Natriuretic Peptide Receptor-C Regulates Coronary Blood Flow and Prevents Myocardial Ischemia/Reperfusion Injury: Novel Cardioprotective Role for Endothelium-Derived C-Type Natriuretic Peptide

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Natriuretic Peptide Receptor-C Regulates Coronary Blood Flow and Prevents Myocardial Ischemia/Reperfusion Injury

Novel Cardioprotective Role for Endothelium-Derived C-Type Natriuretic Peptide

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Background—Ischemia/reperfusion (I/R) injury complicates myocardial infarction and stroke by exacerbating tissue damage and increasing risk of mortality. We have recently identified C-type natriuretic peptide (CNP) as an endothelium-derived hyperpolarizing factor in the mesenteric resistance vasculature and described a novel signaling pathway involving activation of natriuretic peptide receptor C (NPR-C), which plays a pivotal role in the regulation of local blood flow. We tested the hypothesis that CNP/NPR-C signaling is a novel regulatory pathway governing coronary blood flow and protecting against I/R injury.

Methods and Results—CNP and (Cys18)-atrial natriuretic factor (4-23) amide (cANF$^{4-23}$) elicited dose-dependent decreases in coronary perfusion pressure (CPP) that were blocked by Ba^{2+} and ouabain in the isolated Langendorff rat heart. The endothelium-dependent vasodilator acetylcholine elicited the release of CNP from the coronary endothelium. CNP and cANF$^{4-23}$ reduced infarct size after 25 minutes of global ischemia and 120 minutes of reperfusion, maintaining CPP and left ventricular pressure at preischemic values. The vasorelaxant and protective activity of CNP and cANF$^{4-23}$ were enhanced in the absence of endothelium-derived nitric oxide.

Conclusion—Endothelium-derived CNP is involved in the regulation of the coronary circulation, and NPR-C activation underlies the vasorelaxant activity of this peptide. Moreover, this newly defined pathway represents a protective mechanism against I/R injury and a novel target for therapeutic intervention in ischemic cardiovascular disorders. **(*Circulation*. 2004;110:1231-1235.)**

Key Words: endothelium-derived factors ■ myocardial infarction ■ natriuretic peptides ■ cardiovascular diseases ■ ischemia

Restoration of blood flow, or reperfusion, of ischemic tissues is essential for limiting the damage caused by acute myocardial infarction and salvaging organ function. However, reperfusion per se exerts detrimental effects by extending cell death and loss of myocardial and coronary function beyond that achieved by the ischemic insult itself; this phenomenon has been termed ischemia/reperfusion (I/R) injury.[1,2] I/R injury is characterized by microvascular dysfunction, in particular, the loss of endothelium-derived dilators, such as nitric oxide (NO) and prostacyclin, that results in capillary constriction and decreased perfusion, increased fluid and cellular extravasation, and leukocyte plugging.[1,2] Hence, considerable attention has focused on identifying endogenous pathways and therapeutic interventions that prevent or reverse microvascular dysfunction and thereby minimize I/R injury.[1,2]

C-type natriuretic peptide (CNP) represents the paracrine element of the natriuretic peptide axis, complementing the endocrine actions of atrial natriuretic peptide and brain natriuretic peptide to lower blood volume and pressure. We have recently identified CNP as an endothelium-derived hyperpolarizing factor (EDHF) in mesenteric resistance arteries[3] and defined a novel signaling pathway, important in the regulation of vascular tone and local blood flow, involving activation of the natriuretic peptide receptor type C (NPR-C) and opening of a G protein–coupled inwardly rectifying K^+ channel (GIRK).[3] CNP relaxes coronary arteries in vitro,[4,5] and we have demonstrated that endothelial CNP is released in response to endothelium-dependent vasodilators.[3] Intriguingly, activation of inwardly rectifying K^+ channels (K_{IR}) reduces myocardial I/R injury,[6–8] myocardial release of CNP occurs during heart failure,[9] and CNP is found in high concentrations in atherogenic lesions in coronary arteries.[10]

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Herein, we demonstrate that the CNP/NPR-C signaling pathway contributes to the regulation of blood flow and tissue perfusion in the coronary vasculature and that CNP is likely to represent a coronary EDHF. Moreover, we reveal that the CNP/NPR-C pathway exerts a potent protective effect against I/R injury and represents a novel target for the treatment of ischemic cardiovascular disorders.

