Inducible regulation of human brain natriuretic peptide promoter in transgenic mice

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Received 28 April 2000; accepted in final form 9 August 2000

He, Quan, Ding Wang, Xiao-Ping Yang, Oscar A. Carretero, and Margot C. LaPointe. Inducible regulation of human brain natriuretic peptide promoter in transgenic mice. Am J Physiol Heart Circ Physiol 280: H368–H376, 2001.—Studies have shown that brain natriuretic peptide (BNP) gene expression is rapidly induced in the infarcted heart and that plasma BNP levels reflect the degree of left ventricular dysfunction. Our previous in vitro work using transiently transfected neonatal rat cardiac myocytes has shown that the human BNP (hBNP) promoter, in particular a region extending from −127 to −40 relative to the start site of transcription, is more active in cardiac myocytes than in fibroblasts. To study tissue-specific and transcriptional regulation of the hBNP gene in vivo, we generated transgenic mice containing the proximal hBNP promoter (−408 to +100) coupled to a luciferase reporter gene. In four lines of transgenic mice, luciferase activity was ∼33- to 100-fold higher in the heart than in other tissues, including the whole brain. To test whether the transgene responded to a pathophysiologic stimulus, we induced infarction by coronary artery ligation. Luciferase activity was fivefold higher in the infarcted region of the left ventricle at 48 h than in sham-operated animals and remained elevated for 4 wk. Endogenous BNP mRNA was similarly increased in the infarcted hearts of a separate group of mice. We conclude that 1) the proximal 408-bp region of the hBNP promoter confers cardiac-specific expression and 2) myocardial infarction activates the proximal hBNP promoter in vivo. These data suggest that we have a valid model for the study of basal and inducible regulation of the hBNP gene in vivo.

Infarction; myocardial cells; echocardiography

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vivo. These animal models also provide insight into developmental and inducible regulation of genes in response to physiological and pathophysiological stimuli, conditions that cannot be totally mimicked by in vitro analyses. Transgenic mice have been used to study muscle- and cardiac-specific gene expression, whereby the 5'-flanking sequence of myosin light chain-2 (MLC-2), α- and β-myosin heavy chain, and human ANP genes have directed reporter gene expression (7, 21, 28, 32). Since there are differences in regulation of ANP and BNP in the heart, we generated transgenic mice overexpressing the proximal hBNP promoter coupled to a luciferase reporter gene to study its tissue-specific and inducible regulation. Inducible regulation was studied in a model of MI. Our data indicate that the hBNP promoter is expressed primarily in cardiac ventricles, and its activity is induced by MI. Use of this novel model system may provide an understanding of the molecular mechanisms behind changes in the structure and function of the heart during the development of HF.

MATERIALS AND METHODS

Generation of transgenic mice. Standard procedures were used to generate transgenic mice (16). Mice were produced at the Transgenic/Knockout Animal Facility Core at the National Institute of Environmental Health Sciences Center for Molecular and Cellular Toxicology at Wayne State University (Detroit, MI). The proximal hBNP promoter (−408 to +100) was cloned upstream from a luciferase reporter gene in a pUC18 plasmid (20). The transgene was removed from the plasmid by double digestion with Hind III and Pvu II, purified, and microinjected into zygotes from B6C3F1 mice. Viable injected zygotes were transferred into the infundibulum of the oviduct in pseudopregnant CD-1 mice. Three weeks after birth, genomic DNA was extracted from a 1.0-cm piece of tail and subjected to Southern blot for detection of the transgene. Founder mice (transgene-positive) were mated with nontransgenic littermates to test for transmission of the transgene. Male and female heterozygous and homozygous mice were used in these experiments.

