Coronary vasodilator effects of BNP: mechanisms of action in coronary conductance and resistance arteries

CHRISTIAN ZELLNER,1 ANDREW A. PROTTER,2 EITETSU KO,3 MADHUSUDHAN R. POTHIREDDY,1 TERESA DeMARCO,3 STUART J. HUTCHISON,1 TONY M. CHOU,3 KANU CHATTERJEE,3 AND KRISHNANKUTTY SUDHIR3

1The Vascular Research Laboratory, Division of Cardiology, University of California at San Francisco, San Francisco 94143-0124; 2Scios Incorporated, Mountain View, California 94043; and 3The Hormones and Vascular Laboratory, Baker Institute, Melbourne, Victoria 3181, Australia

Zellner, Christian, Andrew A. P rotter, Eitetsu Ko, Madhusudhan R. Pothireddy, Teresa DeMarco, Stuart J. Hutchison, Tony M. Chou, Kanu Chatterjee, and Krishnankutty Sudhir

Coronary vasodilator effects of BNP: mechanisms of action in coronary conductance and resistance arteries. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1049–H1057, 1999.—Brain natriuretic peptide (BNP), a hormone secreted predominantly in ventricular myocytes, may influence coronary vascular tone. We studied the coronary vasodilatory response to BNP under physiological conditions and after preconstriction with endothelin-1 (ET-1) in anesthetized pigs. Average peak-flow velocity (APV) was measured using intracoronary Doppler, and cross-sectional area (CSA) was measured using intravascular ultrasound. Coronary blood flow (CBF) was calculated. Intracoronary BNP induced dose-dependent increases in CSA, APV, and CBF similar in magnitude to those induced by nitroglycerin (NTG). The magnitude of BNP-induced vasodilation was accentuated after preconstriction with ET-1. Pretreatment with either the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester or the cyclooxygenase inhibitor indomethacin attenuated the coronary vasodilator effect of BNP in resistance arteries without influencing epicardial vasodilation. Pretreatment with the ATP-sensitive potassium-channel blocker glibenclamide enhanced epicardial vasodilation in response to BNP. We conclude that BNP exerts coronary vasodilator effects, predominantly in epicardial conductance vessels. An accentuated vasodilatory response to BNP occurs in ET-1-precontracted arteries. BNP-induced vasodilation in coronary resistance arteries may be partially mediated via nitric oxide and/or prostaglandin release.

brain natriuretic peptide; vascular reactivity; pig; endothelin-1; N-nitro-L-arginine methyl ester; indomethacin

BRAIN NATRIURETIC PEPTIDE (BNP) is a cardiac tissue-derived peptide hormone that participates in the maintenance of body fluid and electrolyte homeostasis (3). BNP is structurally and functionally related to atrial natriuretic peptide (ANP); however, it has a distinct pharmacological profile with regard to binding of the known natriuretic peptide receptors (NPRs) (2, 45). Animal and human studies have demonstrated that the diuretic, natriuretic, and hemodynamic effects of BNP (16, 29, 50), whereas the direct vasodilator effects of BNP have recently been reported in isolated human arterial and venous tissue preparations (38). BNP has been suggested to induce vasodilation in vivo in the human forearm and in pulmonary circulation (5, 35), and the density of NPRs in the heart, especially in the coronary vasculature, suggests that BNP may also exert coronary vasoactive effects (7). Okumura et al. (37) showed that administration of BNP induces vasodilation in coronary conductance arteries. In patients with variant angina, hyperventilation-induced anginal attacks and electrocardiogram (ECG) changes are reportedly suppressed by intravenous infusion of BNP in supraphysiological doses (21). However, the mechanisms underlying these observed coronary responses are not clear.

