Vasorelaxing effects of atrial and brain natriuretic peptides on coronary circulation in heart failure

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coronary arteries; nitric oxide; guanosine 3',5'-cyclic monophosphate; downregulation; congestive heart failure

ATRIAL NATRIURETIC PEPTIDE (ANP) and brain natriuretic peptide (BNP) are circulating peptide hormones of cardiac origin with vasorelaxant properties. Natriuretic peptides (NPs) relax blood vessels by increasing intracellular cGMP via activation of particulate guanylate cyclase (2, 40, 42). Nitrovasodilators have been widely used to treat angina pectoris and congestive heart failure (CHF) for >100 years. The vasorelaxing effect of nitroglycerin (NTG) is mediated by elevated intracellular cGMP levels resulting from the activation of soluble guanylate cyclase by an active intermediate, nitric oxide (NO) (9, 16). Previous studies in dogs (4) and humans (5, 8) have shown that the addition of NTG as well as ANP induces a preferential sustained vasodilation in large epicardial coronary arteries.

It has been established that ANP infusion improves cardiac function by reducing preload and afterload in patients with CHF (24, 31). However, in some cases of CHF, ANP infusion has less effect, with regard to renal, hormonal, and hemodynamic changes, than in healthy control subjects (6, 15, 28, 36). We previously demonstrated that, under high concentrations of ANP, ANP receptors coupled to guanylate cyclase may be downregulated in the peripheral and pulmonary vascular beds of patients with CHF (36, 37). Even in dogs with severe CHF induced by rapid ventricular pacing, endogenous ANP suppressed the activation of the renin-aldosterone system and sympathetic nerve activity but did not cause any significant hemodynamic changes through its vasodilator action (39). However, it remains unclear whether reduced vascular responses to ANP in CHF are due to downregulation of ANP receptor, postreceptor uncoupling in the target tissues, or increased cGMP degradation. On the other hand, recent studies on the therapeutic effectiveness of ANP have encompassed the field of coronary circulation in patients with coronary artery disease, such as stable-effort angina pectoris (20, 29) and coronary spastic angina pectoris (19, 34). Thus quantitative comparisons of the vasodilator effects of ANP and NTG on coronary circulation have been made (19, 29).

These findings raise the question of whether ANP has the same therapeutic efficacy as NTG as a coronary vasodilator during CHF, since many cases of CHF are based on myocardial ischemia associated with coronary artery disease. To our knowledge, limited information is available on how the vascular functional responsiveness of isolated vessels to ANP and BNP is altered in the setting of CHF, although previous studies have demonstrated that the number of ANP receptors is decreased by exposure to ANP in cultured vascular smooth muscle cells (1, 14, 30, 32). The present study was designed to examine vasorelaxation of isolated coronary arteries induced by ANP, BNP, and other vasoactive agents in dogs with and without CHF and to clarify the mechanisms of CHF-associated changes in ANP-induced relaxation of isolated dog coronary arteries. Thus we compared the effects of ANP on dog control and CHF coronary arteries with those of NTG with reference to relaxation and cGMP production.

MATERIALS AND METHODS

Reagents. α-Human ANP was obtained from the Peptide Institute (Minoh, J. p.), canine BNP from Peninsula Laboratories (Belmont, CA), NTG from Nihon-Kayaku (Tokyo, J. p.), prostaglandin (PG) F{sub 2α} from Ono (Osaka, J. p.),
beraprost sodium from Yamanouchi (Tokyo, Japan), 8-bromo-cGMP (8-Br-cGMP) from Sigma Chemical (St. Louis, MO), papaverine hydrochloride from Dainippon (Osaka, Japan), and 3-isobutyl-1-methylxanthine (IBMX) from Nacalai Tesque (Kyoto, Japan). Responses to NO were obtained by adding NaN3 solution adjusted to pH 2 (12). The concentrations referred to here are those of freshly prepared acidified NaN3 solution.

