Interleukin-1β Regulation of the Human Brain Natriuretic Peptide Promoter Involves Ras-, Rac-, and p38 Kinase–Dependent Pathways in Cardiac Myocytes

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Abstract—Because both the brain natriuretic peptide (BNP) gene and the cytokine interleukin-1β (IL-1β) are induced in the infarcted myocardium, localized production of IL-1β may regulate the BNP gene. We tested whether (1) IL-1β regulates the human BNP promoter, (2) cis elements in the proximal promoter respond to IL-1β, and (3) mitogen-activated protein kinase (MAPK) signaling pathways [p42/44, c-jun (JNK) and p38 kinase] are involved. We transferred the hBNP promoter coupled to a luciferase reporter gene or constructs with mutations in the proximal promoter GATA and M-CAT elements into neonatal rat ventricular myocytes and treated the cells with IL-1β for 24 hours. IL-1β–stimulated hBNP luciferase activity was eliminated by pretreatment with the transcription inhibitor actinomycin D. Both the p38 kinase inhibitor SB205380 (SB) and cotransfection of a dominant-negative mutant of p38 kinase reduced IL-1β stimulation of the hBNP promoter. Dominant-negative mutants of Ras and Rac inhibited IL-1β–stimulated hBNP luciferase activity by 64% and 90%, respectively. Constitutively active forms of Rac and MKK6, the immediate upstream activator of p38, were stimulatory; however, only the effect of MKK6 was inhibited by SB. Neither the p42/44 nor the JNK pathway was involved in the action of IL-1β. Both IL-1β and MKK6 activation of the hBNP promoter were partially reduced when the promoter contained a mutated M-CAT element. In summary, (1) IL-1β is a transcriptional activator of the hBNP promoter; (2) IL-1β acts through a Ras–dependent pathway not coupled to activation of p42/44 MAPK or JNK; (3) IL-1β acts through a Rac–dependent pathway, but the downstream effector is not known; and (4) IL-1β activation of p38 kinase is partially involved in regulation of the hBNP promoter, targeting the proximal M-CAT element. (Hypertension. 1999;33[part II]:283-289.)

Key Words: cell signaling ■ peptides, natriuretic ■ cytokines ■ M-CAT element

Human brain natriuretic peptide (BNP), one of three members of the natriuretic peptide family, is composed of 32 amino acids and has diuretic, natriuretic, and vasodilator properties.1 BNP is synthesized and secreted constitutively by the ventricles of the heart throughout development, unlike atrial natriuretic peptide (ANP), which is expressed at a low level in adult ventricles except in pathophysiological conditions.1,2 As a consequence of acute myocardial infarction and heart failure, as well as other conditions characterized by left ventricular hypertrophy, plasma BNP can be elevated as much as 100- to 200-fold,2,3 suggesting that synthesis of BNP may play an important compensatory role in these diseases. In addition, plasma BNP levels can be used as a biochemical marker of heart dysfunction.1–6

Inflammatory cytokines such as interleukin-1β (IL-1β) are increased after myocardial infarction and during progression of heart failure.7 Thus IL-1β is a potential regulator of the BNP gene in such pathophysiological conditions. In different types of cells, the effect of IL-1β is mediated by stimulation of a mitogen-activated protein kinase (MAPK), in particular p38 kinase.8–10 Several MAPK pathways have been identified, including p38, c-Jun kinase (JNK), and extracellular signal-regulated protein kinase (p42/44 ERK). Typically, growth factors activate the p42/44 MAPK pathway through the sequential activation of the small GTPase Ras, the kinase Raf (an MAPK kinase kinase), and the tyrosine-threonine kinase MEK (an MAPK kinase). Cytokines and stress activate JNK through the small GTPase Rac, the kinase MEKK (an MAPKKK), and the tyrosine-threonine kinases MKK4 and 7 (also known as JNKKs or SEKs). p38 is phosphorylated on tyrosine and threonine residues by MKK3 and 6 and in turn phosphorylates and activates the transcription factor ATF2, MEF2C, and the kinases MAPKAP2/3, resulting in regulation of target gene transcription.11–13 The identity of the small GTPase and the MAPKKK involved in the p38 kinase cascade is unclear.