Methods

Measurement of Coronary Hemodynamics and Cardiac Function

Male Wistar rats (260 to 340 g; Tuck, Rayleigh, UK) were surgically anesthetized with pentobarbital (45 mg/kg IP) and anticoagulated with heparin (1000 IU/kg IP). Hearts were excised and perfused retrogradely via the aorta, as previously described.[11] Coronary perfusion pressure (CPP) and left ventricular developed pressure (LVDP) were continuously measured by inline pressure transducers (Becton Dickinson), as previously described.[11]

Mechanism of CNP Vasorelaxant Activity

CNP (0.03 to 10 nmol) dose-response curves were constructed in the absence and presence of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 300 μmol/L), the cyclooxygenase inhibitor indomethacin (5 μmol/L), or a combination of barium (30 μmol/L) plus ouabain (1 mmol/L; blockers of K_{IR} and Na^+/K^+-ATPase, respectively). In some experiments, the vasodilator responses of CNP (10 nmol) and the selective NPR-C agonist (Cys18)-atrial natriuretic factor (4-23) amide ($cANF^{4-23}$) (3 nmol) in the absence and presence of the NPR-C antagonist M372049[12] (100 nmol/L, 15-minute pretreatment) were determined.

Bioassay of CNP

Effluent (60 mL) was collected before and during acetylcholine (ACh) application in hearts with an intact endothelium or after endothelium denudation (achieved by using 60 μL of 1% Triton X-100 delivered at 30 μL/min followed by a 20-minute recovery period); endothelial and smooth muscle integrity was tested before and after removal by using ACh (3 nmol) and sodium nitroprusside (10 nmol), respectively. Samples were concentrated on C18AR columns (DRG Diagnostics), and CNP concentrations were determined by using a commercially available radioimmunoassay (DRG Diagnostics) as previously described.[3]

Effects of CNP on I/R Injury

After equilibration, hearts were treated with CNP (30 nmol/L), CNP plus M372049 (100 nmol/L), $cANF^{4-23}$ (30 nmol/L), or saline vehicle (30 minutes) and then subjected to global ischemia (25 minutes) followed by reperfusion (120 minutes). In some experiments, CNP (30 nmol/L, 30 minutes) was administered only at the time of reperfusion. To identify infarcted tissue after I/R insult, hearts were cut into small pieces and incubated with *p*-nitroblue tetrazolium (0.5 mg/mL, 20 minutes at 37°C).[13] Infarct size was expressed as a percentage of the dry weight of the infarcted areas over the total weight of the heart.

Data and Statistical Analysis

All data are expressed as mean±SEM, where n is the number of animals used. For comparison of single points, 1-way ANOVA was used, and for comparison of dose-response curves to CNP, 2-way ANOVA was used. Values were considered significantly different when $P<0.05$.

Results

Regulation of Coronary Perfusion by CNP/NPR-C Signaling

CNP (0.03 to 10 nmol) produced a dose-dependent decrease in CPP (median effective concentration, 9.47±0.71 nmol; E_{max}=3.81±0.29 mm Hg; n≥4). The potency and maximal effect of CNP were significantly enhanced in the presence of L-NAME plus indomethacin but not altered in hearts perfused with the vasoconstrictor U46619 (1 μmol/L) to produce an equivalent increase in CPP (data not shown), and responses were attenuated in the presence of Ba^{2+} plus ouabain (Figure 1). The selective NPR-C agonist $cANF^{4-23}$ also decreased CPP in a Ba^{2+} plus ouabain–sensitive manner (Figure 1, inset). The responses to both CNP and $cANF^{4-23}$ were blocked by the selective NPR-C antagonist M372049 (Figure 1).