Surgical procedure and measurements. This study was approved by the Animal Research Committee of Shiga University of Medical Science. Experiments were randomly conducted in two groups of mongrel dogs (11–17 kg body wt). Dogs that had not been operated on served as the control group (n = 10). The dogs in the CHF group (n = 10) underwent rapid ventricular pacing for 22 days to induce severe CHF. Under pentobarbital sodium anesthesia (25 mg/kg body wt), the dogs were ventilated. Through a left thoracotomy and pericardiectomy, the heart was exposed, and two cardiac unipolar pacing leads (model M-23, Matsuda, Tokyo, Japan) were sutured onto the right ventricular apex. The leads were tunneled to the animal’s back and connected to an external pacemaker (model 540, Seamed).

Cardiovascular monitoring and plasma sampling were conducted as follows. The left femoral vein was cannulated with a thermodilution catheter (model T-047-03, Goodtec), and the catheter was advanced into the pulmonary artery for measurement of pulmonary capillary wedge pressure and cardiac output (CO). The right carotid artery was cannulated with a thermodilution catheter (model T-047-03, Goodtec), and the catheter was advanced into the pulmonary artery for measurement of pulmonary capillary wedge pressure and cardiac output (CO). After 22 days of pacing, mean arterial pressure; CO, cardiac output; PCWP, pulmonary capillary wedge pressure; ANP, atrial natriuretic peptide. Significantly different from control: *P < 0.01; †P < 0.001.

RESULTS

Hemodynamic and hormonal changes during rapid ventricular pacing. After 22 days of pacing, mean arterial pressure and CO were significantly reduced in

| Table 1. Hemodynamic and neurohumoral characteristics in dogs with and without CHF |
|-----------------|------------------|
|                    | Control (n = 10) | CHF (n = 10) |
| MAP, mmHg         | 123 ± 5          | 96 ± 5*      |
| CO, l/min         | 2.7 ± 0.1        | 1.9 ± 0.1†   |
| PCWP, mmHg        | 4 ± 1            | 16 ± 1†      |
| ANP, pg/ml        | 52 ± 5           | 339 ± 40†    |
| cGMP, pmol/ml     | 9 ± 1            | 39 ± 8*      |

Values are means ± SE. CHF, congestive heart failure; MAP, mean arterial pressure; CO, cardiac output; PCWP, pulmonary capillary wedge pressure; ANP, atrial natriuretic peptide. Significantly different from control: *P < 0.01; †P < 0.001.
the CHF dogs compared with the control dogs (Table 1). Pulmonary capillary wedge pressure was significantly higher in the CHF dogs than in the control dogs. Plasma ANP concentrations increased about sixfold higher than in the control dogs. The plasma level of cGMP was also significantly elevated. Additional evidence of heart failure, including anorexia, exertional dyspnea, pleural effusions, and pulmonary and hepatic congestion, was consistently noted at the time of organ harvest in the CHF dogs but not in the control dogs.

Effects of KCl and PGF2α on isolated control and CHF coronary arteries. The addition of 30 mmol/l KCl caused contractions to a similar extent in the control and CHF coronary arteries (1.13 ± 0.10 and 1.11 ± 0.09 g, respectively, n = 10). The addition of PGF2α at 10⁻⁷-10⁻⁵ mol/l contracted the control and CHF coronary arteries in a dose-dependent manner. PGF2α-induced contractions of control and CHF coronary arteries did not differ; the maximal contractions induced by 10⁻⁵ mol/l PGF2α in control and CHF arteries were 1.59 ± 0.09 g (n = 6) and 1.45 ± 0.13 g (n = 6), respectively, and the median effective concentrations for PGF2α in these arteries were (9.2 ± 1.3) × 10⁻⁷ mol/l (n = 6) and (1.1 ± 0.2) × 10⁻⁶ mol/l (n = 6), respectively.