Previous studies have shown that IL-1β can regulate gene expression in cardiac myocytes.14–16 Typically, IL-1β activates transcription factors such as nuclear factor κB and AP-1, which are involved in induction of inflammatory
response genes.\textsuperscript{10,17} Comparison of the proximal hBNP promoter with consensus elements corresponding to known regulatory motifs has identified potential cis elements, including M-CAT (at positions −124 and −97), and GATA-4 (−85). These elements are important in basal, tissue-specific, and inducible regulation of cardiac genes.\textsuperscript{18–20} In this study, we examined whether IL-1β is a direct transcriptional regulator of the hBNP promoter, whether proximal promoter cis elements are targets for its action, and whether the p38 kinase or other MAPK signaling pathways are involved.

**Methods**

**Cell Culture**
Ventricular myocyte–enriched cultures were generated from 1- to 2-day-old Sprague-Dawley rat pups (Charles River Laboratories, Kalazmoo, Mich), as described previously,\textsuperscript{21} according to a protocol approved by the Henry Ford Hospital Committee for Care and Use of Experimental Animals. Ventricular myocytes were cultured in DMEM (Gibco) containing 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L glucose, 0.1 mmol/L bromodeoxyuridine, and 10% fetal bovine serum (HyClone) for 2 days after birth. The cultures were digested with appropriate restriction enzymes to isolate the mutated elements. These elements are important in basal, tissue-specific, and inducible regulation of cardiac genes.\textsuperscript{18–20} In this study, we examined whether IL-1β is a direct transcriptional regulator of the hBNP promoter, whether proximal promoter cis elements are targets for its action, and whether the p38 kinase or other MAPK signaling pathways are involved.

**Transfection and Luciferase Assay**
Transfection and luciferase activity were assayed as described previously.\textsuperscript{21} For the full-length and mutant hBNP-luciferase (hBNPLuc) constructs, 1 μg was transfected by electroporation per 3 × 10\(^6\) cells. For the cotransfection studies, 0.5 μg of constitutively active Rac and MKK6 (or its control) and 10 μg of the dominant-negative (dn) mutants (dnp38, dnRas, dnRaf, dnRac, dnNNK, or a control expression vector) were cotransfected with −1818hBNPLuc. These concentrations were determined in preliminary dose-response studies. After transfection, the cells were divided into aliquots in 3 wells of a 12-well plate, with 0.5 × 10\(^6\) cells per well; 3 × 10\(^5\) cells are originally electroporated, but ≈50% of the cells die in the process. After 40 hours after transfection, the medium was switched to serum-free DMEM. The next day, cells were treated with inhibitors and then IL-1β. Cells were harvested 24 hours later, lysed, and assayed for luciferase activity (Luciferase Assay System, Promega) with an OptoComp 1 luminometer (GMG) according to the manufacturer’s protocol.

Duplicate aliquots of cell lysate from triplicate wells were assayed and averaged. Luciferase activity was normalized to protein levels. Data were expressed as the mean±SE of 4 separate experiments. *P < 0.01 for IL-1β vs ActD+IL-1β.

**Results**

**IL-1β Stimulates the hBNP Promoter**
To test whether IL-1β activates the hBNP promoter in a dose-dependent fashion, we treated transfected cells with 0.05 to 5 ng/mL IL-1β for 24 hours. As shown in Figure 1A, 0.05 to 5 ng/mL IL-1β activated the hBNP promoter. Because 5 ng/mL IL-1β resulted in maximum activation, this dose was used in subsequent experiments.