Southern blots. The tail was digested in 400 μl of lysis buffer [50 mM Tris (pH 8.0), 100 mM EDTA, 100 mM NaCl, 1% SDS, and 200 μg proteinase K] at 55°C overnight. After extraction with phenol and chloroform, the DNA was precipitated and quantified by spectrophotometry. Genomic DNA (5 μg) from each sample was digested with Eco R I. DNA was separated out on a 0.8% agarose gel and then transferred onto a nylon membrane. A 2-kb-pair Eco R I fragment of the firefly luciferase cDNA was radiolabeled with 32P[dCTP for detection of the transgene. Transgene copy number was determined using standard procedures. Briefly, digested genomic DNA and a series of transgene standards (0–32 copies of the transgene) were subjected to Southern blotting, hybridization, and quantification of signal intensity by densitometry.

Northern blot. Total RNA was extracted by homogenization of myocardial samples in TriReagent (Molecular Research Center, Cincinnati, OH) following the manufacturer’s instructions. For Northern blots, 15 μg of total RNA were denatured in glyoxal-DSMO, size fractionated on a 1.2% agarose gel, and transferred to a nylon membrane (GIBCO-BRL, Gaithersburg, MD). cDNA probes for rat BNP and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), as well as hybridization and washing conditions, have been described elsewhere (19). To quantitate changes in BNP mRNA, autoradiographs were analyzed by densitometry (model GS-670, Bio-Rad, Hercules, CA) and corrected to the GAPDH mRNA signal.

Luciferase assay. Tissues were removed from mice and homogenized with a Polytron in 0.6–1.2 ml of 10 mM Tris (pH 7.5) (20). After homogenization, one-fifth volume of 5× reporter lysis buffer (Promega) was added. After freeze-thawing and microcentrifugation to pellet cellular debris, 20 μl of the supernatant were assayed for luciferase activity (Promega system) in a luminometer (OptoComp 1, MGM Instruments). Relative light units were normalized to milligrams of protein for the 20-μl aliquot of tissue lysate using Coomasie reagent (Pierce, Rockford, IL). For analysis of luciferase activity in the heart, the heart was dissected into right and left atrium and ventricles, which were further dissected into right ventricle, free wall of the LV, and septum. To determine which region(s) of the brain expressed hBNPLuc activity, brains were dissected into cerebrum (i.e., right and left cerebral hemispheres), cerebellum, brain stem, hypothalamus, and thalamus. The brain was extracted from the skull and placed ventral side up in a dissecting dish. After removal of the cerebellum, the brain stem (pons and medulla oblongata) was removed and the brain was divided into two hemispheres via a midsagittal section. The left cerebral peduncle was dissected, exposing the thalamus and hypothalamus. Finally, one cerebral hemisphere was removed. For the cerebellum, cerebrum, thalamus, and hypothalamus, the homogenized tissue represented one-half of each region of the brain. Luciferase activity was expressed as mean ± SE, and differences among means were analyzed by Student’s t-test (2 comparisons) or ANOVA with multiple pairwise comparisons made by the Student-Newman-Keuls method (≥3 comparisons). P < 0.05 was considered significant.

Immunocytochemistry. Hearts were removed, washed free of blood, fixed in formalin, and embedded in paraffin. Longitudinal sections (5 μm) were cut and stained for luciferase protein using an immunalkaline phosphatase staining kit (Biomeda) and Fast red (Sigma Chemical) as the substrate. A rabbit anti-luciferase polyclonal antibody was used at a 1:300 dilution (Cortex Biochem, San Leandro, CA). Tissue sections were then counterstained with hematoxylin.

Coronary artery ligation. Mice weighing ≈22 g were anesthetized with pentobarbital sodium (50 mg/kg ip). They were placed supine on a heating pad, and the trachea was exposed by a midline cervical incision. The mouse was intubated and ventilated with a minute volume of 0.2 ml at a rate of 95–110/min (model 680, Harvard Apparatus). The coronary artery was ligated as described previously (38). Briefly, the mouse was placed on its right side, and a thoracotomy was performed, with an incision between the fourth and fifth intercostal spaces. The lungs were retracted, and the pericardium was opened. The left anterior descending coronary artery was ligated with a 9-0 silk suture placed near its origin at the edge of the left atrium. Placement of the ligature was deemed successful when the anterior wall of the LV turned pale. Lungs were inflated, and the thoracotomy site was closed in layers. Sham-operated mice underwent the same procedure except the suture was not tightened. From 48 h to 4 wk after coronary artery ligation, mice were analyzed by echocardiography or anesthetized, and then hearts were removed to assay luciferase activity or BNP and GAPDH mRNA as described above. The Henry Ford Hospital Care of Experi-
Measurement of blood pressure and cardiac function. Blood pressure and heart rate of conscious mice (6.5–36.5 wk of age) were measured by a noninvasive computerized tail-cuff method (model BP-2000, Visitech Systems). To ensure that the mice had adapted to the method, the blood pressure was taken 20 times consecutively, and the average measurement was used.

