulted in a significant increase of CPP at 60 minutes (+229.5% more than prechallenge values, n = 3), accompanied by the formation of relevant amounts of sLT (15.7 ± 2.5 pmol/mL) as well as of LTB₄ (1.31 ± 0.14 pmol/mL) (Fig 3). The challenge of GM-CSF–primed isolated PMNL in the test tube resulted in the production of LTB₄ and its
that had been perfused with MK-886-treated PMNL (group d) induced a significant reduction in the number of PMNL adhering to the EC surface of bath coronary artery and myocardial microvessels in isolated hearts of rabbits well correlated with the data obtained from the analysis of the coronary artery.

Ultrastructural analysis of the capillary vessels showed presence of an intact endothelium and absence of adhering PMNL (Fig 6C and D). No pathological abnormalities were seen in the morphology of capillary vessels. We did not observe any PMNL in the perivascular space nor between the heart muscle fiber areas. Interstitial edema decreased and edema in perivascular spaces was only sometimes observed.

TEM observation of myocardial capillaries from hearts that had been pretreated with the sLT-receptor antagonist SKF 104353 (group d') or infused with MK-886-treated PMNL, and challenged with A-23187 (group d'') well correlated with the data obtained from the analysis of the coronary artery. Ultrastructural analysis of the capillary vessels showed presence of an intact endothelium and absence of adhering PMNL (Fig 6C and D). No pathological abnormalities were seen in the morphology of capillary vessels. We did not observe any PMNL in the perivascular space nor between the heart muscle fiber areas. Interstitial edema decreased and edema in perivascular spaces was only sometimes observed.

The results of the morphometric studies are summarized in Table 1. The density of microvilli present on the luminal surface of EC from coronary artery and myocardial microvessels in isolated hearts of rabbits which had been perfused with PMNL and challenged with A-23187 (group c) was considerably increased in comparison to hearts pertaining to all other experimental groups.

In group a and group b hearts, the presence of PMNL adhering to EC in both coronary artery and microvessels was undetectable. Infusion of PMNL through the coronary vascular bed and their challenge with the Ca-ionophore (group c) resulted in a marked increase in the number of PMNL adhering to the EC of the coronary artery and myocardial microvessels respectively. Pretreatment of the rabbit heart with SKF 104353 (group d') or of the PMNL with MK-886 (group d'') induced a significant reduction in the number of PMNL adhering to the EC surface of both coronary artery and myocardial microvessels.

Hearts that had been challenged with A-23187 in the absence of PMNL (group a) and hearts that had been perfused with PMNL but not challenged with A-23187 (group b) displayed a regular distribution of patent capillary vessels. Infusion of PMNL and challenge with A-23187 (group c) caused a significant reduction in the number of patent capillary vessels per sq mm. On the contrary, group d' and d''
hearts showed a nearly normal density of patent capillary vessels (Table 1).

**DISCUSSION**

The present report describes major morphological alterations of coronary vascular bed and myocardium, accompanying functional changes in a model of transcellular biosynthesis of sulfidopeptide leukotrienes, represented by the isolated, PMNL-perfused heart of rabbit.

Infusion of purified human PMNL through a spontaneously beating Langendorff-heart preparation, and challenge with calcium ionophore A-23187, led to the expected increase in coronary tone and production of sulfidopeptide leukotrienes. We cannot exclude that during preparation of the isolated heart some blood cells have adhered to the vessel walls contributing to the "basal level" of sLT observed. In agreement with previously published data, the amount of sLT contributed by the heart and by isolated PMNL, does not account for the amount detected when the neutrophils are challenged within the vascular bed, thereby supporting the concept of transcellular biosynthesis.