To examine whether the effect of IL-1β is the result of a transcriptional mechanism, transfected cells were pretreated with the transcriptional inhibitor ActD before.
treatment with IL-1β. Figure 1B shows that ActD had no effect on basal hBNP promoter activity but totally inhibited IL-1β–stimulated activity. IL-1β treatment for 3 to 6 hours also stimulated endogenous rat BNP mRNA (data not shown). These data implicate a transcriptional mechanism in IL-1β stimulation of hBNP promoter activity.

p38 kinase, Rac, and Ras Are Involved in IL-1β Regulation of the hBNP Promoter

To investigate whether the p38 kinase pathway is involved in IL-1β–stimulated hBNP promoter activity, transfected myocytes were pretreated with the p38-specific inhibitor SB 1 hour before administration of IL-1β. As shown in Figure 2A, SB reduced IL-1β stimulation of the hBNP promoter by 50% (P<0.05; n=4). To verify this result, we used an alternative approach: cotransfection of a dominant-negative (dn) p38 kinase expression vector. Expression of dn38 also decreased IL-1β–stimulated hBNP promoter activity by 50% (P<0.01; n=6; Figure 2B). It is possible that SB and dn38 have different specificities for the p38 isoforms in cardiac myocytes. Thus we tested them in combination and found that IL-1β–stimulated hBNP promoter activity was reduced by 88% (control, 1; SB+dn38, 0.7±0.3-fold; IL-1β, 4.3±0.6-fold; SB+dn38+IL-1β, 1.4±0.7-fold; n=3). Thus activation of p38 kinase partially mediates the effect of IL-1β on the hBNP promoter.

If IL-1β signal transduction involves the p38 pathway, then the effect of IL-1β should be mimicked by overexpression of the upstream activator of p38, MKK6. Constitutively active MKK6 [MKK6b(E)] potently stimulated hBNP promoter activity compared with the 4-fold increase stimulated by IL-1β (Figure 2C). Addition of IL-1β failed to increase MKK6-stimulated activity further, suggesting that p38 kinase...
is maximally activated. We verified that M KK6 stimulation of the hBNP promoter was mediated by p38 kinase by addition of SB, which decreased hBNP promoter activity by 86% (Figure 2D).

It is not clear how binding of IL-1β to its receptor is coupled to activation of the p38 MAPK signaling pathway. We tested the ability of small GTPases of the Ras family (Ras, Rac, and Rho) to either activate the hBNP promoter or inhibit IL-1β–stimulated activity. When we cotransfected −1818hBNPLuc with dominant-negative mutants of Ras and Rac, IL-1β–stimulated luciferase activity was decreased by 65% and 90%, respectively (Figure 3A). Overexpression of constitutively active Rac increased hBNP-luciferase activity 5-fold, and IL-1β increased this effect to 8-fold (Figure 3B). The effect of Rac overexpression was specific, since constitutively active RhoA had no effect on hBNP promoter activity (data not shown). However, Rac activation of the hBNP promoter may not involve p38 kinase, as SB was unable to inhibit this effect (Figure 3B). These data suggest that signaling pathways downstream from activated Ras and Rac are involved in IL-1β regulation of the hBNP promoter.

Involvement of p42/44 MAPK and JNK
Scherle et al.23 report that IL-1β activates all three MAPKs (p42/44 ERK, JNK, and p38) in rabbit articular chondrocytes, whereas other studies report that IL-1β selectively activates MAPKs.8,9,10,24 To test whether p42/44 MAPK is involved in IL-1β stimulation of the hBNP promoter, we used a specific inhibitor, PD, which inhibits MEK, the upstream activator of p42/44 MAPK. Figure 4A shows that PD failed to inhibit the effect of IL-1β. In fact, PD by itself increased hBNP promoter activity in −1818hBNPLuc-transfected myocytes and enhanced the effect of IL-1β. This effect of PD was also observed in separate experiments examining endothelin-1 regulation of the hBNP promoter (data not shown). Because the effect of PD was contrary to our expectations, we used a second approach to inhibit the p42/44 MAPK signaling pathway, overexpression of dominant-negative Raf, the MAPK kinase kinase associated with activation of MEK and thus p42/44. As shown in Figure 4B, dnRaf had no effect on either basal or IL-1β–induced hBNP promoter activity. These data suggest that Ras and Rac activation of the hBNP promoter involves a mechanism(s) independent of p42/44.

