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C-Reactive Protein Relaxes Human Vessels In Vitro

Leonid Sternik, Saquib Samee, Hartzel V. Schaff, Kenton J. Zehr, Lilach O. Lerman, David R. Holmes, Joerg Herrmann, Amir Lerman

Objective—C-reactive protein (CRP) is a sensitive marker of inflammation and a prognostic marker in cardiovascular disease. Evidence suggests direct biological activities of CRP within the vascular wall. The study was designed to examine the vasoreactive effects of CRP.

Methods and Results—Human internal mammary artery rings were obtained during cardiovascular bypass surgery and suspended in an organ bath chamber. The rings were precontracted with endothelin-1, and response to cumulative concentrations of CRP was obtained. Experiments were repeated after initial incubation with 20, 40, and 60 mmol/L KCl, the potassium channel blockers BaCl2, tetraethylammonium chloride, and glibenclamide, and the NO synthase inhibitor N-monomethyl-L-arginine or removal of the endothelium. CRP caused dose-dependent relaxation of human internal mammary artery rings, which was not affected by preincubation with N-monomethyl-L-arginine or removal of the endothelium. Maximum relaxation response to CRP (79.5 ± 10%) was attenuated by KCl (2.5 ± 11.5%, P < 0.001), BaCl2 (24.5 ± 7.5%, P < 0.001), and tetraethylammonium chloride (34.9 ± 8.25%, P < 0.01) but not by glibenclamide.

Conclusions—The present study demonstrates that CRP exerts an endothelium-independent vasorelaxing effect via potassium channels. Thus, the study suggests a role of CRP in the regulation of vascular tone. (Arterioscler Thromb Vasc Biol. 2002;22:1865-1868.)

Key Words: atherosclerosis ■ C-reactive protein ■ potassium channels ■ vasorelaxation ■ inflammation

C-reactive protein (CRP) has been recognized as a sensitive diagnostic marker for inflammation and has substantiated the view of atherosclerosis as an inflammatory disease in recent years.1,2 Importantly, elevated levels of CRP are associated with increased future risk of cardiovascular disease.2,3 Furthermore, there is increasing evidence of direct proinflammatory effects of CRP in the vascular wall.4 Thus, once considered to be only an innocent bystander, CRP might be actively involved in inflammation, including the mediation of its manifestations.1

CRP is expressed by liver hepatocytes after stimulation by interleukin-6 in an acute-phase response.5 It binds to altered self and foreign molecules, including LDLs.6 Within atherosclerotic diseased vessels, CRP deposition, which is normally completely absent, can be found at the intima-media border.7 Colocalization with complement factors within the diseased wall pointed toward a role in complement activation and enhancement of phagocytic activity in atherosclerosis.7-9 Recent findings extended this classical view by demonstrating that CRP can actively stimulate the expression of adhesion molecules by endothelial cells.10-12 Thus, CRP deposition within the vascular wall might contribute not only to the regulation of the inflammatory process but also to its manifestations, such as leukocyte recruitment.

Although vascular smooth muscle cells (VSMCs) are also in proximity to CRP deposition in the vascular wall and although reduction in vascular tone (and thereby hyperemia) is an integral part of inflammation, the vasoreactive properties of CRP are still unknown. Thus, the present study was designed to determine whether CRP is a vasoreactive substance and, if so, to determine the potential modes of action involved.

Methods

Tissue Sampling
The study was approved by the Mayo Institutional Review Board. Residual segments of IMA were obtained during surgery from 16 patients undergoing coronary artery bypass grafting at the Mayo Clinic, Rochester, Minn, and immediately placed in a cold, serum-free Krebs-Ringer bicarbonate solution of the following millimolar composition (control solution): NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, Ca-EDTA 0.026, and glucose 11.1. Directly before the experiment, the vessels were carefully cleaned of adherent fat and connective tissue and cut into rings (4 mm long). Endothelium negative (E−) vessel rings were obtained by gentle rubbing with a metal wire. No more than 2 rings per tissue sample were used for one organ bath experiment.

Organ Bath Experiments
As described before,13 rings of IMA were transferred to organ chambers filled with 25 mL control solution (37°C, pH 7.4) and...
gassed with 94% oxygen and 6% carbon dioxide. Rings were mounted between 2 hooks attached to an isometric force transducer with continuous recording of tension. After stabilization at resting tension for 45 minutes, viability of the tissue was documented by a point of relaxation that was considered to be the maximal relaxation of the vessel (100% relaxation). Recombinant, Escherichia coli–derived CRP was purified by two-stage affinity column chromatography with the use of phosphoryl choline. The purity of CRP preparations was confirmed by SDS-PAGE; no contaminating proteins were detected by silver staining of overloaded gels. CRP was confirmed to be endotoxin-free twice by the limulus test (Sigma Chemical Co, sensitivity 0.125 EU/mL). Precautions were taken to avoid polysaccharide contamination during the experiments.

Determination of Role of Endothelium in Vasoactive Effect of CRP
To elucidate the role of endothelium in the vasoactive properties of CRP in human IMA, the endothelium was mechanically removed as previously described, and after precontraction with ET-1 and 30 minutes of equilibration, the dose response to CRP was assessed.14,15 To determine the role of the NO pathway in the vasoactive properties of CRP and to study the effect of the endogenous NO pathway, 10−8 mol/L of N-nomonomethyl-L-arginine (L-NMMA, Sigma) was added 20 minutes before the precontraction with ET-1.