These potent vasoactive autacoids appeared to be the mediators responsible for the functional changes observed, because a significant protection occurred following pretreatment of the heart with the LTD₄-receptor antagonist SKF 104353 as well as by PMNL exposure to the potent and slowly reversible 5-LO inhibitor MK-886. The use of A-23187 to challenge human blood cells perfusing a rabbit heart might raise the problem of its pathophysiological relevance, but was selected because of its reproducibility to study morphological and functional consequences, at the organ level, of PMNL 5-LO activation, resulting in "in situ" generation of sLT. Nevertheless similar results, in terms of increase of CPP and production of sLT, were also obtained.
Fig 5. Morphological features of myocardial microvessels from isolated, perfused rabbit hearts. (A) SEM of isolated heart challenged with A-23187 (0.5 μmol/L) showed a normal pattern of microvessels (*), with absence of perivascular and intermuscle edema (OM × 3,000). (B) SEM of isolated heart perfused with PMNL and challenged with A-23187, showed a reduced number of patent (nonstick) capillary vessels (+) with presence of perivascular tissue (−) and intermuscle fiber edema (⊥) (× 2,000). (C) and (D) SEM of isolated heart infused with PMNL pretreated with MK-886 (1 μmol/L) and activated with A-23187, and of isolated heart pretreated with SKF 104353 (10 μmol/L), infused with PMNL and challenged with A-23187, respectively. A morphology similar to that of (A) was observed ([C], × 3,000, [D] × 5,000) (+ indicate microvessels).

Ultrastructural changes observed in heart tissue in the presence of challenged neutrophils involved an increased number of microvilli on the luminal surface of EC, a greater number of leukocytes adhering to coronary vessels and myocardial microvessels, the presence of a large number of desquamated, fusiform EC, reduced density of patent capillary vessels, and a generalized edema of perivascular spaces and intermuscle fiber areas.

These changes were fully prevented by pretreatment of PMNL only, with the leukotriene biosynthesis inhibitor MK-886, supporting the concept that, by blocking key steps involved in the process of transcellular biosynthesis and therefore attenuating the formation of mediators, tissue infiltration and cardiac damage can be prevented. Similar prevention was obtained using the sLT end-organ antagonist SKF 104353, indicating the existence of a causal relationship between presence of sLT and degree of tissue injury.

The pronounced sLT-dependent, PMNL-dependent ischemia observed is able to affect EC in different ways. First, cells try to increase the surface available for gas and nutrient exchange by expression of cytoplasmic microvilli or by ex-
tending membrane protrusions into the vessel lumen, likely in the attempt to increase gas exchange with circulating blood. These microvascular changes may represent an adaptive response of EC to altered hemodynamic conditions, and is related to their duration. The occurrence of an increased expression of microvilli on the endothelial surface seems to be a cellular response to different kind of stimuli and has been described, using SEM, in cerebral ischemia in the brain of hypertensive rats, in human and rat thoracic aorta after sudden death, in the rabbit hindlimb microvessels after prolonged ischemia and reperfusion, and in the isolated coronary artery and myocardial microvessels of the rat after infusion with activated PMNL.

In samples taken from hearts that had been perfused with PMNL and challenged with A-23187 (group c), cytoplasmatic protrusions directed towards the vessel lumen were sometimes found on EC. These structures could also represent a cellular response to severe hypoxia in areas where an increased expression of cytoplasmic microvilli is insufficient to guarantee an adequate exchange surface. Histologic studies have shown that EC of coronary vessels are highly susceptible to ischemic injury; extensive EC swelling and derangement has been reported after as little as 10 min of myocardial ischemia in the capillary vascular bed.

Leukocyte activation during ischemia is a very relevant phenomenon in the microcirculation, in which blood cells may aggregate forming mixed microaggregates that can impede blood perfusion in the capillary bed. The initial alterations in the morphology of the vascular bed may be counteracted by the presence of endogenous defense systems, including vasodilatory autacoids (PGI2, NO) and the antioxidant reserve (SOD, glutathione peroxidase and catalase).
EC may contract in response to LTs, with a reduction of their cell surface and exposure of subendothelial matrix. The last phase of endothelial injury development is characterized by severe alterations, i.e., areas of EC are denuded, PMNL migrate into the extravascular matrix, some EC die. Clusters of aggregated PMNL are present on the EC surface as well as on the subendothelial lining and both PMNL and EC play an active role in orchestrating the interactive process of PMNL adhesion and migration through the vessel wall. In light of a recent report showing the importance of PMNL-EC adhesion for sLT transcellular biosynthesis, the close contact between PMNL and endothelial cells of coronary vasculature, shown by morphological analysis, provide evidence for a facilitated intercellular transfer of LTA₄.