Because Ras activates Rac in some types of cells25 and Rac is involved in activation of the JNK pathway,11,12 we next tested whether IL-1β stimulation of the hBNP promoter involves JNK. To do this we used a dominant-negative mutant of JNKK, an upstream activator of JNK. Figure 4C shows that dnJNKK had no effect on IL-1β–stimulated hBNP promoter activity in transfected cardiac myocytes. We also cotransfected a JNKK expression vector with the hBNP promoter and then stimulated the myocytes with IL-1β, but activated JNKK had no effect on the hBNP promoter (data not shown), confirming the results of the dnJNKK experiments. These data suggest that Ras and Rac activation of the hBNP promoter involves a mechanism(s) independent of JNK, although they do not exclude the involvement of JNK isoforms insensitive to dnJNKK.

IL-1β Regulation of the hBNP Promoter Targets the Proximal M-CAT Element
The proximal hBNP promoter contains cis elements involved in tissue-specific expression in cardiac myocytes, including GATA-4 (−85) and M-CAT (−124 and −97) (Q.H., G.W., M.L., unpublished observations, 1998). To
investigate whether these elements are involved in IL-1β regulation of the hBNP promoter, we mutated each of them and transfected these mutated constructions into myocytes. Mutation of the M-CAT element at –97 reduced IL-1β stimulation of the hBNP promoter by 60%; however, there was no effect of mutation of the other elements (Figure 5A). To test whether p38 kinase targets the M-CAT element at position –97, we cotransfected 1818(M97)hBNPLuc with the MKK6 expression vector. MKK6 activation of the mutated hBNP promoter was decreased 35% relative to the wild-type promoter (Figure 5B). Thus a target for p38 kinase regulation of the hBNP promoter is the M-CAT element.

Discussion

Our data are the first to demonstrate that IL-1β regulates the hBNP promoter through a transcriptional mechanism targeting the proximal M-CAT element and that the effect of IL-1β is mediated by Ras-, Rac-, and p38-dependent pathways. Moreover, the M-CAT element is a target for the p38 kinase signaling pathway in cardiac myocytes.

IL-1β signal transduction is complex and can include activation of JNK, p42/44, and p38 kinases in different types of cells.8–10,23,24 Activation of p38 kinase can have opposite effects on IL-1β–stimulated genes in the same cell type5 or regulate some (but not all) responses to IL-1β.9 Our data using both pharmacological and molecular approaches indicate that p38 kinase is involved in IL-1β regulation of the hBNP promoter, a result not supported by data of Zechner et al,26 who showed that MKK6 activates the rat ANP and BNP promoters and that SB inhibits this effect by 70%. They also showed that the α-adrenergic agonist phenylephrine (PE) stimulated the rat ANP and BNP promoters through a p38 kinase–dependent pathway and that SB inhibited PE stimulation of the rat BNP promoter by only 40%, similar to our results.

Our data indicate that IL-1β signaling in cardiac myocytes involves the small GTPases Ras and Rac but not Rho. Ras activates the p42/44 pathway and Rac the JNK pathway in cardiac myocytes,26 but neither pathway seems critical for IL-1β regulation of the hBNP promoter in myocytes. Similarly, neither pathway is involved in PE stimulation of the rat ANP and BNP promoters.26–28 Additionally, we found that the MEK inhibitor PD activated the hBNP promoter and potentiated the effect of IL-1β, suggesting that either MEK itself or p42/44 negatively regulates the promoter, as shown for PE regulation of the rat ANP promoter.27,29

How Ras and Rac couple IL-1β binding to its receptor with activation of downstream signaling pathways is unclear. In preliminary studies, we have found that a dominant-negative mutant of the nonreceptor tyrosine kinase Src inhibits IL-1β stimulation of the hBNP promoter by 50% (Q.H., M.L., unpublished data, 1998), suggesting that Src may transmit signals from the IL-1β receptor to downstream effectors, including p38 kinase. Based on our preliminary pharmacological data, other kinases may also be involved, including protein kinase C (PKC) and phosphatidylinositol-3-kinase (PI3K).