Two-dimensional M-mode transthoracic echocardiography was performed on conscious mice using an Acuson 256 system (Mountain View, CA) with a 15-MHz linear transducer according to previously documented procedures (37). The heart was first imaged in the two-dimensional mode from the parasternal long-axis view, and then an M-mode cursor was positioned perpendicular to the ventricular septum and posterior wall of the LV at the level of the papillary muscles. M-mode images were used to determine posterior LV wall thickness, interventricular septum thickness, LV end-diastolic dimension, LV end-systolic dimension, and aortic root dimension. For diastolic dimensions, measurements were made at the maximum LV cavitary dimension, while systolic parameters were measured at the time of maximum anterior motion of the posterior wall. Images were stored in digital format on magneto-optical disks, and all measurements were made using the software installed in the echocardiography machine. Three beats were averaged for each measurement. LV mass (mg) and shortening fraction were calculated as described previously (37). LV mass was normalized for body weight and expressed as milligrams per 10 g body wt. Echocardiographic data were analyzed by one-way ANOVA. Bonferroni’s method was used to analyze multiple comparisons vs. control. \( P < 0.05 \) was considered significant.

RESULTS

Generation of founder lines of hBNP luciferase transgenic mice. Transgenic mice were generated using the proximal hBNP promoter coupled to luciferase (408hBNPLuc). Eleven transgene-positive founder mice were detected by Southern blot. Eight of the founders (F202, F203, F63, F75, F77, F83, F86, and F92) were bred to control B6C3F1 mice, and all lines transmitted the transgene to their offspring. Four lines with high levels of luciferase activity in the heart (Tg203, Tg83, Tg86, and Tg75) were maintained. Tg203 offspring were bred to generate a line that was homozygous at the transgene locus, while the other lines were maintained as heterozygous by breeding with nontransgenic litters. There were \( \sim 17 \) copies of the transgene in lines 83 and 86, 11 copies in line 75, and 12 copies in line 203.

Tissue-specific expression of the 408hBNPLuc transgene. To study the activity of the hBNP promoter in different tissues in vivo, F1 offspring of four lines of 408hBNPLuc mice were assayed for luciferase activity in the ventricle (LV, right ventricle, and septum), brain, kidney, liver, lung, and skeletal muscle. As shown in Fig. 1, luciferase activity was much higher in total ventricle (Fig. 1A) or LV (Fig. 1, B–D) than in the

![Fig. 1. Tissue-specific expression of 408hBNPLuc transgene in mice. y-Axis, luciferase activity [expressed as percentage of total ventricle (V) or left ventricle (LV), which is arbitrarily set to 100%]; x-axis, tissue tested for luciferase activity. A: Tg203; for ventricle, \( 3.6 \pm 0.7 \times 10^6 \) actual relative light units (RLU)/mg protein (\( n = 9 \) mice from 3 separate litters). B: Tg75; \( 2.7 \pm 0.6 \times 10^5 \) actual RLU/mg protein (\( n = 6 \) mice). C: Tg83; \( 1.6 \pm 0.3 \times 10^6 \) actual RLU/mg protein (\( n = 7 \) mice). D: Tg86; \( 3.5 \pm 1.3 \times 10^6 \) actual RLU/mg protein (\( n = 8 \) mice). V, ventricles (right + left + septum); LV, left ventricle (free wall + septum); BR, brain; KID, kidney; LIV, liver; Lu, lung; SKM, skeletal muscle (gastrocnemius or quadriceps). The brain (including cerebellum and brain stem) was removed intact, and one-half was homogenized and assayed. Each bar represents mean \( \pm \) SE.](http://ajpheart.physiology.org/doi/10.1152/ajpheart.00035.2017)
other tissues in all four lines. Compared with luciferase activity in the ventricle, activity in the brain, kidney, liver, lung, and skeletal muscle was 1–3%. Thus high heart-specific luciferase activity was seen in all four lines.

