Bone Morphogenic Protein-4 Induces Hypertension in Mice
Role of Noggin, Vascular NADPH Oxidases, and Impaired Vasorelaxation

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Background—Recent in vitro studies have shown that disturbed flow and oxidative conditions induce the expression of bone morphogenic proteins (BMPs 2 and 4) in cultured endothelial cells. BMPs can stimulate superoxide production and inflammatory responses in endothelial cells, raising the possibility that BMPs may play a role in vascular diseases such as hypertension and atherosclerosis. In this study, we examined the hypothesis that BMP4 would induce hypertension in intact animals by increasing superoxide production from vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and an impairment of vasodilation responses.

Methods and Results—BMP4 infusion by osmotic pumps increased systolic blood pressure in a time- and dose-dependent manner in both C57BL/6 mice (from 101 to 125 mm Hg) and apolipoprotein E–null mice (from 107 to 146 mm Hg) after 4 weeks. Cotreatment with the BMP antagonist noggin or the NADPH oxidase inhibitor apocynin completely blocked the BMP4 effect. In addition, BMP4 infusion stimulated aortic NADPH oxidase activity and impaired vasorelaxation, both of which were prevented either by coinfusing noggin or by treating the isolated aortas with apocynin. BMP4, however, did not cause significant changes in maximum relaxation induced by the endothelium-independent vasodilator nitroglycerin. Remarkably, BMP4 infusion failed to stimulate aortic NADPH oxidases, increase blood pressure, and impair vasodilation responses in p47phox-deficient mice.

Conclusions—These results suggest that BMP4 infusion induces hypertension in mice in a vascular NADPH oxidase–dependent manner and the subsequent endothelial dysfunction. We suggest that BMP4 is a novel mediator of endothelial dysfunction and hypertension and that noggin and its analogs could be used as therapeutic agents for treating vascular diseases. (Circulation. 2006;113:2818-2825.)

Key Words: blood flow ■ blood pressure ■ endothelium-derived factors ■ free radicals ■ hypertension ■ nitric oxide ■ vasodilation

Increased vascular production of reactive oxygen species (ROS), including superoxide and H2O2, commonly occurs in hypertension, atherosclerosis, hypercholesterolemia, aging, and diabetes.1–3 The major potential sources of vascular ROS production are nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, uncoupled endothelial nitric oxide (NO) synthase, and mitochondria. Of these, the membrane-associated vascular NADPH oxidases have been shown to be the major source of superoxide production in the vessel wall.5–8 An important consequence of increased production of superoxide in the vascular wall is its diffusion-limited reaction with endothelium-derived NO, which reduces NO bioavailability and in turn leads to endothelial dysfunction and hypertension.1

Although bone morphogenic protein (BMP) was originally discovered as a protein that mediates bone growth9 and ectopic vascular and valvular calcification, it has other critical roles in embryonic development, patterning, cartilage formation, and cell differentiation as well.10,11 The BMPs are members of the transforming growth factor-β superfamily and are secreted as active proteins. Their activities are counterbalanced by secreted specific antagonists, such as noggin and chordin.12 Because of its relatively specific effect, noggin has proved to be a valuable tool to define BMP function in cells and tissues.13

BMP4 and the closely related protein BMP2 are found in calcified atherosclerotic plaques,14 and they play a role in calcification involving medial smooth muscle cells.15–17 Recent studies, however, suggest that BMPs have a novel role in vascular inflammation in an endothelium-dependent manner. Using a DNA microarray, we discovered that BMP4 is a shear-sensitive gene in cultured endothelial cells.18 On the
other hand, BMP2 but not BMP4 has been shown to be upregulated in endothelial cells by tumor necrosis factor-α, H2O2, and high pressure.19 Although the upstream stimuli that regulate the expression of BMP2 and BMP4 may be different, they both induce similar proinflammatory effects.13,18,19 BMP4 released by endothelial cells in response to proatherogenic oscillatory shear stress stimulates intercellular adhesion molecule expression and monocyte adhesion to these cells.18 Moreover, BMP4 was shown to induce and activate NADPH oxidases in cultured endothelial cells, thus producing superoxide, which in turn led to inflammatory responses.13 Despite these in vitro data, it is unclear whether BMP4 has any effect on vascular pathophysiology in intact animals.