Effects of vasorelaxing agents on isolated control and CHF coronary arteries. Typical recordings of the responses to ANP, NTG, and NO in isolated control and CHF dog coronary arteries are illustrated in Fig. 1. In control coronary arteries that had been partially contracted with PGF2α, the addition of 10⁻⁷ mol/l ANP produced moderate relaxation. In CHF coronary arteries contracted with PGF2α, the relaxation response to ANP (10⁻⁷ mol/l) was abolished. On the other hand, NTG and NO produced similar concentration-dependent relaxations in control and CHF coronary arteries. The quantitative concentration-response curves are shown in Fig. 2. ANP and BNP at 10⁻⁸ and 10⁻⁷ mol/l caused stepwise relaxations in control coronary arteries (Fig. 2). The vasorelaxant effects of ANP and BNP at 10⁻⁸ and 10⁻⁷ mol/l were suppressed in CHF coronary arteries compared with control coronary arteries, respectively. The relaxation responses of CHF coronary arteries to 10⁻⁷ mol/l ANP were markedly suppressed to 17% of those in control arteries (Fig. 2). However, the responses of CHF coronary arteries to 10⁻⁸ mol/l BNP,
which produced relaxation similar to that produced by 10^{-7} \text{ mol/l} ANP in control coronary arteries, were suppressed to 46% of those in control arteries (Fig. 2).

As shown in Fig. 3, the concentration-dependent vasorelaxing effects of NTG and NO were unchanged in CHF coronary arteries compared with control coronary arteries.

Furthermore, as shown in Fig. 4, the concentration-dependent vasorelaxing effects of 8-BrcGMP and beraprost sodium were unchanged in CHF coronary arteries compared with control coronary arteries.

Effects of CHF on tissue cGMP production. Increases in tissue cGMP were examined by adding 10^{-7} \text{ mol/l} ANP and 10^{-8} \text{ mol/l} NTG, which produced comparable degrees of relaxation, in isolated control coronary arteries. In preliminary experiments, maximal increases in tissue cGMP production were observed 2 min after exposure to ANP or NTG (data not shown). Therefore, tissue cGMP measurements were performed 2 min after exposure to ANP or NTG in subsequent experiments. The results are summarized in Fig. 5. In control and CHF coronary arterial strips, basal levels of tissue cGMP averaged 61 \pm 8 (n = 6) and 66 \pm 8 pmol/g frozen tissue (n = 6), respectively; no differences were observed (Fig. 5). In control coronary arteries, the level of tissue cGMP was increased by the addition of 10^{-7} \text{ mol/l} ANP and by the addition of 10^{-8} \text{ mol/l} NTG (Fig. 5). In CHF coronary arteries the stimulatory effect of ANP was abolished. However, NTG (10^{-8} \text{ mol/l}) induced similar increases in tissue cGMP in control and CHF coronary arteries (Fig. 5).

**DISCUSSION**

The present study demonstrated for the first time a specific reduction in the vasorelaxation responses of coronary arteries to ANP and BNP in dogs with severe CHF induced by rapid ventricular pacing, which suggests the possibility of the downregulation of NPs coupled to guanylate cyclase in dog coronary arterial smooth muscle cells. In contrast, the vasorelaxant effects of NTG and NO in CHF coronary arteries were similar to those in control coronary arteries.

**Hemodynamic and hormonal changes.** On the basis of hemodynamic and neurohormonal profiles, in comparison with those in a previous study (39), we assumed that this experimentally induced CHF model is equivalent to severe CHF. The plasma cGMP level has been demonstrated to be useful as a biological marker of endogenous ANP activity (17, 28, 39). Kanamori et al. (17) observed significant increases in plasma ANP and plasma cGMP levels in dogs with mild CHF induced by 6 days of rapid ventricular pacing. However, in dogs...
with severe CHF induced by rapid pacing for longer periods, the plasma cGMP level did not increase further, despite a progressive increase in plasma ANP. The increase in plasma cGMP after ANP infusion was greater in normal dogs than in those with severe CHF (17). On the other hand, Riegger et al. (28) reported no difference between normal and CHF dogs with respect to the secretion of cGMP during ANP infusion in relation to the plasma levels of ANP. We previously reported that the increase in endogenous ANP observed in severe CHF dogs produced no significant systemic vasodilator effect (39). Therefore, we assumed that the attenuation of endogenous ANP activity on hemodynamics observed in CHF may be due to the downregulation of guanylate cyclase-coupled ANP receptors in vascular beds (39). However, there is no previous evidence of the downregulation of ANP receptors in vascular beds of pacing-induced CHF dogs. Previous clinical studies have reported a positive correlation between the plasma ANP and cGMP concentrations in patients with mild CHF but not in patients with severe CHF, i.e., the cGMP concentration reached a plateau, despite high concentrations of ANP (36). In addition, previous studies of ANP infusion have demonstrated attenuated hemodynamic and renal excretory effects in patients with CHF (6, 15, 25, 36). Thus it has been suggested that ANP receptors may be downregulated in the vascular beds of patients with chronic severe CHF.