BNP activates the membrane-bound guanylyl cyclase-A (GC-A) receptor, which results in the accumulation of intracellular cGMP in target tissues (27). It is generally believed that cGMP mediates the vascular smooth muscle relaxant effect of BNP, presumably in a manner similar to nitric oxide donors such as nitroglycerin (NTG). Increases in coronary resting tone, induced by the novel natriuretic peptide antagonist HS-142–1 (46, 48), suggest that natriuretic peptides also play a role in the regulation of basal coronary blood flow and vascular tone. In addition, human BNP relaxes human arteries and veins precontracted with phenylephrine or the endogenous peptide endothelin-1 (ET-1) (4, 38). Although secretion of ET-1 is reportedly inhibited by both ANP and BNP (12), the effects of BNP on ET-1-mediated coronary vasoconstriction remain unclear.

In this study we investigated the coronary vasodilator properties of BNP in domestic swine in vivo. We examined the effects of BNP on coronary conductance and resistance arteries under conditions of both normal vascular tone and ET-1-induced preconstriction. We also studied the possible contributions of nitric oxide, prostaglandins, and ATP-sensitive potassium channels to BNP-induced coronary vasodilation.

METHODS

Twenty-three female domestic swine (mean wt 42 ± 0.8 kg) were anesthetized with Innovar (0.04 mg/kg sc) and α-chloralose (100 mg/kg iv), with additional doses of α-chloralose given as needed to maintain the level of anesthesia. Animals were mechanically ventilated with room air. Heart rate was monitored from the ECG, and blood pressure was monitored from a canula placed in the left femoral artery. All studies conformed to the “Position of the American Heart Association (AHA) on Research Animal Use” adopted November 11, 1984, by the AHA. The protocol was approved by the University of California at San Francisco Committee on Animal Research (approval no. A7916–12776–01).

Catheterization procedures. Under fluoroscopic guidance, the left main coronary artery was cannulated via the transfemoral approach using an 8-Fr Amplatz R-1 guiding catheter (Advanced Cardiovascular Systems, Temecula, CA). As previ-
ously described (9), a 0.014-in. Doppler wire (Endosonics, Rancho Cordova, CA) was first introduced through the guiding catheter, after which a 3.2-Fr 30-MHz ultrasound imaging catheter (Boston Scientific, Sunnyvale, CA) was introduced directly over the Doppler wire into the circumflex coronary artery. The Doppler transducer was positioned 2 cm distal to the tip of the imaging catheter (9). Transvenous atrial pacing at a rate of 110 beats/min was used during the entire study to prevent changes in heart rate.

Experimental protocols. Unless otherwise indicated, pharmacological agents were administered directly into coronary circulation through the guiding catheter in the ostium of the left main coronary artery. Measurements of coronary artery cross-sectional area (CSA) and flow velocity were made at 30-s intervals after each administration. Intracoronary drug infusions were made over a 1-min period unless otherwise specified; final concentrations in the coronary artery are indicated, assuming a flow rate of 50 ml/min, as previously described (9, 44). Saline infusions given before drug administration served as control. Human BNP was obtained from Scios (Mountain View, CA), and, unless otherwise noted, other pharmacological agents were obtained from Sigma Pharmaceutical (St. Louis, MO).

In 11 pigs, BNP was infused at concentrations increasing from 1 pM to 0.1 µM. NTG was infused at concentrations increasing from 0.1 nM to 10 µM. For each infusion concentration, sufficient time (range 5–9 min) was allowed for epicardial coronary dimensions and flow velocity to return to baseline before the next dose was administered. The vasodilator effects of BNP and NTG were also assessed after preconditioning with 10 nM ET-1 (n = 11 for BNP; n = 8 for NTG) as a continuous intracoronary infusion over 10 min. In an additional five pigs, the effects of concentrations of BNP at the peak of the dose-response curve (10 nM to 0.1 µM) were assessed after the following three pharmacological interventions, performed in random order. 1) Nitric oxide synthesis was inhibited by intracoronary administration of N-nitro-L-arginine methyl ester (L-NAME) to obtain a final concentration of 10 mM in the coronary artery (43). In a separate set of eight pigs, NTG was infused at concentrations increasing from 0.1 nM to 10 µM before and after L-NAME. 2) Prostaglandin synthesis was inhibited by intravenous infusion of indomethacin (2 mg/kg iv over 5 min; Dupont-Merck Pharmaceuticals, West Point, PA). Although a dose of 3 mg/kg body wt was used to block prostaglandin production in previous studies of the coronary vasculature in pigs (17), we used a lower dose to minimize systemic effects, particularly a rise in blood pressure. 3) ATP-sensitive potassium channels were inhibited by intracoronary administration of glibenclamide (15, 31, 33, 42) to obtain a final concentration of 10 µM in the coronary artery. A washout period of at least 60 min was allowed between the administration of antagonists.