Determination of Role of Potassium and Chloride Channels
To determine whether potassium channels mediate the vasoconstriction effects of CRP, additional arteries with intact endothelium were incubated with 20, 40, and 60 mmol/L KCl before precontraction with ET-1 and cumulative concentrations of CRP. To exclude the possibility that the effect of KCl was mediated through chloride channels, additional arteries were exposed to 10−6 mol/L DIDS (Sigma) 20 minutes before contraction with ET-1 and relaxation with CRP. To specifically block different potassium channels, the following potassium channel inhibitors were added 20 minutes before contraction with ET-1 and relaxation with CRP: 10−6 mol/L glibenclamide (Research Biochemicals International), an inhibitor of ATP-sensitive potassium channels; 10−4 mol/L tetraethylammonium chloride (TEA, Sigma), an inhibitor of calcium-activated potassium (KCa+) channels; and 10−7 mol/L BaCl (Sigma), an inhibitor of inward rectifier potassium (Kir) channels.13,14

Data Analysis
Vasorelaxation was expressed relative to the maximum contraction induced by ET-1 (n indicates the number of IMA rings used for a certain experiment). The maximum vasorelaxation response in each group was expressed as mean±SEM. Group comparison was made by 2-way ANOVA. Statistical significance was accepted at a value of P<0.05.

Results
Patient Population
The demographic and clinical characteristics of the patient population are outlined in the Table.
This is further supported by the absence of complement system within the atherosclerotic vasculature, which might conceivably imply additional biological activity beyond its role as a marker and mediator of inflammation, CRP seems to be a functional significance to these pathological findings has been suggested by colocalization with components of the complement system within the intima without CRP. Indeed, CRP does activate the expression of adhesion molecules, pointing toward a direct role of CRP in leukocyte recruitment. Furthermore, it has been identified as an important modulator in the inflammatory process. As demonstrated by immunohistological studies, deposition of CRP can be found in the subendothelial space in atherosclerotic vessels. A first functional significance to these pathological findings has been suggested by colocalization with components of the complement system within the atherosclerotically diseased wall. This is further supported by the absence of complement in the intima without CRP. Indeed, CRP does activate the classical complement pathway and enhances phagocytic activity in fulfillment of its function as an important mediator of innate immunity. Within the atherosclerotically diseased vessel, this mode of CRP-mediated opsonization might be important for LDL uptake by macrophages and foam cell formation. Yet CRP deposition in the atherosclerotic vascular wall might conceivably imply additional biological activity. For example, recent in vitro studies have demonstrated that incubation of endothelial cells with CRP results in a direct endothelium-independent vasorelaxing effect on VSMCs, thereby potentially contributing to vasodilation and hyperemia in inflammation.

Indeed, vasodilation is one of the elements of the inflammatory response leading to an increase in tissue perfusion and temperature. Histamine and bradykinin have been considered to be central mediators in this response because they are both potent endothelium-dependent vasodilators. According to this classical concept, no role was attributed to CRP other than that of exerting a regulatory function in the inflammatory cascade, leading to the production of these vasodilatory mediators. Yet beyond this concept, the present findings of a direct vasorelaxing effect of CRP suggest that CRP might have a more direct, rather than a solely indirect, role in the hyperemic response characterizing inflammation. This might even add further pathophysiological significance to increased CRP levels, inasmuch as they are observed in acute coronary syndromes. Given a role in inflammatory vasodilation, increased CRP levels and deposition in the plaque might enhance plaque perfusion through neovessels and, thereby, plaque metabolism and temperature. Indeed, a recent study has demonstrated that plaque temperature is related to systemic CRP levels. However, how this relates to plaque stability and the confirmation of these putative direct biological effects of CRP on atherosclerotic lesion remains the subject of future studies. Thus, CRP might be not only a marker for plaque inflammation but also a modulator of plaque inflammation and plaque stability.
infection, inflammation, or tissue injury. The most important prognostic implications of elevated serum concentrations of CRP in cardiovascular disease were first recognized for patients with acute coronary syndromes. Unstable angina patients with serum concentrations of CRP >3 μg/mL at the time of admission are at increased risk of major adverse cardiac events during the hospital stay, and this association is even stronger with admission serum concentrations of CRP >10 μg/mL. In the present study, we used cumulative concentrations of 10−10 to 10−6 mol/L of CRP, which corresponds to serum concentrations of 0.0023 to 23 μg/mL. Of note, an accentuation of the vasorelaxation was observed at concentrations ≥10−7 mol/L and thus at clinically relevant, corresponding serum concentrations.

Hyperpolarization of VSMCs in response to activation of potassium channels has been recognized as an important major mechanism of vasodilation. Among the 4 existing potassium channels involved in this response, KCa+ and Kir channels are of utmost importance. Of note, these are the same potassium channels which were identified to mediate the direct vasodilatory effect of CRP in the current study based on the findings that coinubcation with either TEA or BaCl abolished the vasodilatory effect of CRP. On the contrary, glibenclamide, a selective inhibitor of ATP-sensitive potassium channels, did not affect the CRP-mediated vasorespons; neither was coinubcation with DIDs, a selective chloride channel inhibitor, associated with inhibition of the CRP response. Second messengers and intracellular signaling pathways, which might be involved in this biological action of CRP, will have to be identified by future studies. Thus, CRP may exert a relaxing effect on VSMCs via activation of KCa+ and Kir channels with further underlying molecular mechanisms remaining to be determined.

In conclusion, the present in vitro study demonstrates that CRP mediates vasorelaxation in human IMAs via the effect on VSMCs, involving KCa+ and Kir channels. Therefore, beyond its status as a serum marker and modulator of the inflammatory process, CRP might have a role in the regulation of vascular tone, with significance, for instance, for hyperemia in inflammation.

Acknowledgments

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