In the present study, we examined the hypothesis that chronic infusion of BMP4 in mice would stimulate vascular NADPH oxidase activity and increase superoxide production, which would decrease endothelial NO bioavailability and lead to hypertension. Our results indicate that BMP4, previously thought to play proatherogenic roles in response to unstable flow conditions, can also cause hypertension by altering endothelial function. The findings could provide a common mechanism leading to hypertension and atherosclerosis and might help explain the common coexistence of these 2 diseases.

Methods

Animal Studies

Male C57BL/6J (wild type) and apolipoprotein E–null (apoE−/−) mice from Jackson Laboratory (Bar Harbor, Me) and male p47phox-deficient mice (p47phox−/−) from Taconic Farms (Albany, NY) were purchased at the age of 4 to 5 weeks. All experimental protocols were approved by the institutional Animal Care and Use Committee at Emory University. Mice (5 to 6 weeks of age) were anesthetized with 2.5% 2,2,2-trifluoroethanol (Avertin) IP (0.3 mL per 25 g of body weight) and subcutaneously implanted with single osmotic micro-pumps (Alzet Osmotic Pumps, DURECT Corporation, Cupertino, Calif) for delivery of vehicle, recombinant human BMP4, or recombinant human noggin (both from BD Biosciences, San Jose, Calif) for 4 weeks. For BMP4-noggin groups, the animals were implanted with 2 pumps, each delivering BMP4 or noggin in separate locations in mouse flanks to prevent direct mixing of the 2 proteins. Four groups were studied as follows (n=5 mice per group): (1) vehicle (0.1% bovine serum albumin in 4 mmol/L HCl); (2) BMP4 (2 delivery rates were studied: 0.45 and 0.90 mg/kg for 4 weeks); (3) noggin (0.45 or 0.90 mg/kg for 4 weeks); and (4) BMP4+noggin double implants (each pump delivered 0.45 or 0.90 mg BMP4 or noggin per kilogram for 4 weeks). In some studies, the vehicle- and BMP4-treated mice were also treated with the NADPH oxidase inhibitor apocynin (1.5 mmol/L) added daily to the drinking water for 4 weeks: vehicle+apocynin or BMP4+apocynin (BMP4 0.90 mg/kg for 4 weeks with apocynin). C57BL/6J mice were fed a normal chow diet throughout the study period, and the apoE mice were fed a normal chow diet exactly as described.22

Blood Pressure Measurements

Systolic blood pressure was measured weekly during the 4-week period during infusion by a computerized tail-cuff plethysmograph (model BP-2000; VisiTech Systems, Apex, NC).21 Each mouse was accustomed to the apparatus for 4 days before measurements were recorded, and on each day of blood pressure determination, 10 measurements were obtained and averaged for each mouse.

Measurement of NADPH-Dependent Superoxide Production

Abdominal aortas were obtained after the 4-week treatment, dissected free of adventitia, snap-frozen, and then used to prepare membrane fractions, as described previously,22 with minor modifications. In brief, the frozen aortic segments were homogenized in 350 µL of 50 mmol/L phosphate buffer (treated for 2 hours with 5 g/100 mL Chelex-100 and filtered containing the protease inhibitors aprothin (10 µg/mL), leupeptin (0.5 µg/mL), pepstatin (0.7 µg/mL), and phenylmethylsulfonyl fluoride (0.5 mmol/L) (pH 7.4 at 4°C). The homogenate was centrifuged at 400g for 10 minutes, and the supernatant was sonicated (at 4 W power) for 10 seconds on ice and centrifuged at 28 000g for 15 minutes at 4°C. The membrane pellet was resuspended in 150 µL of the homogenization buffer, and 20-µg protein aliquots were used for electron spin spectroscopy (ESR) for quantitative measurement of superoxide radicals induced by NADPH with 1 mmol/L 1-hydroxy-3-carboxypyrrolidine (CPh), 200 µmol/L NADPH, and 0.1 mmol/L diethylenetriaminopentaacetic acid in a total volume of 100 µL of Chelex-treated phosphate-buffered saline. The hydroxyamine spin probe CPh provides quantitative measurements of superoxide radicals with high sensitivity.23 The ESR spectra were recorded with an EMX ESR spectrometer (Bruker, Billerica, Mass) and a super-high-Q micro-wave cavity exactly as described.22

Vascular Reactivity Study

Thoracic aortas were rapidly removed, cleaned of adventitia, cut into 3-mm ring segments, and studied as previously described.24 After contraction by application of prostaglandin F2α, relaxations to cumulative concentrations of acetylcholine, the calcium ionophore A23187, and nitroglycerin (to determine endothelium-independent vasorelaxation) were examined. The degree of precontraction to prostaglandin F2α was chosen to approximate 80% of the maximal response to KCl (80 mmol/L). To examine the role of ROS produced by NADPH oxidase in inhibiting relaxation, isolated vessels were incubated in an organ chamber with apocynin (0.05 mmol/L) for 20 minutes before these dose-response experiments were performed.