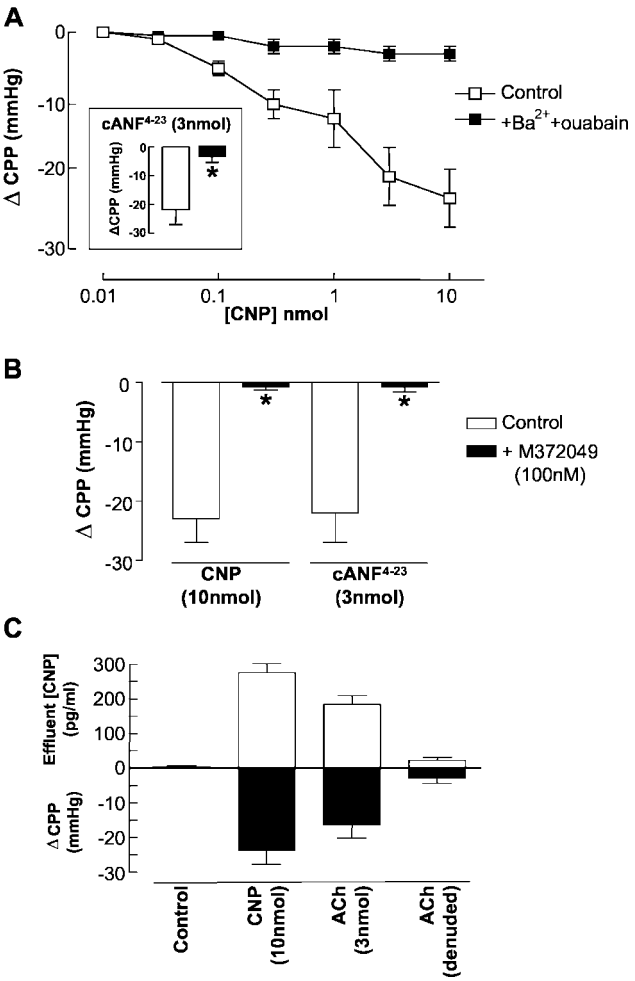

Figure 1. A, Dose-dependent decrease in CPP by CNP (0.03 to 10 nmol) in isolated, perfused rat heart (Langendorff) in presence and absence of Ba^{2+} (30 μmol/L) plus ouabain (1 mmol/L). Inset: Decrease in CPP induced by selective NPR-C agonist $cANF^{4-23}$ (3 nmol) in presence and absence of Ba^{2+} plus ouabain. B, Blockade of CNP- (10 nmol) and $cANF^{4-23}$- (3 nmol) induced vasodilation by NPR-C antagonist M372049 (100 nmol/L). C, Changes in CPP and concomitant release of CNP into coronary effluent in response to CNP (10 nmol) and ACh (3 nmol) in presence and absence of endothelium. All experiments were conducted in presence of L-NAME (300 μmol/L). *$P<0.05$ vs corresponding control, n≥5. All abbreviations are as defined in text.

Release of CNP by the Coronary Vascular Endothelium

CNP concentrations in the effluent increased significantly after administration of ACh and mirrored the changes in CPP;