BNP mRNA has been detected in all four compartments of the rat and human heart (5, 17). Thus we determined whether luciferase activity was detectable in all four regions of the mouse heart. Data in Fig. 2 show that luciferase activity was very high in the right ventricle and LV of all four lines, almost undetectable in the left atrium of all four lines, and varied in the right atrium from 7–8% of the LV value (Tg203 and Tg75) to 70–90% (Tg83 and Tg86).

To determine the cell type in which luciferase was expressed, hearts from the Tg203 line were fixed, em-

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Fig. 2. Heart regions expressing the 408 hBNPLuc transgene. RA, right atrium; LA, left atrium; RV, right ventricle; LV, free wall + septum. A: Tg203 (n = 9, P < 0.01, RA and LA vs. LV). B: Tg75 (n = 6, P < 0.01, RA, LA, and RV vs. LV). C: Tg83 (n = 7, P < 0.01, LA vs. LV). D: Tg86 (n = 8, P < 0.01, LA vs. LV, and P < 0.05, RV vs. LV). **P < 0.01; *P < 0.05. Each bar represents mean ± SE.

Fig. 3. Immunocytochemical localization of luciferase in the ventricle. Sections of hearts from nontransgenic (A) and Tg203 mice (B) were immunostained for luciferase protein. Red staining in B represents luciferase protein. Magnification ×400.
bedded, sectioned, and stained with an anti-luciferase antibody. Figure 3 shows positive staining (red color) for luciferase in LV myocytes (B), while a control non-transgenic mouse heart is without luciferase staining (A). To further confirm that luciferase protein was produced in cardiac myocytes, we prepared primary cultures of myocytes from neonatal mouse hearts. Luciferase activity was $1.62 \pm 0.13 \times 10^5$ relative light units/mg protein ($n = 3$).

Studies of dog and pig brains have shown that BNP is detectable in the brain stem, hypothalamus, and spinal cord (31). Data in Fig. 4 indicate that luciferase was higher in the brain stem and thalamus/hypothalamus than in the cerebellum and cerebrum of Tg203 mice and that activity was $<5\%$ of that of the ventricle.

**Effect of MI on BNP expression.** As described in the introduction, BNP levels correlate with LV dysfunction in humans. To test whether infarction results in up-regulation of the endogenous mouse BNP gene, a group of nontransgenic mice was subjected to coronary artery ligation or sham operation, and 48 h later the heart was removed for isolation of RNA. Northern blot indicated increased endogenous BNP mRNA in the infarcted LV compared with sham-operated controls (Fig. 5A), while GAPDH mRNA was not affected. Densitometry of the Northern blot showed a greater than fivefold increase in BNP mRNA normalized to GAPDH mRNA in the infarcted hearts (Fig. 5B).

To test whether the 408hBNPLuc transgene contains regulatory elements that would respond to the pathophysiological stimulus of MI, we ligated the coronary artery of Tg203 mice and assayed luciferase activity in the LV 48 h later. Figure 5C indicates that luciferase activity was increased fivefold in the infarcted hearts compared with sham-operated Tg203 mice. We also produced an infarct in Tg86 and Tg83 mice and found at least fivefold stimulation of luciferase activity compared with sham-operated mice (data not shown). Luciferase activity increased in the right ventricle as well as the septum in all three lines.

