Polymorphonuclear Leukocytes Induced Vasoconstriction in Isolated Canine Coronary Arteries

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To assess how polymorphonuclear leukocytes (PMNs) act on coronary vasoactivity, we measured the changes in isometric tension of isolated rings of canine coronary arteries upon addition of autologous PMNs to organ chambers in which the rings were suspended. When PMNs isolated by the colloidal polyvinylpyrrolidone-coated silica (Percoll) gradient method were added to the chambers, ring preparations of left circumflex coronary arteries developed isometric tension. The increase in tension was dependent on the amount of PMNs (1×10^4 to 5×10^5 cells/ml). Maximal tension obtained by an optimal amount of PMNs (5×10^5 cells/ml) was almost comparable to that produced by prostaglandin F_2α (5 μM). Integrity of endothelial cells was not disrupted after the addition of PMNs because the developed tension could be reversed by the addition of acetylcholine in an endothelium-dependent manner. Mechanical rubbing of endothelium abolished the PMN-induced vasoconstriction, which was regained by placing an endothelium-unrubbed ring inside a rubbed ring (“sandwich preparation”). When PMN suspensions were pretreated with 5-lipoxygenase inhibitors of arachidonate, PMN-induced vasoconstriction was greatly suppressed, although the pretreatment of vascular preparations did not alter the development of isometric tension. These findings indicate that PMNs induce the contraction of coronary arterial rings in the presence of intact endothelial cells. The mechanism by which PMNs induce the contraction is the release of vasoconstrictive substances by metabolic interaction between PMNs and endothelial cells. Vasoconstrictive substances produced by the PMN-endothelial system, such as 5-lipoxygenase metabolites through a "leukotriene A_4 steal" mechanism, may contribute to the contraction of vascular smooth muscle. (Circulation Research 1990;66:253–258)

Blood corpuscles and endothelial cells regulate blood flow by a complex metabolic interaction. Aggregating platelets, which produce potent vasoactive substances such as thromboxane A_2, serotonin, and adenosine nucleotides, induce the contraction of blood vessels with defective endothelial cells because these vasoconstrictive substances act directly on vascular smooth muscle cells. On the other hand, blood vessels with intact endothelial cells are relaxed by aggregating platelets because vasoconstrictive substances produced by platelets act on vascular endothelial cells and induce the production of prostacyclin (PGI_2) through prostaglandin H_2 steal phenomenon or endothelium-derived relaxing factor (EDRF), which are potent vasodilative factors.

In addition to their adherence to the vascular wall and migration to tissue, leukocytes are known to have an intimate interaction with endothelial cells. The leukocyte-endothelial interaction has been suggested to play a pivotal role in the pathogenesis of vascular events of inflammatory reaction such as extravascular migration of leukocytes, increased vascular permeability, and leukocyte-mediated endothelial injury. Recent studies indicate that leukocytes augmented the extent of myocardial injury, particularly in reperfused myocardial tissue. There are many possible mechanisms by which leukocytes exacerbate the ischemic damage of myocardium. The development of vasotonicity induced by the altered leukocyte-endothelial interaction may be involved. Leukocytes release potent vasoactive substances, such as leukotrienes and active oxygen species that cause vascular contraction and endothelial injury, while endothelial...
lial cells produce potent vasodilators, such as PGI₂ and EDRF, and prevent leukocyte activation. Thus, leukocyte-endothelial interaction is implicated not only in the inflammatory reaction, but also in the pathogenesis of ischemic heart disease. Therefore, it is important to assess how leukocytes act on coronary vasoactivity through their interaction with endothelial cells. In this study, we investigated the changes in isometric tension of canine coronary arterial rings induced by the addition of autologous polymorphonuclear leukocytes (PMNs), the major class of leukocytes, which appear in the initial stage of myocardial infarction and inflammatory reaction, in the presence or absence of endothelial injury.