It is important to remember that leukotrienes are local hormones and their effects are likely to be exerted in paracrine rather than endocrine conditions. High local bioavailability of sLT generated in situ, in close proximity to the appropriate target cells (vascular smooth muscle cells), may lead to functional effects of higher efficacy than those obtained after exogenous perfusion and is consistent with the autacoid nature of the paracrine signaling of these molecules.

In PMNL-perfused heart of rabbit, the simple infusion of unchallenged neutrophils, as well as challenge of buffer-perfused rabbit hearts, was unable to cause any appreciable change in vascular tone as well as in coronary artery or myocardial microvessel ultrastructure. However, activation of PMNL 5-LO by A-23187 challenge, led to alterations in coronary vascular resistance and in heart ultrastructure, suggesting onset of an ischemic process. The severity of the ischemic event, is likely the result of an efficient uptake of PMNL-derived LTA₄ by the endothelial cells, favored by adhesion of myeloid leukocytes to the vascular wall, as shown by SEM. Evidence for transcellular leukotriene synthesis in vitro, in a complex model of pulmonary leukostasis and "in vivo" in the hamster microcirculation, have been presented and the occurrence of transcellular metabolic events that might contribute to eicosanoid formation in pathological situations, have already been evoked.

Leukocytes are present in necrotic myocardium and seem to be determinant in the final formation of the necrotic area of postischemic myocardium. In fact, depletion of neutrophils or administration of antibodies for CD11/CD18 glycoprotein adhesive receptors of PMNL are able to reduce the extent of necrotic tissue injury as well as to reduce sLT synthesized by transcellular biosynthetic mechanisms.

It seems logical to hypothesize that inhibitors of adhesion molecules between the PMNL and the EC, by hampering cell-cell contact, and making intercellular metabolic events much less efficient, may represent, in cooperation and synergy with sLT synthesis inhibitors (or receptor antagonists), the most efficient way to control cardiac inflammatory disorders.

REFERENCES


Table 1. Morphometric Characteristic of Coronary Artery and Myocardial Microvessels Obtained From Isolated Hearts of Rabbit

<table>
<thead>
<tr>
<th>Coronary artery</th>
<th>Group a</th>
<th>Group b</th>
<th>Group c</th>
<th>Group d'</th>
<th>Group d'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3 ± 0.12</td>
<td>0.4 ± 0.11</td>
<td>0.7 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.31 ± 0.11</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>11.7 ± 1.4</td>
<td>2.9 ± 0.7*</td>
<td>2.0 ± 0.88*</td>
</tr>
</tbody>
</table>

Myocardial microvessels

| 1               | 0.06 ± 0.01 | 0.06 ± 0.01 | 0.1 ± 0.01 | 0.06 ± 0.01 | 0.06 ± 0.01* |
| 2               | 0       | 0       | 8.9 ± 1.1 | 1.01 ± 0.3* | 0.9 ± 0.3* |
| 3               | 18.02 ± 1.0 | 18.0 ± 0.5 | 9.1 ± 1.1 | 17.8 ± 1.1* | 17.9 ± 1.0* |

Group a: isolated, perfused hearts of rabbit, challenged for 30 minutes with the calcium ionophore A-23187 (0.5 μmol/L) (n = 3); Group b: hearts perfused with unstimulated PMNL (10⁶ cells/mL) (n = 3); Group c: hearts perfused with PMNL and challenged with A-23187 (n = 4); Group d': hearts that had been pretreated with the sLT-receptor antagonist SKF 104353 (10 μmol/L), perfused with PMNL and challenged with A-23187 (n = 3); Group d": hearts that had been perfused with MK-886-treated PMNL (MK-886 1 μmol/L) and challenged with A-23187 (n = 3).

Abbreviations: 1, number of microvilli on the luminal surface of EC/μm²; 2, number of adherent leukocytes/mm²; 3, number of patent capillaries/mm².

Results are expressed as mean ± SE, where each sample was the mean of 50 observations.

* P < 0.05 v group c.


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