![Figure 5](http://example.com/figure5.png)

**Fig. 5.** Effect of coronary artery ligation on brain natriuretic peptide (BNP) mRNA and luciferase activity. At 48 h after ligation of the coronary artery, the heart was removed. A: BNP mRNA in sham-operated (Sham) vs. infarcted (INF) nontransgenic mouse hearts. BNP and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNAs are shown. GAPDH mRNA shows equal loading and transfer of RNA to the nylon membrane. B: Northern blot data were scanned by laser densitometry and quantitated. DU, densitometry units, corresponding to BNP mRNA normalized to GAPDH mRNA ($n = 4$ Sham and 6 INF). C: luciferase activity vs. treatment ($n = 3$). **$P < 0.01$ vs. Sham. Each bar in $B$ and $C$ represents mean ± SE.

Thus an increase in hBNP promoter activity (i.e., transcription) is likely the cause of increased levels of BNP mRNA in infarcted hearts.

We next tested whether the hBNP promoter was chronically activated after ligation in Tg203 mice. Figure 6 shows that hBNP promoter activity was increased 2.5- and 1.7-fold in infarcted hearts compared with sham-operated controls at 3 and 4 wk, respectively. Thus the proximal 408-bp region contains reg-

![Figure 6](http://example.com/figure6.png)

**Fig. 6.** Luciferase activity vs. treatment ($n = 3$). **$P < 0.01$ vs. Sham. Each bar represents mean ± SE.
ulotary elements that respond to the chronic pathophysiological stimuli associated with infarction.

Echocardiography of Tg203 mice. We measured blood pressure and heart rate using a computerized tail-cuff system. The transgenic mice were 6.5–36 wk old; the nontransgenic controls, which were genetically similar, were 6.5–24 wk old. There were no detectable differences in blood pressure or heart rate between nontransgenic mice and the Tg203 line of transgenic mice (data not shown).

To study cardiac function in Tg203 mice, we used two-dimensional M-mode echocardiography. As expected, there was no difference in cardiac mass, chamber dimensions, or function between the control Tg203 and nontransgenic mice (data not shown) and between Tg203 and sham-operated mice (Table 1). At 3 and 4 wk after coronary artery ligation, echocardiography indicated that cardiac remodeling had occurred, including dilatation (increase in LV end-diastolic and end-systolic dimensions) and hypertrophy (increase in cardiac mass and posterior wall thickness) of the LV.

In addition, cardiac function was diminished, as evidenced by the decrease in ejection and shortening fractions. These changes in cardiac structure and function after MI were accompanied by stimulation of hBNP-driven luciferase activity (Fig. 6).

DISCUSSION

Our studies are the first to examine the hBNP promoter in vivo. We have generated lines of transgenic mice in which the proximal 408 bp of the hBNP promoter overexpressed the firefly luciferase reporter gene primarily in the cardiac ventricles. Expression of luciferase in myocytes was detected by immunocytochemistry and assay of luciferase activity in primary cultures of neonatal mouse myocytes. These results confirm our in vitro studies, wherein the hBNP promoter was found to be more active in ventricular myocytes than in atrial myocytes and fibroblasts (20).

Moreover, using a model of MI, we have shown that the proximal 408-bp region of the hBNP promoter responds to this pathophysiological stimulus, as does endogenous mouse BNP mRNA. Thus we have created a model by which we can study regulation of hBNP gene expression in response to pathophysiological stimuli that result in ischemia, hypertrophy, and HF.

In our tissue-specific expression studies, we found that the 408hBNPLuc transgene was expressed primarily in the heart. Luciferase activity was almost undetectable in the brain stem and thalamus/hypothalamus. These data are consistent with studies on the detection of BNP mRNA in rodent and human tissues. By using quantitative RT-PCR, Dagnino et al. (5) showed that rat BNP mRNA is highest in the heart, with extracardiac expression in the lung, hypothalamus, and whole brain being <1% of that in the right atrium. A similar approach was used to evaluate BNP transcripts in human cardiac and extracardiac tissues at autopsy, and BNP mRNA in the LV was 10 times higher than in the brain and lung (10). These data suggest greater extracardiac expression of BNP in human tissues. In contrast to the rat and human, mouse BNP mRNA has been detected by Northern blot (25), RNase protection (30), and in situ hybridization (3) in the atria and ventricles but not the brain and other extracardiac tissues. The absence of extracardiac ex-