Materials and Methods

PMN Preparation

Autologous arterial blood was collected into heparinized plastic syringes and centrifuged at 180g for 10 minutes to obtain the buffy coat. Two milliliters of the buffy coat was layered over 3 ml of 55% isotonic colloidal polynvinylpyrrolidone-coated silica (Percoll, Pharmacia, Uppsala, Sweden) solution and centrifuged at 1,600g for 20 minutes. The PMN-rich band on the erythrocyte layer was taken, and contaminating erythrocytes were lysed with 0.15 M NH₄Cl. PMNs were resuspended in saline in the range of 4x10⁵ to 2x10⁷ cells/ml. Suspensions prepared in this method contained >92% PMNs. The contamination of mononuclear cells and platelets was <7% and <4%, respectively.

Tissue Preparation

Adult mongrel dogs of either sex (9–12 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Beating hearts were excised and immersed in cold Tyrode’s solution composed of (g/l) NaCl 8.0, KCl 0.2, CaCl₂ 0.2, MgCl₂ 0.1, NaH₂PO₄ 0.05, NaHCO₃ 1.0, and glucose 1.0. The left circumflex coronary artery was dissected free and cleaned of adherent connective tissue, then cut into ring preparations 4 mm long. Care was taken to avoid stretching and to keep endothelial cells intact or unrubbed. Most of the arteries used in this experiment had an outer diameter of 1.5–2.0 mm. In some vascular rings, endothelial cells were mechanically damaged by gentle rubbing of the intimal surface with a stainless steel tube (“rubbed rings”). A “sandwich preparation” was made by placing an unrubbed ring inside a rubbed ring (Figure 4, inset).

Organ Chamber Experiments

Arterial rings were suspended in water-jacketed organ chambers filled with 20 ml Tyrode’s solution maintained at 37°C and gassed continuously with 95% O₂-5% CO₂. Each ring was suspended on a pair of stainless steel hooks, one of which was fixed to the bottom of the chamber and the other to a force-displacement transducer (model UL-10GR, Shinkoh, Tokyo, Japan). The changes in isometric tension were recorded on a polygraph (model RM-6000, Nihon Kohden, Tokyo, Japan). Arterial rings were stretched gradually to a resting force of 7 g, during which the solution filling the organ chamber was replaced every 20 minutes. After equilibration for 60 minutes, all arterial rings were contracted with 5 μM prostaglandin F₂α (PGF₂α), and 5 μM acetylcholine (ACh) was subsequently added to the organ chamber for estimation of endothelial damage. Endothelium-unrubbed rings relaxed in response to ACh, whereas endothelium-rubbed rings did not. After the presence or absence of intact endothelial cells was thus confirmed, 0.5-ml portions of PMN suspension were added to the organ chamber containing vascular rings in 20 ml Tyrode’s solution. Antagonists of vasoactive substances were added to the organ chamber 10 minutes before the addition of PMNs in another series of experiments. We also pretreated PMN suspensions or vascular preparations with some antagonists for 10 minutes, washed them, and then added PMNs to the vascular rings. In these experiments, no stimulants were added to the organ chambers. In some experiments, 1 ml PMN suspension (1×10⁷ cells/ml) was incubated with or without calcium ionophore A23187 (2 μM) at 37°C for 5 minutes. After centrifugation at 1,600g for 10 minutes, PMN-free supernatant was added to the organ chamber instead of the PMN suspension. At the end of all experiments, the arterial rings were recontracted with PGF₂α (5 μM). The data obtained from any experiments were excluded if the initial and final contractile responses to PGF₂α differed by >10%.

Intimal surfaces of vascular rings were also examined by means of a scanning electron microscope for assessment of the endothelial damage of endothelium-rubbed arterial rings. The vascular rings were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3), dehydrated in graded series of ethanol and water mixtures, and critical-point dried. Subsequently, dried rings were dissected for exposure of the lumen, sputter-coated with gold, and examined. The unrubbed rings had smooth-surfaced and regularly arrayed endothelial cells. Although the denudation of endothelial cells was avoided, the rubbed rings had scraped and rough-surfaced endothelial cells.