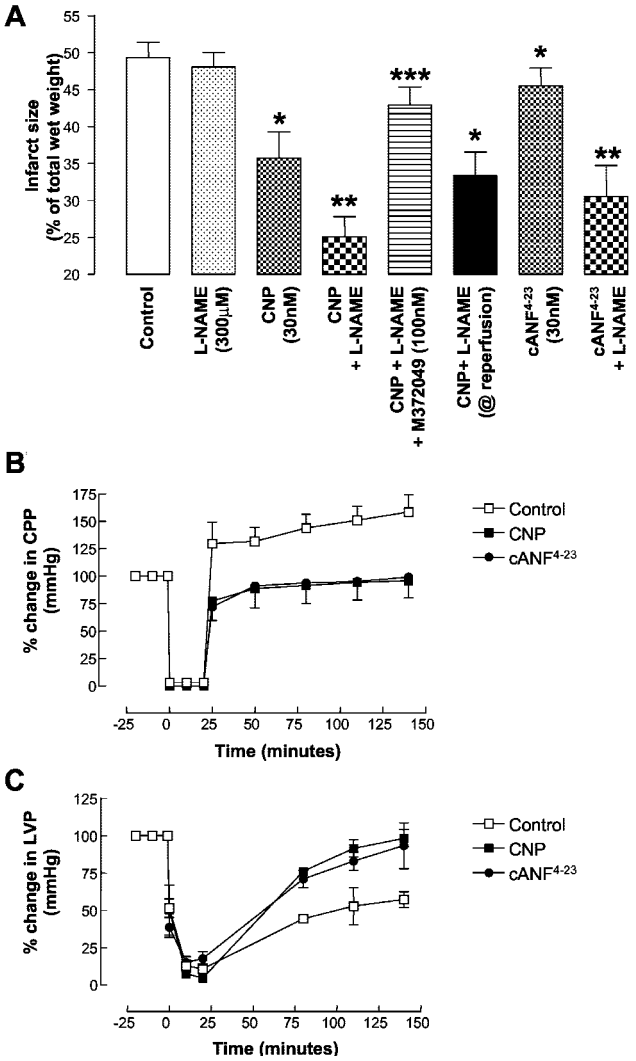

Figure 2. A, Infarct size as percentage wet weight in isolated, perfused rat heart (Langendorff) subjected to 25 minutes of global ischemia followed by 120 minutes of reperfusion in presence and absence of CNP (3 nmol), $cANF^{4-23}$ (3 nmol), L-NAME (300 μmol/L), and M372049 (100 nmol/L). B, CPP and C, LVDP in isolated, perfused rat heart (Langendorff) subjected to 25 minutes of global ischemia followed by 120 minutes of reperfusion in presence and absence of CNP (3 nmol) or $cANF^{4-23}$ (3 nmol). *$P<0.05$ vs control, **$P<0.05$ vs CNP or $cANF^{4-23}$ alone, ***$P<0.05$ vs CNP+L-NAME. n≥5. All abbreviations are as defined in text.

responses were absent after endothelial denudation (Figure 1; n≥5).

CNP/NPR-C Signaling Protects Against I/R Injury

The I/R insult in control hearts produced an infarct of ≈50% (Figure 2) and a concomitant increase in CPP and a reduction in LVDP (Figure 2). In the presence of CNP (during ischemia and reperfusion), CPP and LVDP were maintained at preischemic levels (Figure 2), with a significant reduction in infarct size that was more pronounced in hearts pretreated with L-NAME (Figure 2). In addition, in the presence of CNP, LVDP (85.9±12.4 mm Hg), $-dP/dt$ (94.8±8.0 mm Hg/s), and left ventricular end-diastolic pressure (29.8±1.9 mm Hg) were significantly improved (ie, nearer to preischemic values) compared with controls (53.9±7.8 mm Hg, 80.2±7.4 mm Hg/s, and 49.8±1.9 mm Hg, respectively; $P<0.05$ for each, n≥5) after 120 minutes of reperfusion. Infusion of CNP during reperfusion only also caused a significant reduction in I/R injury (Figure 2).

Similarly, $cANF^{4-23}$ protected the heart from I/R injury, reducing infarct size and maintaining CPP and LVDP (Figure 2), an effect significantly enhanced in the presence of L-NAME. Moreover, M372049 attenuated the beneficial effects of CNP (Figure 2).

Discussion

We have recently described a novel signal transduction pathway in the vasculature involving endothelium-derived CNP, activation of NPR-C, and opening of a GIRK to bring about smooth muscle hyperpolarization and relaxation; such a pathway is likely to underlie the action of EDHF.[3] In the present study, we have demonstrated that endothelial CNP also contributes to the regulation of coronary blood flow and, moreover, that the mechanism of action of CNP in the coronary vasculature occurs through an identical NPR-C coupling that we have characterized in the mesenteric vasculature. Not only is CNP/NPR-C signaling important in regulating coronary perfusion, but also its activation represents a protective mechanism against I/R injury by reducing infarct size and maintaining CPP and LVDP at preischemic levels. Thus, CNP/NPR-C signal transduction is likely to represent a widespread mechanism for the regulation of local blood flow and tissue perfusion and a novel therapeutic target for the treatment of ischemic cardiovascular disorders.

In isolated, perfused hearts, CNP elicited a potent, dose-dependent relaxation of coronary arteries, an effect blocked by treatment with Ba^{2+} plus ouabain, a combination of inhibitors that is generally accepted to block EDHF-dependent hyperpolarization.[14] Moreover, the actions of CNP were mimicked by the selective NPR-C agonist $cANF^{4-23}$, and the actions of both CNP and $cANF^{4-23}$ were abolished by the selective NPR-C antagonist M372049[12]; such observations confirm that NPR-C activation underlies the vasorelaxant activity of CNP. Furthermore, the endothelium-dependent dilator ACh evoked the release of CNP into the coronary circulation. Together, these observations suggest that endothelium-derived CNP is likely to act as a coronary EDHF and to play a role in the regulation of blood flow in the coronary vasculature. This hypothesis is supported by previous publications indicating that CNP can mediate hyperpolarization of coronary arteries[4,15] and that shear stress upregulates CNP expression in human endothelial cells.[16,17]