Data Analysis

Results are presented as mean±SEM. Comparisons of dose-response curves between groups were made by a repeated-measures ANOVA or 1-way ANOVA followed by Bonferroni test, when appropriate. A value of P<0.05 was considered statistically significant. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

BMP4 Induces Hypertension in C57BL/6J and ApoE−/− Mice

To examine whether BMP4 affected blood pressure, C57BL/6J (wild type) and apoE−/− mice were chronically infused with vehicle or BMP4 (0.9 mg/kg for 4 weeks). In wild-type mice, the BMP4 infusion significantly increased blood pressure by 13 mm Hg above control values (from 100 to 113 mm Hg) as early as the second week, and this was increased further after 4 weeks (from 101 to 125 mm Hg, P<0.05; Figure 1A). The hypertensive effect of BMP4 occurred earlier and was more pronounced in the apoE−/− mice than in the wild-type group. By the first week, BMP4 had already increased blood pressure by ≈10 mm Hg above controls, which increased further by 39 mm Hg above controls after 4 weeks (from 107±3.6 to 146±3.2 mm Hg, P<0.01; Figure 1B).

We next examined the specificity of the hypertensive effect of BMP4 by infusing the BMP antagonist noggin (0.9 mg/kg for 4 weeks) either alone or with BMP4 (noggin+BMP4, each delivered at a rate of 0.9 mg/kg by 2 separate pumps). As shown in
BMP4 Stimulates Vascular NADPH Oxidase Activity

We examined whether BMP4 infusion stimulates vascular NADPH oxidase activity by measuring NADPH-dependent superoxide production in membrane fractions prepared from the aortas of wild-type and apoE−/− mice that were treated with vehicle, BMP4, noggin, and BMP4+noggin for 4 weeks, as shown in Figure 1. As shown in Figure 2, BMP4 infusion significantly increased aortic membrane NADPH oxidase activity by 66% and 60% above that of vehicle control in both wild-type and apoE−/− mice (P<0.001), respectively, although the membranes from the apoE−/− mice produced slightly more superoxide than did the wild type. In contrast, aortic NADPH oxidase activity from noggin- and BMP4+noggin-treated mice was almost identical to that of vehicle controls, demonstrating that noggin completely prevented the stimulatory effect of BMP4 in both wild-type and apoE−/− mice (Figure 2). Moreover, addition of apocynin to the drinking water during BMP4 infusion significantly blunted the aortic membrane NADPH oxidase activity to levels similar to those of controls (P<0.001 versus BMP4). Addition of superoxide dismutase (SOD) during the ESR assay to the membranes from BMP4-treated mice significantly reduced superoxide production to levels similar to those of controls (Figure 1E), providing strong support for the role of NADPH oxidases in BMP4-induced hypertension. Taken together, these results demonstrate that BMP4 induces and noggin prevents hypertension in an NADPH oxidase–dependent manner.

BMP4 Impairs Endothelium-Dependent Vasorelaxation: Improvement by Noggin, Apocynin, and p47phox Knockout

Effect of BMP4 on Endothelium-Dependent Relaxation

To evaluate the effect of BMP4 on endothelial function, we examined relaxation evoked by acetylcholine and calcium
Vascular Reactivity

Effect of NADPH Oxidase Inhibition on relaxations to nitroglycerin (Tables 1 and 2). Noggin infusion alone had no effect on relaxations to nitroglycerin (Figure 3I and Table 1). Apocynin had no effect on relaxations to nitroglycerin (Figure 3I and Table 1). More important, BMP4 infusion into p47phox<sup>−/−</sup> mice failed to induce an impairment of endothelium-dependent vasorelaxation in response to acetylcholine, A23187, and nitroglycerin. These results suggest an essential role for p47phox-dependent NADPH oxidases in BMP4-induced endothelial dysfunction.