Two-dimensional ultrasound system description and image analysis. The ultrasound catheter (3.2 Fr) has a fixed 30-MHz transducer and a rotating mirror assembly. Images were displayed on a video monitor; axial resolution was <150 µm, and lateral resolution was <250 µm. Gain, contrast, and reject settings were adjusted by the operator to yield a well-balanced gray-scale appearance on the video display. Real-time images were stored on high-quality super-VHS (S-VHS) videotape for subsequent off-line analysis. As previously described (9), selected portions of the videotape were digitized (12 bits, Rasterops 324, Santa Clara, CA) in real time (30 frames/s) and stored on a computer disk for off-line determination of luminal area.

Doppler ultrasound system description. Doppler-derived blood flow velocities were measured using a 0.014-in. steerable Doppler guide wire (FloWire, Endosonics). This guide-wire system has a miniature Doppler ultrasound crystal that transmits signals at a carrier frequency of 15 MHz and receives pulsed-wave ultrasound signals, sampled at a distance of 5 mm from the guide-wire tip. The Doppler signals are analyzed by a FloMap instrument (Endosonics) in which dedicated digital signal-processing chips perform the fast Fourier transformation required for the spectral display. The signals are transformed into gray scale, and the resultant spectrum is displayed on a monitor. In our study, the ECG was simultaneously displayed on the monitor. Also displayed were quantitative measurements of average peak velocity (APV) throughout the cardiac cycle. The monitor display was continuously recorded on an S-VHS videotape for further off-line analysis and for comparison with corresponding cross-sectional ultrasound images.

Calculations and statistical analysis. Luminal CSAs at baseline and after administration of drugs were determined by computer-assisted planimetry using specialized software. Volumetric coronary blood flow (CBF) was calculated from the relationship: CBF = CSA × APV × 250/12.02, as previously validated (9). Dose-response relationships with BNP, NTG, and ET-1 were examined by ANOVA for repeated measures followed by a post hoc Student-Newman-Keuls test. Overall effects of L-NAME, glibenclamide, and indomethacin on BNP-induced vasodilation were analyzed by two-way repeated-measures ANOVA. Specific comparisons between the maximal effect before and after the antagonist were also assessed using a Student’s t-test for paired observations. Values are means ± SE.

RESULTS

Effect of BNP and NTG on coronary artery dimensions and flow. Baseline measurements are shown in Tables 1 and 2. With concentrations of 1 pM and higher, BNP caused significant increases in CSA, APV, and CBF as shown in Fig. 1. No significant changes in systemic arterial pressure or heart rate were observed with any dose. NTG also caused significant increases in CSA and CBF, without changes in systemic arterial pressure. The maximum response to BNP over the range examined was similar to that induced by NTG (Fig 1).