Table 1. Echocardiography of Tg203 mice

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>Sham (n = 15)</th>
<th>3 wk (n = 8)</th>
<th>4 wk (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>29 ± 0.6</td>
<td>26.3 ± 1.2</td>
<td>29.7 ± 0.8</td>
<td>28.9 ± 2.7</td>
</tr>
<tr>
<td>LVEDd, mm</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>3.9 ± 0.4*</td>
<td>3.4 ± 0.3*</td>
</tr>
<tr>
<td>LVDs, mm</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>2.8 ± 0.3*</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>Cardiac mass/body wt, mg/10 g</td>
<td>11.2 ± 1.1</td>
<td>15.3 ± 1.7</td>
<td>29.7 ± 0.8*</td>
<td>30.9 ± 5.6*</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>1 ± 0.03</td>
<td>1.1 ± 0.02*</td>
<td>1.2 ± 0.04*</td>
<td>1.3 ± 0.02*</td>
</tr>
<tr>
<td>EF, %</td>
<td>86.5 ± 2.5</td>
<td>82.6 ± 1.3</td>
<td>40.8 ± 4*</td>
<td>53 ± 6.8*</td>
</tr>
<tr>
<td>SF, %</td>
<td>53.2 ± 1.6</td>
<td>55.2 ± 0.6</td>
<td>30.6 ± 3.6*</td>
<td>35.3 ± 2.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MI, myocardial infarction; LVEDd, left ventricular diastolic dimension; LVDs, left ventricular systolic dimension; PWT, posterior wall thickness in systole; SF, shortening fraction; EF, ejection fraction. Sham values include mice from 3- and 4-wk groups. *P < 0.05 vs. control.
expression may simply reflect the inability of these methods to detect low levels of BNP mRNA. Since the luciferase assay system is a very sensitive indirect measurement of transcriptional regulation (i.e., promoter activity), we expected to detect luciferase activity in some extracardiac tissues, particularly the brain. Thus, in our studies, the hBNP promoter was expressed primarily in the mouse heart, consistent with studies examining BNP mRNA or peptide in rodents and humans.

Regarding luciferase expression in different regions of the mouse heart, all four lines of 408hBNPLuc mice showed high levels of activity in the LV and right ventricle and very low levels in the left atrium. Highly variable activity was detected only in the right atrium. Based on the weight of the ventricles compared with the atria, transgene expression is highly ventricle enriched. BNP mRNA has been detected in all chambers of human hearts obtained at autopsy (17). In the mouse, BNP mRNA, detected by in situ hybridization, is higher in the developing ventricles than atria, but levels in the adult ventricles and atria are equivalent (3). One explanation for the difference in the pattern of transgene expression vs. BNP mRNA is a positional effect resulting from the site of transgene integration. Although the site of integration of the transgene can affect transgene expression (27), it is unlikely that this is the reason for the variable expression in the right atrium, since the pattern of expression was similar for the other heart regions in all four lines.

A number of genes are expressed in a chamber-specific fashion during embryogenesis and in the adult heart (see Ref. 8 for review). MLC-3F regulatory sequences resulted in transgene expression in the right and left atria and LV (18), while a 250-bp fragment of the MLC-2v promoter directed expression of a marker transgene exclusively to the right ventricle (21, 29). An MEF2-binding site (AT-rich region) is critical for expression of the MLC-2v gene in the ventricle (21, 29). A similar regulatory element has not been identified in the BNP promoter, suggesting that a different element or combination of elements in the proximal hBNP promoter is highly ventricle specific. In transient transfection studies, we have identified elements involved in basal and inducible regulation of the hBNP promoter, including GATA and M-CAT-like sites (12, 13, 20). Transcription factors binding these elements participate in heart development and regulation of many cardiac-specific genes (see Ref. 8 for review). The lack of expression of the hBNPLuc transgene in the left atrium and variable expression in the right atrium may result from the absence of regulatory elements upstream from position −408. The proximal 500 bp of the human ANP promoter confer atria-specific expression to a transgene (7), and the arrangement of cis elements in this region is different from that of the proximal hBNP promoter. Finally, it is possible that chamber-specific regulation cannot be maintained by the transgene, because the natriuretic peptide gene locus in the mouse and human genome is organized with the BNP gene 12–15 kb upstream from the ANP gene (33). This physical linkage may be critical for transcriptional regulation of both genes; however, to our knowledge, neither the presence nor location of a locus control region, similar to that described for the β-globin gene locus (9), has been identified for the regulation of the natriuretic peptide genes.