**Discussion**

In this report, we show for the first time that chronic infusion of recombinant BMP4 to either C57BL/6J (wild type) or apoE<sup>−/−</sup> mice induces 3 significant vascular effects: (1) activation of p47phox-dependent NADPH oxidases in the aorta, (2) impairment of endothelium-dependent vasorelaxation, and (3) hypertension. Furthermore, our results indicate that BMP4-induced hypertension is dependent on activation of vascular NADPH oxidases, which in turn impair endothelium-dependent vasorelaxation. These conclusions are supported by these findings as well as our previous in vitro data. First, the BMP antagonist noggin infused together with BMP4 completely prevented hypertension, NADPH oxidase activation, and endothelial dysfunction induced by BMP4 infusion. For the BMP4+noggin studies, we infused BMP4 and noggin in 2 separate pumps implanted in separate locations to prevent their mixing before delivery into the circulation. Noggin prevented virtually all of the effects of BMP4, clearly suggesting that the effects of BMP4 were due to its specific action on its receptor. It remains to be resolved, however, whether noggin could be used as a general antihypertensive agent. Second, inhibition of NADPH oxidases by either apocynin provided in the drinking water concurrently with BMP4 infusion or p47phox knockout also prevented BMP4-induced hypertension and ROS production by NADPH oxidases, supporting the role for NADPH oxidases, especially the p47phox-dependent forms, in the actions of BMP4. Third, the present results suggest that BMP4 infusion stimulates activity of vascular NADPH oxidases, because it can be inhibited not only by noggin infusion but also by apocynin feeding and p47phox knockout. The effects of BMP4 on hypertension, NADPH oxidase activity, and endothelium-dependent vasodilation were exacerbated in apoE<sup>−/−</sup> mice fed a high-fat diet, reflecting a potential additive effect of hypercholesterolemic conditions in apoE<sup>−/−</sup> mice.

The critical role of increased vascular superoxide production in hypertension has been demonstrated in several experimental animal models and human patients. For example, treatment of rat and mouse models of hypertension with antioxidants such as SOD and vitamins C and E, or NADPH oxidase inhibitors effectively reduced blood pressure.

Although xanthine oxidase, uncoupled endothelial NO synthase, and mitochondria are additional sources, the nonphagocytic, membrane-bound NADPH oxidases are known as a major source of superoxide production in the vascular system. NADPH oxidases can impair endothelium-dependent vasodilation, we sought to inhibit NADPH oxidase activity in organ chambers by incubating vessels with apocynin. Apocynin completely normalized relaxations to acetylcholine and calcium ionophore A23187 in wild-type mice infused with BMP4 (Figure 3G and 3H and Table 1). Apocynin had no effect on relaxations to nitroglycerin (Figure 3I and Table 1). More important, BMP4 infusion into p47phox<sup>−/−</sup> mice failed to induce an impairment of endothelium-dependent vasorelaxation in response to acetylcholine, A23187, and nitroglycerin. These results suggest an essential role for p47phox-dependent NADPH oxidases in BMP4-induced endothelial dysfunction.

**Effect of BMP on Relaxations to Nitroglycerin**

The endothelium-independent agonist nitroglycerin induced >90% relaxation in aortic segments from both wild-type and apoE<sup>−/−</sup> mice. Maximal relaxation was not altered by BMP4; however, the sensitivity to nitroglycerin, as reflected by the ED₅₀, was significantly shifted to the right in vessels from both strains of animals after BMP4 infusion (Figure 3C and Tables 1 and 2). This shift in ED₅₀ was eliminated in mice that also received noggin concurrently with BMP4 (Figure 3F and Tables 1 and 2). Noggin infusion alone had no effect on relaxations to nitroglycerin (Tables 1 and 2).

**Effect of NADPH Oxidase Inhibition on Vascular Reactivity**

As noted earlier (Figure 2), BMP4 infusion increased p47phox-dependent NADPH oxidase activity in mouse aortas. Because increased superoxide production from aortic NADPH oxidases can impair endothelium-dependent vasodilation, we sought to inhibit NADPH oxidase activity in organ chambers by incubating vessels with apocynin. Apocynin completely normalized relaxations to acetylcholine and calcium ionophore A23187 in wild-type mice infused with BMP4 (Figure 3G and 3H and Table 1). Apocynin had no effect on relaxations to nitroglycerin (Figure 3I and Table 1). More important, BMP4 infusion into p47phox<sup>−/−</sup> mice failed to induce an impairment of endothelium-dependent vasorelaxation in response to acetylcholine, A23187, and nitroglycerin. These results suggest an essential role for p47phox-dependent NADPH oxidases in BMP4-induced endothelial dysfunction.