Table 1. MAP and HR changes induced by NTG and BNP before and after pharmacological antagonism of prostaglandins, nitric oxide synthase, and ATP-sensitive potassium channels

<table>
<thead>
<tr>
<th>Agent (Dose)</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>95 ± 4</td>
<td>111 ± 1</td>
</tr>
<tr>
<td>BNP (0.1 µM)</td>
<td>96 ± 5</td>
<td>114 ± 1</td>
</tr>
<tr>
<td>NTG (10 µM)</td>
<td>99 ± 9</td>
<td>111 ± 4</td>
</tr>
<tr>
<td>Indo (2 mg/kg)</td>
<td>112 ± 8</td>
<td>111 ± 2</td>
</tr>
<tr>
<td>BNP (0.1 µM) after Indo</td>
<td>104 ± 8</td>
<td>111 ± 2</td>
</tr>
<tr>
<td>L-NAME (0.1 mM)</td>
<td>93 ± 9</td>
<td>113 ± 2</td>
</tr>
<tr>
<td>BNP (0.1 µM) after L-NAME</td>
<td>93 ± 9</td>
<td>113 ± 2</td>
</tr>
<tr>
<td>Glib</td>
<td>92 ± 1</td>
<td>112 ± 2</td>
</tr>
<tr>
<td>BNP (0.1 µM) after Glib</td>
<td>88 ± 4*</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>ET-1 (10 nM)</td>
<td>88 ± 4*</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>BNP (0.1 µM) after ET-1</td>
<td>86 ± 5</td>
<td>113 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; NTG, nitroglycerin; BNP, brain natriuretic peptide; Indo, indomethacin; L-NAME, N-nitro-L-arginine methyl ester; Glib, glibenclamide; ET-1, endothelin-1. *Significant change vs. Glib; †significant change vs. baseline.
Effect of ET-1 on BNP- and NTG-induced coronary vasodilation. After intracoronary infusion of ET-1, there was a significant reduction in coronary artery CSA (8.6 ± 1.8% decrease; \( p < 0.05 \)), baseline APV (13.2 ± 1.7% decrease; \( p < 0.05 \)), CBF (15.3 ± 4.2% decrease; \( p < 0.05 \)), and mean arterial pressure (Table 1). The magnitude of the BNP-induced increase in epicardial coronary CSA was significantly (\( p < 0.05 \)) enhanced after pretreatment with ET-1 (%increases: CSA, 13.0 ± 6.1; APV, 12.1 ± 5.0; and CBF, 27.7 ± 6.2) (Fig. 2). However, in a separate group of animals, response to NTG before and after administration of ET-1 remained unchanged (Fig. 3).

Effect of L-NAME on BNP- and NTG-induced coronary vasodilation. L-NAME did not induce any significant changes in the baseline CSA [0.38 ± 8.4% decrease, \( p = \) not significant (NS)], APV (16.1 ± 10.7% decrease, \( p = \) NS), CBF (22.7 ± 18.5% decrease, \( p = \) NS), blood pressure, or heart rate (Table 1). L-NAME did not attenuate the epicardial vasodilator response to BNP but did result in significant attenuation of BNP-induced increases in APV and CBF (Fig. 4). In contrast, the vasodilator response to NTG remained unchanged before and after administration of L-NAME (Fig. 5).

Effect of indomethacin on BNP-induced coronary vasodilation. After intravenous infusion of indomethacin, there was a significant decrease in APV (19.2 ± 6.0% decrease, \( p < 0.05 \)) and a tendency to a decreased baseline CBF (28.9 ± 9.9% decrease, \( p = 0.07 \)) associated with a transient increase in blood pressure (Table 1). Baseline CSA (1.3 ± 9.6% increase, \( p = \) NS) remained unchanged. To avoid the confounding effect of the indomethacin-induced increase in blood pressure, the vasodilator effect of BNP was examined when blood pressure changes had returned to baseline. The magnitudes of BNP-induced increases in CSA, APV, and CBF were all attenuated by indomethacin; the attenuation of
**DISCUSSION**

This study demonstrates that human BNP exerts direct vasodilatory effects on coronary conductance and resistance arteries in vivo (37). At the highest dose of BNP, there was an increase in coronary blood flow in the absence of any significant change in the determinants of myocardial oxygen demand, namely, in blood pressure or in heart rate. Consistent with previous studies of differential effects of vasoactive agents on coronary conductance and resistance arteries (43), BNP caused dilation of resistance vessels at lower doses, whereas at higher doses epicardial coronary vasodilation appeared to be predominant. The magnitudes of coronary epicardial vasodilation and augmentation in coronary blood flow in response to BNP were comparable to those of NTG.