An intriguing result of our study is that IL-1β stimulation of the hBNP promoter is Rac-dependent but that Rac activation of the promoter is not p38 kinase–dependent. This suggests that other Rac-mediated signals, such as reactive oxygen species,30 may be involved in IL-1β stimulation of the hBNP promoter. Alternately, Rac may activate a form of p38 kinase not inhibited by SB, such as p38γ (also called SAPK3 or ERK6)31 or p38δ.32 Because MKK6 activates both of these novel p38 kinases whereas SB does not inhibit them,1,13,33 this could explain why SB only inhibits MKK6 activation of the hBNP promoter by 86% (Figure 2D).

We have shown previously that the full-length hBNP promoter is more active in myocytes than in fibroblasts and that a region in the proximal promoter located between

Figure 5. IL-1β regulation of the hBNP promoter involves the –97 M-CAT cis element. The y axis represents IL-1β–stimulated luciferase activity, and the x axis is unstimulated cells (arbitrarily set to 1). A, Effect of mutation of M-CAT and GATA elements. Each bar represents the mean ± SE of 5 to 6 separate experiments. MGATA indicates 1818hBNPLuc with mutated GATA element at –85; M97, 1818hBNPLuc with mutated M-CAT at –97; and M124, 1818hBNPLuc with mutated M-CAT at –124. P < 0.05 vs IL-1β-stimulated 1818hBNPLuc. B, Effect of MKK6 on 1818(M97)hBNPLuc. The y axis is luciferase activity in MKK6-transfected cells, and the x axis is construct tested. Fold increase is calculated relative to cells transfected with plasmids in the absence of MKK6. Each bar represents the mean ± SE of 4 separate experiments. P < 0.01 vs 1818hBNPLuc.
−127 and −40 consists of potential cis elements arranged in tandem: M-CAT (−124 and −97) and GATA (−85), which are critical for cardiac-specific expression (Reference 21; Q.H., M.L., unpublished data, 1998). The present study indicates that inducible regulation of the hBNP promoter also targets the proximal promoter region, in particular the M-CAT element at −97. We believe this effect is specific for IL-1β, since the hypertrophic growth factor endothelin-1 did not target this region (Q.H., M.L., unpublished observations, 1998). Our data would also suggest that IL-1β targets the M-CAT element through a p38 kinase–dependent pathway. A similar mechanism may be involved in PE regulation of the rat BNP promoter.

Thuerauf and Glembockii13 have shown that PE regulation of the rat BNP promoter targets a proximal M-CAT element and that this effect is Ras- and PKC-dependent. The same group has also shown that PE regulation of the rat BNP promoter involves p38 kinase.26 Thus it is interesting to speculate that p38 may target either (1) a protein binding to the M-CAT element, (2) a protein binding to an adjacent site, or (3) another kinase or phosphatase, which in turn targets the element. This would represent an important, novel action of p38 kinase. Previous studies have implicated p38 in the activation of other transcription factors, including ATF2, ELK1, MEF2C, and ATF611–13,34; whether these elements also participate in the regulation of the hBNP promoter is unknown.

In summary, our results demonstrate that IL-1β regulation of the hBNP promoter is Ras- , Rac-, and p38-dependent and that p38 kinase may target the proximal M-CAT element. Since BNP synthesis is induced in the infarcted myocardium and is high in the failing heart, and plasma BNP level is a marker of left ventricular dysfunction, elucidation of the complex molecular signals that regulate its synthesis may lead to understanding how ischemic injury progresses into heart failure.

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References

27. Post GR, Goldstein D, Thuerauf DJ, Glembocki CC, Brown JH. Dissociation of p44 and p42 mitogen-activated protein kinase activation from...


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