**Figure 2.** Chronic BMP4 infusion in mice stimulates vascular NADPH oxidases, and noggin prevents it. Membrane fractions prepared from aortas of wild-type (A), apoE<sup>−/−</sup> (B), and p47phox<sup>−/−</sup> mice infused with vehicle, BMP4, noggin, or BMP4+noggin for 4 weeks (0.9 mg/kg) as described in Figure 1 were used to determine NADPH oxidase activity. Some mice were treated with apocynin (1.5 mmol/L in drinking water concurrently with BMP4 or vehicle (in vivo). Superoxide production was determined by monitoring the oxidation of CPH to the stable 3-carboxypropyl (CP) nitroxide radical. Treatment of membranes with SOD (50 U/mL) during ESR measurement was used to determine specific superoxide production (portions above the horizontal broken lines). Values are mean±SD, *<P<0.001, n=3 or 4 each.

- **A. Wild-type**
  - Con
  - BMP
  - Nog
  - Apo
  - Ex

- **B. ApoE<sup>−/−</sup>**
  - Con
  - BMP
  - Nog
  - Apo
  - Ex

- **C: p47<sup>−/−</sup>**
  - Con
  - BMP
  - Nog
  - Apo
  - Ex

P<sub>0.001</sub>
wall under conditions such as hypertension, atherosclerosis, diabetes, and aging.1–3,8 Recently, 5 NADPH oxidases have been cloned (nox1 through 5); nox1 is expressed in endothelial and smooth muscle cells; nox2 is expressed in endothelial cells and adventitial fibroblasts; and nox4 is found in endothelial cells, smooth muscle cells, and fibroblasts.3,7,8

The pathophysiological significance of NADPH oxidases in hypertension has been most elegantly illustrated by studies of genetic deletions of NADPH oxidase components. Mice deficient in p47phox, a cytosolic component necessary for optimum activation of nox1 and nox2,30 have been shown to be resistant to angiotensin II–induced hypertension.6 In contrast, genetic deletion of nox2 lowered basal blood pressure, but it failed to prevent angiotensin II–induced hypertension while inhibiting vascular remodeling in knockout mice.31,32 In contrast, angiotensin II–induced hypertension was blunted in nox1-deficient mice, whereas it was enhanced in nox1-overexpressing transgenic mice.33,34 These results strongly suggest a critical role for nox1-based NADPH oxidase in blood pressure regulation, at least in response to certain stimuli, including angiotensin II.

The present study strongly suggests that BMP4-induced hypertension is mediated by nox1- and p47phox-based NADPH oxidase.
Responses in C57BL/6J Mice

<table>
<thead>
<tr>
<th>Maximal relaxation, %</th>
<th>Ach</th>
<th>NTG</th>
<th>A23187</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild-type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>95.32 ± 1</td>
<td>94.61 ± 1</td>
<td>88.59 ± 3</td>
</tr>
<tr>
<td>BMP4</td>
<td>74.31 ± 1*</td>
<td>95.46 ± 2</td>
<td>62.28 ± 6†</td>
</tr>
<tr>
<td>BMP4 + noggin</td>
<td>91.16 ± 4</td>
<td>95.32 ± 3</td>
<td>77.41 ± 01</td>
</tr>
<tr>
<td>Noggin</td>
<td>93.54 ± 1</td>
<td>93.28 ± 1</td>
<td>73.89 ± 5</td>
</tr>
<tr>
<td>Vehicle + apocynin</td>
<td>100.2 ± 2</td>
<td>98.72 ± 3</td>
<td>80.78 ± 2</td>
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<tr>
<td>BMP4 + apocynin</td>
<td>95.33 ± 1‡</td>
<td>97.88 ± 2</td>
<td>85.59 ± 2§</td>
</tr>
<tr>
<td><strong>p47(phox)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>90.37 ± 3</td>
<td>96.80 ± 2</td>
<td>88.47 ± 1</td>
</tr>
<tr>
<td>BMP4</td>
<td>91.02 ± 3¶</td>
<td>96.98 ± 2</td>
<td>81.40 ± 5</td>
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ED50 (log M)