Cellular mechanisms: receptors and signaling. NTG and BNP are thought to act via the second messenger cGMP on vascular smooth muscle cells, but with distinct differences in cGMP activation. The GC-A receptor, a biological receptor for BNP, is part of a receptor class of proteins termed particulate guanylyl cyclase. It is a membrane-bound protein with guanylyl cyclase activity. Binding of BNP to the extracellular domain of the GC-A receptor activates the intracellular guanylyl cyclase domain, resulting in the catalysis of cGMP from GTP (28). NTG, which also acts via the second messenger cGMP in vascular smooth muscle cells (19), appears to activate an intracellular cytosolic form called soluble guanylyl cyclase (39). The so-called particulate guanylyl cyclase is distinct from the cytosolic soluble form of guanylyl cyclase, which may provide one possible
BNP effects in ET-1-preconstricted arteries. The vaso-
dilator effects of BNP were accentuated when coronary
arteries were preconstricted with ET-1. This accentu-
ated response to BNP might be explained by either
optimal-receptor presentation of the transmembrane
receptor or participation of an allosteric binding site of
a ligand such as ATP. ATP increases guanylyl cyclase
activity of natriuretic peptides in the rat (23). Recently,
natriuretic peptides have been shown to inhibit local
production of ET-1 by protein kinase C inactivation
(14). Another possible explanation is that the cGMP-
dependent pathway used by BNP opposes a cellular
state associated with constriction but has less effect on
cellular states associated with less tone. Within the
range of doses examined, ET-1 did not enhance coro-

Fig. 4. BNP-induced increases in CSA (A), APV (B), and calculated
CBF (C) before (pre) and after (post) administration of 100 µM
Nω-nitro-L-arginine methyl ester (L-NAME). BNP-induced increase
in APV and CBF is attenuated after L-NAME (n = 5). *Significant
difference vs. pre-L-NAME (P < 0.05).

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Fig. 5. NTG-induced increases CSA (A), APV (B), and calculated
CBF (C) before and after administration of 100 µM L-NAME (n = 5).
Administration of L-NAME did not alter responses to NTG.
appear to induce endothelium-independent vasorelaxation in a variety of species, there is evidence that ANP could act as a stimulus for nitric oxide production. In cultured human proximal tubular cells, ANP stimulated nitric oxide production in a dose-dependent manner, which was apparently related to binding to the natriuretic peptide clearance receptor (NPR-C) (30). These effects may vary depending on the tissue studied. In our study, BNP appears to induce differential vasodilator effects in conductance and resistance arteries. In resistance arteries, BNP-induced vasodilation was

Role of nitric oxide in BNP-induced vasodilation. Although both ANP (34, 47, 49) and porcine BNP (51)
largely blocked by L-NAME, suggesting that endothelium-derived nitric oxide contributes to such vasodilation. In conductance vessels, the preserved vasodilation after L-NAME suggests that release of nitric oxide does not participate in BNP-induced vasodilation. These observations are consistent with in vitro studies showing that the vasodilatory response to BNP in conductance arteries is largely endothelium independent (51). However, the administration of L-NAME induced non-significant decreases in APV and CBF; it is therefore possible that L-NAME exerts a constricting effect that supersedes the vasodilator effect of BNP.

Role of potassium channels in BNP-induced vasodilation. In addition to nitric oxide, potassium channels contribute to regulation of coronary vascular tone. Potassium channels are present in vascular smooth muscle and in high density in the coronary microcirculation (41) and mediate vasodilation. Increased potassium conductance and hyperpolarization in response to BNP have been shown in mesangial cells, the main target cell for natriuretic peptides in the kidney (6). In the present study, however, glibenclamide did not attenuate the vasodilator effects of BNP in the microcirculation. BNP-induced vasodilation in epicardial coronary arteries was enhanced after pretreatment with glibenclamide, which is consistent with accentuated effects of BNP in constricted arteries and previously described coronary vasoconstrictor effects observed with glibenclamide (13). The maximum response to BNP (in terms of absolute effects on CSA) was not increased after glibenclamide, which also suggests that the percentage increase in epicardial coronary dimensions after glibenclamide administration probably resulted from a decrease in baseline values.