Organ chamber studies. In CHF coronary arteries that had been partially contracted with PGF$_{2\alpha}$, the responses to ANP and BNP were markedly suppressed compared with those in control coronary arteries. ANP and BNP have been shown to selectively activate particulate guanylate cyclase associated with the membrane via NP receptors located in vascular smooth muscle cells, which in turn stimulates cGMP formation and thus dilates vessels (2, 40, 42). In concert with previous reports (10, 26, 27), the contractions induced by PGF$_{2\alpha}$ and KCl in CHF arteries did not differ from those in control arteries. Therefore, it is unlikely that the CHF-related reduction of ANP- and BNP-induced relaxation observed in the present study is due to differences in the absolute level of preconstrictions in response to PGF$_{2\alpha}$. In vivo studies with dogs and humans have shown that intravenous or intracoronary ANP dilates large epicardial coronary arteries and increases coronary blood flow, and these coronary effects are similar to those of NTG (4, 5, 8). Recent studies have examined whether ANP infusion has the same coronary vascular effects as nitrovasodilators in patients with coronary artery diseases (19, 29). ANP infusion has been shown to have a beneficial effect in exercise-induced myocardial ischemia in patients with stable-effort angina (20) and in coronary spasm induced by hyperventilation in patients with variant angina (34). Thus, similar to NTG, the infusion of ANP may have the same beneficial effects on coronary circulation as in normal cardiac function. Herrmann et al. (13) demonstrated that intravenous ANP infusion had no deleterious effects on coronary vascular resistance in patients with CHF. However, they assumed that its effects may occur indirectly as a result of coronary autoregulation, rather than by a direct vasodilatory effect of ANP. It has been reported that BNP, in addition to ANP, may also play a pathophysiological role in CHF (38, 43). Marcus et al. (21) showed that human BNP infusion had potent vasodilative effects in patients with severe CHF. Recently, we reported the possibility that BNP may downregulate NP receptors coupled to guanylate cyclase in patients with CHF (38).

NTG produced similar concentration-response curves in control and CHF coronary arteries. NTG activates soluble guanylate cyclase, which results in increased cGMP production (9, 16, 22); NPs and NTG share a final common pathway through cGMP production. The present results suggest that in isolated dog coronary arteries, CHF does not influence the mechanisms of NTG action, i.e., 1) the conversion of NTG to NO, 2) the activation of soluble guanylate cyclase, and 3) the intracellular action of cGMP. The present results are consistent with those of Forster et al. (10), in that the relaxant effects of NTG in the coronary arteries of dogs did not change with pacing-induced severe CHF. The vasorelaxing effects of NO in CHF coronary arteries were similar to those in control coronary arterial strips. The role of endothelium-derived relaxing factors in coronary circulation in dogs with pacing-induced CHF remains controversial (10, 26, 41). O’Murchu et al. (26) demonstrated that the sensitivity of vascular smooth muscle in isolated dog coronary arteries to NO was not...
altered during pacing-induced severe CHF, which is consistent with the present results. To investigate whether CHF affected steps beyond cGMP production in vascular smooth muscle cells, we examined the responses to 8-BrcGMP, a cGMP analog, in control and CHF coronary arteries. The vasorelaxing effects of 8-BrcGMP in CHF coronary arteries were similar to those in control coronary arterial strips. The present data confirmed that once cGMP is formed, its effects are not changed by the induction of CHF. On the basis of these results, it is suggested that 1) soluble guanylate cyclase-mediated vasorelaxation in CHF coronary arteries is intact and 2) it is unlikely that a step beyond cGMP production is responsible for the diminished responsiveness of CHF coronary arteries to ANP and BNP.