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<th>Maximal relaxation, %</th>
<th>Ach</th>
<th>NTG</th>
<th>A23187</th>
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</thead>
<tbody>
<tr>
<td><strong>Wild-type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>−7.54 ± 0.36</td>
<td>−7.66 ± 0.18</td>
<td>−7.53 ± 0.13</td>
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<tr>
<td>BMP4</td>
<td>−7.01 ± 0.29</td>
<td>−7.13 ± 0.13</td>
<td>−7.38 ± 0.25</td>
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<td>BMP4 + noggin</td>
<td>−7.43 ± 0.29</td>
<td>−7.58 ± 0.30</td>
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<tr>
<td>Noggin</td>
<td>−7.76 ± 0.25</td>
<td>−7.42 ± 0.16</td>
<td>−7.45 ± 0.12</td>
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<tr>
<td>Vehicle + apocynin</td>
<td>−7.51 ± 0.25</td>
<td>−7.80 ± 0.27</td>
<td>−8.38 ± 0.15</td>
</tr>
<tr>
<td>BMP4 + apocynin</td>
<td>−7.36 ± 0.18</td>
<td>−7.77 ± 0.20</td>
<td>−7.88 ± 0.26</td>
</tr>
<tr>
<td><strong>p47(phox)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>−6.87 ± 0.22</td>
<td>−7.35 ± 0.29</td>
<td>−7.94 ± 0.26</td>
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<tr>
<td>BMP4</td>
<td>−6.84 ± 0.17</td>
<td>−7.45 ± 0.19</td>
<td>−8.32 ± 0.22#</td>
</tr>
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</table>

Ach indicates acetylcholine; NTG, nitroglycerin; and A23187, calcium ionophore A23187. BMP4 and noggin were given at a dose of 0.9 mg/kg each unless stated otherwise. 

*P<0.001 vs vehicle, noggin, and BMP4 + noggin. 
†P<0.05 vs vehicle, noggin, and BMP4 + noggin. 
‡P<0.0001 vs BMP4. 
§P<0.05 vs BMP4. 
¶P<0.005 vs C57BL/6J BMP4. 
||P<0.05 vs vehicle. 
#P<0.05 vs C57BL/6J BMP4.

Several observations support this notion. Of the 3 nox isoforms expressed in endothelial cells, nox1 and nox2 require p47(phox) for their optimal activation, whereas nox4 activity is believed to be independent of p47(phox).35 Apocynin inhibits NADPH oxidases by blocking assembly of the cytosolic p47(phox) to the membrane.36 Therefore, nox1 and nox2 are the most likely NADPH oxidases that would be affected by either genetic deletion of p47(phox) in mouse aortas or apocynin treatment in vivo, ex vivo, or in vitro. Recently, we have shown that treatment of cultured endothelial cells with oscillatory shear stress or BMP4 induces ROS production and inflammatory responses, and these effects can be prevented either by treating the cells with noggin or nox1 short interfering RNA or by using endothelial cells obtained from p47(phox)−/− mice.31,32 Consistent with these in vitro findings, the present study has demonstrated that BMP4’s effect on the activation of NADPH oxidases, impairment of vasorelaxation, and hypertension can be prevented by either apocynin treatment or p47(phox) knockout. Taken together, our present and previous in vitro data indicate that BMP4 upregulates and activates NADPH oxidases dependently on nox1 and p47(phox). It remains to be resolved, however, which arterial cell types are the targets of BMP4. Given our previous in vitro studies, which showed that BMP4 stimulated superoxide production in endothelial cells by activating nox1- and p47(phox)-dependent NADPH oxidases,3 it is reasonable to speculate that endothelial nox1 NADPH oxidase is the most probable target of BMP4. These questions need to be clarified by further studies.

In the present study, we found that BMP4 infusion markedly altered endothelium-dependent relaxations to both acetylcholine and calcium ionophore A23187. This finding is consistent with the observation that BMP4 induced an increase in vascular superoxide production. Superoxide reacts with NO at nearly a diffusion-limited rate, leading to a reduction in NO bioavailability and the formation of the strong oxidant peroxynitrite.37,38 As discussed earlier, the increase in vascular superoxide caused by BMP4 was likely due to activation of NADPH oxidase. In keeping with this, incubation of vessels with apocynin (or vessels from p47(phox)−/− mice) completely normalized relaxation in BMP4-infused, wild-type mice.