Role of prostaglandins in BNP-induced vasodilation. Inhibition of prostaglandins attenuated BNP-induced vasodilation in coronary resistance arteries in the current study. These findings suggest that BNP may exert its coronary effects, in part, via a mechanism involving prostaglandin release. In the rat kidney, C-type natriuretic peptide-induced arteriolar vasodilation was abolished by pretreatment with either L-NAME or indomethacin (1). Additionally, ANP fragments have been shown to inhibit Na⁺-K⁺-ATPase by release of PGE₂ in the kidney (8). Similar mechanisms might contribute to BNP-induced coronary vasodilation and thus require further investigation.

Limitations. The structure of BNP is poorly conserved across species (22), which is consistent with reports of species-specific potencies of BNPs from different species in various activity assays (20). For example, human BNP has potent hemodynamic and renal effects in rabbits (11) and dogs (10), but not in rats (20). Compared with the effects of rat BNP, human BNP exerts more potent effects on porcine vessels. Human BNP can relax porcine coronary artery tissue preparations in vitro with an estimated EC₅₀ of 0.02 nM, whereas rat BNP is much less potent in this assay (EC₅₀ = 1.1 nM) (20). This suggests that human BNP will have potent cardiovascular effects in pigs, consistent with the coronary vasorelaxant effects described in this report.

BNP may compete with ANP for metabolism by the natriuretic peptide clearance receptor (32) or neutral endopeptidase (26), resulting in higher ANP plasma levels, as reported after infusion of pharmacological doses of BNP (50). Thus ANP might contribute to the vasodilation in response to administration of BNP. Part of the increase in epicardial CSA and CBF might be the result of flow-mediated, endothelium-dependent vasodilation (18) rather than a direct effect of BNP. An inhibitory influence of L-NAME is consistent with this possibility. However, L-NAME did not influence NTG-induced epicardial coronary vasodilation. Finally, the vascular and humoral alterations that occur in patients with coronary artery disease and heart failure might modify coronary response to BNP and limit the implications of this study.

Clinical significance. The vasodilator effects of BNP in coronary conductance and resistance arteries raise the possibility of its use in patients with epicardial coronary vasospasm and in patients with microvascular angina. Kato et al. (21) found that intravenous infusion of synthetic BNP suppressed hyperventilation-induced angina. ET-1 contributes to coronary spasm in conductance and resistance vessels (36) and may play a role in variant angina. In this study, after pretreatment with ET-1, BNP increased CBF substantially and reversed endothelin-mediated vasoconstriction completely. In heart failure, BNP levels have been shown to increase with the severity of the disease (50) and plasma levels are reportedly elevated (40). In two double-blind, placebo-controlled randomized studies involving patients with congestive heart failure, BNP treatment was reported to reduce right atrial pressures, pulmonary capillary wedge pressures, and systemic vascular resistance and to increase cardiac output (29, 50). Recent studies also suggest that low-dose infusion of BNP causes favorable hemodynamic changes without influencing cardiac output (25). The direct vasodilator effects of BNP probably contributed to the reduction in preload and afterload observed in these patients. These findings, observed even in the presence of the vasoconstricting peptide ET-1, suggest that coronary vasodilator effects of synthetic BNP, the enhanced natriuretic effects of BNP (24, 29), and the prolonged half-life compared with ANP might favor BNP as a future treatment option in patients with this syndrome. Further studies in humans are required to examine the applicability of our findings to pathophysiological states, such as angina and heart failure.

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Address for reprint requests: K. Chatterjee, Box 0124, Univ. of California at San Francisco, 505 Parnassus Ave., San Francisco, CA 94143-0124.

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