Drugs

The following drugs were used: phenolamine mesylate from CIBA-Geigy Japan, Takarazuka, Japan; propranolol hydrochloride and atropine sulfate from Wako Pure Chemical Industries, Osaka, Japan; and catalase, superoxide dismutase, pyrilamine maleate salt, indomethacin, and esculetin from Sigma Chemical, St. Louis, Missouri. CV398811 and AA86112 were gifts from Takeda Chemical Industries, Osaka, Japan. Indomethacin and esculetin were dissolved in 50% ethanol, and AA861 was dissolved in 50% dimethyl sulfoxide. All other drugs were dissolved in 0.9% saline shortly before use.
Statistical Analysis

All data are expressed as mean±SEM and p values. Statistical analysis was performed by use of paired and unpaired t tests.

Results

Contractile Responses of Canine Coronary Arterial Rings to PMNs

When autologous PMNs (5×10⁵ cells/ml in final concentration) were added to the organ chamber, isometric tension increased substantially to 1.4 g in endothelium-unrubbed vascular rings. In the same experiment with endothelium-rubbed rings, PMNs did not affect isometric tension of vascular preparations (Figure 1, inset). PMNs were probably activated during the experiment, because the initially turbid solution gradually became clear and because, upon scanning electron microscopic observation, PMN aggregates were found to adhere to the intimal surface of vascular rings. These contractile responses of endothelium-unrubbed rings to PMNs were consistently observed in all vascular preparations tested. The observed PMN-induced vasoconstriction reached maximal tension in 5 minutes and lasted at least 20–30 minutes. The sustained contraction returned to basal tension after repeated washing. As the amount of added PMNs was increased from 1×10⁴ to 5×10⁵ cells/ml in final concentration, isometric tension of arterial rings increased linearly from 17±12% to 80±21%, expressed as percent of PGF₂α (5 μM)-induced contraction, above which a plateau value of approximately 90% was reached. The addition of similar amounts of PMNs did not induce the development of isometric tension in endothelium-rubbed arterial rings (Figure 1).

Characteristics of PMN-Induced Contraction of Arterial Rings

To assess the possibility that the endothelial damage was caused by the addition of PMNs and might predispose the rings to PMN-induced vasoconstriction, we added ACh (5 μM) to the organ chamber after endothelium-unrubbed vascular rings were contracted by PMNs (5×10⁵ cells/ml). In the vascular rings with intact endothelium, PGF₂α-induced precontraction was reversed in endothelium-dependent manner as the amount of ACh increased and reached the basal level at 5 μM ACh. In the endothelium-rubbed rings, precontraction was not reversed by ACh because damaged endothelial cells are deprived of EDRF-releasing activity in response to ACh. The vascular rings with intact endothelium precontracted by PMNs were relaxed immediately after the addition of ACh, suggesting that the arterial rings still possessed functional endothelial cells and endothelium-dependent relaxation (Figure 2).