Of interest, we found that BMP4 also altered vasorelaxation sensitivity to nitroglycerin, as reflected by a rightward shift in the dose-response curve, although maximal relaxations did not change. It has recently been proposed that there are low- and high-potency pathways for bioconversion of nitroglycerin to its active vasodilator metabolite.39,40 The high-potency pathway, responsible for relaxations to low concentrations (<10−7 mol/L), has been attributed to the action of the mitochondrial aldehyde dehydrogenase in vascular smooth muscle.41 Furthermore, it has been shown that this pathway can be inactivated by peroxynitrite.42 It is interesting to speculate...
that the shift in ED₅₀ caused by BMP4 might be mediated by oxidative inactivation of aldehyde dehydrogenase and that this was corrected by apocynin treatment.

In addition to shear, hypoxia has been shown to induce BMP4 secretion in pulmonary endothelial cells, although its role in pulmonary smooth muscle cell proliferation and pulmonary hypertension is controversial.⁴³–⁴⁵ On the other hand, it is now well established that the loss-of-function mutations of 1 of the BMP4 receptors (BMPR-II) are linked to familial primary pulmonary hypertension and sporadic primary pulmonary hypertension in humans and animal models.⁴⁶–⁴⁹ Given these in vivo findings, it seems unlikely that the increase in systemic blood pressure effected by BMP4 infusion observed in the present study can be accounted for by the pulmonary effect.

On the basis of these concepts and findings, we suggest the following working hypothesis, as shown in Figure 4. We propose that endothelial cells exposed to disturbed blood flow produce BMP4, which is secreted abuminally to the subendothelial layer and luminally to the blood. BMP4, in an autocrine- and a paracrine-dependent manner, then activates arterial NADPH oxidases, leading to increased superoxide production, which then interacts with NO and reduces its bioavailability, leading to an impairment of endothelium-dependent vasorelaxation and eventual hypertension.

In summary, we have shown that chronic BMP4 infusion activates arterial NADPH oxidases and that this in turn leads to endothelial dysfunction and hypertension. Noggin prevents these effects. The novel actions of BMP4 and noggin raise the possibility that noggin and its related compounds might be therapeutic agents and that BMP4 is a novel mediator of endothelial dysfunction and hypertension.

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Disclosures
None.

References

Figure 4. We propose that an increase in BMP4 stimulates vascular NADPH oxidases, which in turn increase ROS production, leading to reduction of NO bioavailability, endothelial dysfunction, and hypertension. The shaded area is the pathway examined in the present study. Noggin prevents BMP4-induced vascular effects, suggesting its potential use as a therapeutic agent to treat and prevent endothelial dysfunction and hypertension. BMP4 expression is regulated by shear stress conditions that could change in response to physical activity levels.
BMP4 Induces Hypertension in Mice

Miriya et al.

**CLINICAL PERSPECTIVE**

Bone morphogenic proteins 2 and 4 (BMP2/4) are well-known bone and cartilage morphogens, but it is unclear whether they also play a role in the pathophysiology of cardiovascular diseases. Using a microarray analysis, we recently discovered that BMP4 is produced in endothelial cells by stimulating superoxide production from nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. In this study, we hypothesized that BMP4 would induce hypertension in intact animals by increasing superoxide production from vascular NADPH oxidases, which in turn would impair vasodilation responses. Mice were chronically infused with BMP4 for 4 weeks by osmotic pump implants. BMP4 increased systolic blood pressure in a time- and dose-dependent manner in both wild-type C57BL/6 mice (from 101 to 125 mm Hg) and apolipoprotein E-null mice (from 107 to 146 mm Hg) for 4 weeks. Cotreatment with the BMP antagonist noggin or the NADPH oxidase inhibitor apocynin completely blocked the BMP4 effect. In addition, BMP4 infusion stimulated aortic NADPH oxidase activity and impaired endothelium-dependent vasorelaxation, both of which were prevented by noggin coinfusion. Remarkably, BMP4 infusion failed to stimulate aortic NADPH oxidase increases, increase blood pressure, and impair vasodilation responses in NADPH oxidase-null mice lacking the p47phox gene. These results show that BMP4 causes hypertension in mice by activating vascular NADPH oxidases and subsequently inducing endothelial dysfunction. BMP4, which could be secreted into the blood from the endothelium by disturbed flow conditions, is a novel mediator of endothelial dysfunction and hypertension, and noggin and its analogs could be used as therapeutic agents in treating vascular diseases.
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