Interestingly, the vasoactivity of CNP was increased in the presence of NO synthase inhibition, in accord with previous observations made in endothelium-denuded coronary arteries[5] and with the requirement for NO blockade to reveal EDHF bioactivity.[14] This phenomenon implies complementary cytoprotective roles for CNP and NO in the coronary vasculature, whereby the loss of one pathway

might be compensated for by upregulation of the alternative system. This is of particular significance for ischemic cardiovascular disorders because they are characterized by loss of endothelium-derived NO bioactivity.[1] Under such conditions, the influence of CNP/NPR-C may be heightened, and moreover, drugs mimicking the biological activity of CNP (ie, NPR-C agonists) may prove to be important new medicines to treat these diseases. This proposal is supported by the recent report that CNP release from the (coronary) endothelium is increased during cardiovascular disease.[9,18]

Having demonstrated the existence and vasorelaxant activity of a CNP/NPR-C transduction system in the coronary circulation, we performed further experiments to assess whether this novel signaling pathway represents a protective mechanism against I/R injury. Global ischemia followed by reperfusion produced a characteristic increase in CPP, a decrease in LDVP, and an associated area of infarction corresponding to ≈50% of total tissue weight. Treatment with either CNP (at concentrations comparable to that produced endogenously by the endothelium[3]) or $cANF^{4-23}$ protected hearts against the damaging effects of I/R injury with suppression of both infarct size and myocardial dysfunction. This protective effect was enhanced in the absence of endothelium-derived NO (ie, in the presence of L-NAME) but suppressed by NPR-C blockade with M372049. Moreover, administration of CNP during the reperfusion period alone also reduced I/R injury, suggesting that NPR-C activation may prove beneficial in patients presenting with an acute ischemic episode. Together, these data confirm that CNP/NPR-C represents a protective mechanism against I/R injury and add further weight to the importance of this newly defined transduction system in regulating cardiovascular homeostasis. Interestingly, several recent publications indicate that the bioactivity of EDHF is unaffected or actually upregulated after I/R injury,[19,20] perhaps linking the bioactivity of CNP as an EDHF with a protective mechanism limiting microvascular dysfunction.

The mechanism by which CNP/NPR-C exerts a protective effect against I/R injury remains unclear and merits further attention. Activation of NPR-C in the coronary vascular bed (as we have demonstrated in the mesenteric vasculature) results in the opening of a (G protein–gated) K_{IR}, because responses to CNP were sensitive to Ba^{2+}. Reduced K_{IR} channel activity has been demonstrated previously to exacerbate I/R injury,[6–8] providing evidence that preserved activity of such channels, as would be achieved by NPR-C activation, is beneficial in minimizing I/R injury. Moreover, evidence accumulated in recent years provides considerable support for a common mechanism underlying the protective effect of several mediators (eg, NO, bradykinin, and adenosine) in I/R injury.[21] Although the processes involved are far from clear, it is generally accepted that the opening of a K_{ATP} channel, either on the cytoplasmic and/or mitochondrial membrane, underlies the protective effects.[22] Thus, the beneficial effect of CNP in I/R injury may be mediated via opening of a GIRK, which results in a similar change in net K^+ flux as that achieved by K_{ATP} channel opening. Alternatively, as the myocardial K_{ATP} channel belongs to the K_{IR} superfamily,[23,24] direct activation of the K_{ATP} channel by CNP cannot be excluded.

In sum, we have provided strong evidence that CNP modulates perfusion of the heart by activation of NPR-C and is likely to represent a coronary EDHF. We have also demonstrated that CNP/NPR-C signal transduction represents a novel protective mechanism against I/R injury. Such observations add considerable weight to the thesis that activation of NPR-C represents an important, cGMP-independent activity of CNP in the regulation of vascular tone. These actions, in combination with the protective effect of CNP/NPR-C in preventing I/R injury, highlight this signaling pathway as a novel therapeutic target to treat ischemic vascular disease (ie, myocardial infarction, stroke) and other cardiovascular disorders (eg, hypertension, atherosclerosis, restenosis).

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