To examine whether vasoconstrictive substances contained in PMN suspensions induced the contraction of vascular rings, we incubated 1-ml portions of PMN suspension (1×10⁵ cells/ml) with or without A23187 (2 μM) for 5 minutes at 37°C. After centrifugation at 1,600g for 10 minutes, PMN-free supernatants were successively added to the organ chamber containing endothelium-unrubbed arterial rings (Figure 3). In the absence of A23187 there was subtle or no contraction of coronary rings (9.2±4.3% of PGF₂α-induced contraction), while the addition of A23187 produced slight relaxation (−12.5±3.0%) due to the possible action of A23187 on vascular walls. Subsequent addition of 0.5 ml PMN suspension (2×10⁷ cells/ml) to the preparation containing A23187 induced pronounced contraction (75.6±27.2%, n=5).
The necessity of the presence of intact endothelial cells in PMN-induced vasoconstriction was confirmed by use of sandwich preparation (Figure 4, inset). PGF$_{2\alpha}$-induced contraction was reversed by the addition of ACh in sandwich preparations, indicating that the endothelial cells of unrubbed rings serve to supplement the functions of rubbed rings (data not shown). PMN suspensions were then added to the chambers containing unrubbed, rubbed, and sandwich preparations. The endothelium-rubbed preparation, in which PMNs induced little or no isometric tension (5.6$\pm$2.3% of PGF$_{2\alpha}$-induced contraction) when compared with the endothelium-unrubbed ring (80.5$\pm$12.5%), regained 29.4$\pm$7.0% contraction when a vascular ring with intact endothelium was placed inside the lumen (Figure 4).

To determine the chemical mediator that caused PMN-induced contraction, we added antagonists of vasoactive substances to the organ chamber 10 minutes before addition of PMNs. Receptor antagonists of $\alpha$-adrenergic (phenolamine, 3 $\mu$M), $\beta$-adrenergic (propranolol, 0.5 $\mu$M), cholinergic (atropine, 0.5 $\mu$M), histaminergic (pyrilamine, 10 $\mu$M), and platelet-activating factor (CV3988, 1 $\mu$M) did not attenuate this PMN-induced vasoconstriction, nor did radical scavengers (catalase, 130 units/ml, and/or superoxide dismutase, 60 units/ml) and arachidonate cyclooxygenase inhibitor (indomethacin, 1 $\mu$M). Among the inhibitors we used, only the arachidonate 5-lipoxygenase inhibitors, esculetin (1 $\mu$M) and AA861 (5 $\mu$M), decreased the development of isometric tension (24.7% and 19.7%, respectively, $p<0.05$, $n=8$).

To clarify whether these lipoxygenase inhibitors act on PMNs or vascular rings, we pretreated PMN suspensions or vascular preparations with incremen-
TABLE 1. Effect of Arachidonate Lipooxygenase Inhibitors on PMN-Induced Contraction of Vascular Rings

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<th>Contraction (%)</th>
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<tr>
<td>PMN suspensions</td>
<td>Vascular rings</td>
</tr>
<tr>
<td>Control</td>
<td>77.5±12.1</td>
</tr>
<tr>
<td>Esculetin (50 μM)</td>
<td>36.3±5.4*</td>
</tr>
<tr>
<td>AA861 (10 μM)</td>
<td>33.4±5.9*</td>
</tr>
</tbody>
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Contraction was expressed as percent of prostaglandin F2α (5 μM)-induced contraction (n=8). PMN, polymorphonuclear leukocyte.
*p<0.05 vs. control.

Discussion

We demonstrated that autologous PMNs induced sustained contraction of canine coronary arterial rings in a concentration-dependent manner. This PMN-induced contraction was abolished by rubbing of the intimal surfaces of vascular rings and was regained by the insertion of endothelium-unrubbed rings inside rubbed rings, suggesting that the presence of intact endothelial cells was essential in the PMN-induced contraction.

It is important to define the mechanism by which PMN suspensions induce contraction of canine coronary arteries with intact endothelial cells. There is a possibility that endothelial cells of vascular rings may be injured by potent cytotoxic substances, such as active oxygen species and lysosomal enzymes, released by PMNs. In fact, cultured endothelial monolayers have been shown to sustain injury by adhered leukocytes. Because endothelial cells release vasodilative substances such as EDRF and PGl2, endothelial damage caused by PMNs may result in the development of resting tension of vascular rings. However, in the present investigation, we demonstrated that endothelium-dependent relaxation induced by ACh, which is a sensitive indicator of functional endothelial cells, was preserved in the vascular rings contracted by PMNs, suggesting that endothelial integrity was maintained in this experiment.