Early studies demonstrated an increase in the production of PG, such as PGI2 and PGE2, in patients with CHF complicated by hyponatremia (7). In the present study the vasorelaxing effects of beraprost sodium in CHF coronary arteries were similar to those in control coronary arterial strips. The present data confirmed that once cGMP is formed, its effects are not changed by the induction of CHF. On the basis of these results, it is suggested that 1) soluble guanylate cyclase-mediated vasorelaxation in CHF coronary arteries is intact and 2) it is unlikely that a step beyond cGMP production is responsible for the diminished responsiveness of CHF coronary arteries to ANP and BNP.

Tissue cGMP studies. We examined tissue cGMP production in coronary arterial strips under treatment with 0.5 mmol/l IBMX, a cGMP phosphodiesterase inhibitor, to exclude the possibility of more rapid degradation of cGMP by the upregulation of cGMP phosphodiesterase in CHF. The basal levels of tissue cGMP in control and CHF coronary arteries were similar. In control coronary arteries the level of tissue cGMP was increased by the addition of ANP and by the addition of NTG. In CHF coronary arteries the increase in the tissue cGMP level by ANP was abolished, whereas that by NTG was unchanged. These findings strongly suggest that the downregulation of guanylate cyclase-coupled NP receptors plays a role in the attenuated responses of CHF coronary arteries to ANP. Among the receptors for NPs, the NP-A receptor (NPR-A) and the NP-B receptor (NPR-B) contain guanylate cyclase domains in their structures and are considered to be biologically active (3, 33). Another type of receptor, called the C-type receptor, is not coupled to guanylate cyclase and has been postulated to serve as a specific clearance receptor for NPs (11). Enzymatic degradation by neutral endopeptidase and binding of NPs to C-type receptors in vascular smooth muscle cells (7, 30). Receptor downregulation by NPs must be distinguished from prior NP receptor occupation. Because the mean plasma ANP level in CHF dogs in the present study was 395 ± 96 pg/ml, i.e., in the range of 0.1 nmol/l, prior receptor occupation appears to play a minimal role, if any, in coronary arterial smooth muscle cells in severe CHF dogs.

In conclusion, our results indicated a specific reduction in the relaxant effects of ANP and BNP on the coronary arteries in severe CHF dog. The attenuated responses to ANP and BNP may be responsible for the downregulation of NP receptors coupled to guanylate cyclase, although the mechanism of other mechanisms is not excluded. In contrast, the relaxant effects of NTG and NO on CHF coronary arteries were similar to those on control coronary arteries. Thus ANP and BNP do not have the same therapeutic efficacy as NTG as coronary vasodilators in the setting of CHF. Our findings may provide a new insight into the clinical usefulness of ANP and BNP in patients with CHF on the basis of coronary artery disease.

Study limitations. Using receptor binding studies and Northern blot studies, we did not evaluate whether the number of binding sites for NP and the NPR-A mRNA levels are altered in coronary arterial smooth muscle cells of CHF dogs in comparison with those of control dogs. Furthermore, we did not measure the guanylate cyclase activity, either particulate or soluble, stimulated by guanylate cyclase activators. Therefore, we could not provide direct evidence of the downregulation of NPR-A in CHF coronary arteries. In addition, it is unclear whether the vasodilative effects of C-type NP, which binds to NPR-B (18), differ in control and CHF coronary arteries. Further studies are needed to determine how NP receptor subtypes undergo differential downregulation during CHF.

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