To test whether our transgenic mice could be used as a model to study regulation of the hBNP promoter in an in vivo pathophysiological setting, we induced ischemia and infarction by coronary artery ligation. The hBNPLuc transgene was activated up to 4 wk after MI, indicating that the proximal promoter region contains regulatory elements responsive to the pathophysiological stimuli induced by coronary artery ligation. The hBNP promoter was activated at least fivefold at an early time point (48 h) and nearly twofold at 3 and 4 wk. The decline in activation of the promoter with time likely reflects the remodeling and decompensation of the heart after MI. In these mouse hearts, LV hypertrophy and dilatation were accompanied by a decrease in function, which probably resulted from a continuous loss of myocytes and progressive interstitial fibrosis in the noninfarcted region of the LV.

Proinflammatory mediators such as interleukin-1β and tumor necrosis factor (14), vasoactive peptides such as endothelin-1 (4), mechanical factors (stretching of the ventricle), and β-adrenergic stimulation could contribute to activation of the hBNP promoter after MI. Interleukin-1β and endothelin-1 activate the hBNP promoter in transient transfection experiments in vitro (12). Moreover, interleukin-1β, acting in part through p38 kinase, targets the M-CAT element at position −97 of the hBNP promoter (12), and endothelin-1 targets a GATA element at −85 (unpublished data). The β agonist isoproterenol also activates the hBNP promoter through proximal M-CAT elements (13). Studies have suggested that GATA, AP-1, and M-CAT elements play a role in inducible expression of cardiac-specific genes in models of hypertrophic growth of myocytes in vitro (34) and in hearts subjected to pressure overload (11, 15, 36). Molkentin et al. (22) identified the calcineurin-activated transcription factor NF-AT3 as being important in the development of cardiac hypertrophy, HF, and regulation of natriuretic peptide genes. In particular, the NF-AT3 binding site at −927 of the hBNP promoter is synergistically activated by NF-AT3 and GATA-4. However, our present studies would indicate that the −927 NF-AT3 site is not required for upregulation of the hBNP promoter after MI, inasmuch as luciferase activity is induced in the 408hBNPLuc mice. Thus the hBNP promoter is likely regulated in vivo by a complex combination of cis elements and transcription factors. We are presently defining the roles of these elements in regulation of the hBNP gene in vitro, and future studies will focus on whether these elements are involved in cardiac-specific and inducible regulation of the hBNP gene in vivo.

In conclusion, the proximal 408 bp of the hBNP promoter confer cardiac muscle-specific expression of a luciferase reporter gene. Overall ventricular expression is higher than atrial expression, suggesting
that the proximal promoter could be used to target genes of interest to the ventricular myocardium. In addition, the proximal 408-bp region of the hBNP promoter is responsive to pathophysiological stimuli associated with MI. By studying hBNP promoter activity after MI and during the subsequent 3–4 wk when HF develops in mice, we may gain insight into the molecular mechanisms that underlie infarction and HF. Moreover, since hBNP promoter activity is associated with LV dysfunction after MI, we may be able to use these mice to test the efficacy of drugs and gene transfer therapies for improvement of cardiac function.

The authors thank Dr. Yun-He Liu for expert assistance with the echocardiograph studies and FangFei Wang for performing the surgical procedures.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-28982 and HL-03188.

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