Since PMNs release vasoconstrictive substances such as leukotrienes, histamine, and active oxygen species, these vasoactive substances contained in PMN suspension may act on the vascular wall, resulting in the observed PMN-induced vasoconstriction. However, such a mechanism does not gain support from the present finding that cell-free supernatant of PMN suspension, stimulated by A23187, did not induce vasoconstriction. The fact that PMNs subsequently added to the organ chamber induced the contraction of vascular rings indicates that the cell-to-cell interaction between PMNs and endothelial cells is essential in this PMN-induced contraction of vascular smooth muscle.

The importance of PMN-endothelial interaction was confirmed by sandwich preparations. PMN-induced contraction abolished by the rubbing of endothelial cells was regained by attachment of functional endothelial cells to rubbed surfaces of vascular rings. These findings indicate that a vasoconstrictive substance is released through the intercellular metabolism between PMNs and endothelial cells and is transmitted to vascular smooth muscle cells, thus inducing the contraction of vascular preparations.

There are many reports that the coexistence of PMNs and endothelial cells augments each other's cellular metabolism and enhances the production of vasoactive substances. Endothelial cells per se cannot synthesize vasoconstrictive leukotriene C4 (LTC4) due to the lack of leukotriene A4 (LTA4), a precursor of LTC4, while a mixed incubation of endothelial cells with PMNs significantly synthesized LTC4. These findings were interpreted by an "LTA4 steal" mechanism, in which LTA4 synthesized by PMNs was transferred intercellularly to endothelial cells and served as the substrate of the LTC4 synthetase system that exists in endothelial cells. The LTC4 steal phenomenon was also reported between PMNs and platelets. Our finding that 5-lipooxygenase inhibitors significantly attenuated PMN-induced contraction, especially when PMNs were pretreated with these drugs, indicates that 5-lipooxygenase metabolites of PMNs play a pivotal role in the PMN-induced contraction. It is possible that under these conditions LTC4 is produced by the endothelium-PMN system through an LTA4 steal mechanism and serves to contract vascular smooth muscles.

PMN-induced contraction of vascular rings with intact endothelial cells was reduced by about 60% with 5-lipooxygenase inhibitors; 60% of PMN-induced contraction may be explained by the LTA4 steal mechanism mentioned above. But the mechanism of the residual 40% of contraction remains unexplained. Besides LTC4 production through LTA4 steal phenomenon, endothelial cells were recently reported to produce vasoconstrictive substances. Rubanyi and Vanhoutte demonstrated that isolated canine coronary arterial rings with endothelium were contracted in an endothelium-dependent manner under anoxic conditions, suggesting production of vasoconstrictive factors from endothelial cells in anoxia. Cultured endothelial cells of bovine aorta were also reported to release vasoconstrictive substances that contracted isolated porcine coronary arteries and coronary arteries of perfused rabbit hearts. The vasoconstrictive activity in culture media was abolished by treatment with proteolytic enzymes.
Yangisawa et al. discovered a novel vasoconstrictive peptide, endothelin, from conditioned medium of porcine aortic endothelial cells and determined the structure of the peptide. We could not investigate the involvement of these peptidergic vasoconstrictive substances in our organ chamber system because treatment with proteolytic enzymes changed the contractile responses of vascular rings (data not shown). The contraction that was not abolished by the pretreatment with lipooxygenase inhibitors may be derived from the release of these vasoconstrictive peptides from endothelial cells stimulated by PMNs.

We demonstrated that the tonicity of vascular smooth muscle cells was regulated, at least partly, by the interaction of vascular endothelial cells with PMNs through intercellular leukotriene metabolism. For resolution of complicated metabolic interactions that regulate vascular tone, it is of utmost importance to determine the chemical nature of the substances that mediate the cell-to-cell relation in this system.

References

KEY WORDS • polymorphonuclear leukocyte • endothelial cell • endothelium-derived vasoconstrictive factor • LTA4, steal
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