Atrial natriuretic peptide inhibits endothelin-1-induced activation of JNK in glomerular mesangial cells

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Atrial natriuretic peptide inhibits endothelin-1-induced activation of JNK in glomerular mesangial cells. Atrial natriuretic peptide (ANP) has been shown to counteract various actions of endothelin-1 (ET-1) in mesangial cells. We have reported that both extracellular signal-regulated kinase (ERK) and c-Jun NH$_2$-terminal kinase (JNK) are activated by ET-1 and ET-1-induced activation of ERK is inhibited by ANP. To further clarify the action of ANP, we examined the effect of ANP on ET-1-induced activation of JNK. ANP inhibited ET-1-induced activation of JNK in a dose-dependent manner. This inhibitory effect of ANP was reversed by HS-142-1, an antagonist for biological receptors of ANP, while C-ANP, an analog specific to clearance receptors of ANP, failed to inhibit ET-1-induced activation of JNK. 8-Bromo-cGMP and sodium nitroprusside were also able to inhibit ET-1-induced activation of JNK, suggesting cGMP-dependent action of ANP. In contrast, ANP failed to inhibit interleukin-1β (IL-1β)-induced activation of JNK. Since an increase in intracellular calcium ([Ca$^{2+}$]$_i$) was shown to be necessary for ET-1-induced activation of JNK in mesangial cells, we measured [Ca$^{2+}$]$_i$ using fura-2. ANP attenuated the ET-1-induced increase in [Ca$^{2+}$]$_i$ in concentrations enough to inhibit ET-1-induced activation of JNK. Finally, ANP was able to inhibit ET-1-induced increase in DNA-binding activity of AP-1 by gel shift assay. These results indicate that ANP is able to inhibit ET-1-induced activation of AP-1 by inhibiting both ERK and JNK, suggesting that ANP might be able to counteract the expression of AP-1-dependent genes induced by ET-1.

Endothelin-1 (ET-1), one of the potent vasoconstrictive peptides, is known to contribute to the regulation of glomerular filtration rate by inducing the contraction of glomerular mesangial cells [1]. In addition, ET-1 has been shown to induce the proliferation and extracellular matrix (ECM) protein production in mesangial cells [2–5]. In contrast, atrial natriuretic peptide (ANP), one of the vasorelaxing substances, has been found not only to induce the relaxation of mesangial cells, but also to counteract the contraction, proliferation, and ECM protein production induced by ET-1 [6–9]. Therefore, the interaction between vasoconstrictive and vasorelaxing substances is considered to be important in the regulation of various functions of glomerular mesangial cells. However, the mechanisms of the interaction between these substances are not fully elucidated yet.

**Key words:** endothelin-1 (ET-1), atrial natriuretic peptide (ANP), c-Jun NH$_2$-terminal kinase (JNK), gene expression, signal transduction.

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Recently, the mitogen-activated protein kinase (MAPK) family, including extracellular signal-regulated kinase (ERK), c-Jun NH$_2$-terminal kinase (JNK) and p38 MAP kinase, was identified in mammalian cells [10]. These kinases were shown to be activated in response to diverse extracellular stimuli including vasoconstrictive peptides [11–15], and thus play a key role in the signal transduction leading to various cellular functions such as contraction, proliferation and protein synthesis [13–17]. We and others have reported that ET-1 is able to activate ERK in mesangial cells [11, 12, 18], and we have recently reported that JNK is also activated by ET-1 in mesangial cells [19]. Activation of both ERK and JNK is considered to induce the phosphorylation of several transcription factors such as Elk-1, c-Jun, ATF-2 [16, 20, 21], resulting in the formation of the AP-1 complex [22]. Indeed, we and others have reported that ET-1 enhances the DNA-binding activity of AP-1 in mesangial cells [19, 23], indicating that the activation of both ERK and JNK by ET-1 might contribute to the regulation of the expression of a number of genes through the activation of AP-1.

From these observations, we hypothesized that ANP could counteract the effect of ET-1 by inhibiting the ET-1-induced activation of the MAPK family in mesangial cells. In fact, we found that ANP was able to inhibit ET-1-induced activation of ERK in cultured rat mesangial cells [18, 24]. To further clarify the action of ANP, in the present study we examined whether ANP could inhibit the ET-1-induced activation of JNK and the ET-1-induced increase in DNA-binding activity of AP-1 in mesangial cells.

**METHODS**

**Materials**

Rat ET-1 and ANP were obtained from Peptide Institute (Osaka, Japan). 8-Bromo-cGMP (8-Br-cGMP), sodium nitroprusside (SNP) and rat des-[Glu18-Ser19-Gly20-Leu21-Gly22]ANP$_{23-37}$NH$_2$ (C-ANP) were purchased from Sigma (St. Louis, MO, USA). HS-142-1 was kindly supplied by Kyowa Hakko Kogyo (Tokyo, Japan). The glutathione-S-transferase (GST) fusion protein expression vector containing the transactivation domain of c-Jun (amino acids 1 to 79), pGEX2t-c-Jun, was a gift of Dr. Michael Karin (University of California, San Diego, CA, USA). Glutathione Sepharose 4B was purchased from Pharmacia Biotechnology (Uppsala, Sweden). Recombinant human interleukin 1β (IL-1β) was purchased from Genzyme (Cambridge, MA, USA). [γ$^{32}$P]ATP (6000 Ci/mmol) was bought from NEN Research Products (Boston, MA, USA). Anti-ACTIVE$^\text{ty}$-JNK antibody and anti-JNK1 antibody...
were purchased from Promega (Madison, WI, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Fura-2 acetoxymethylester (fura-2/AM) was obtained from Dojin Chemicals (Kumamoto, Japan). All other reagents were of chemical grade and purchased from standard suppliers.

Mesangial cell culture and experimental protocol

Glomerular mesangial cells were obtained from a culture of glomeruli isolated from male Sprague-Dawley rats weighing 100 to 150 g by a sieving method as previously described [25]. Isolated glomeruli were cultured in the RPMI 1640 medium containing 20% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml selenious acid. Cultured cells were identified as mesangial cells by morphological and biochemical characters as previously described [26].

Subconfluent cells were made quiescent by reducing the concentrations of FBS to 0.4% for 24 hours. Quiescent mesangial cells were incubated in an incubation medium (RPMI 1640 medium with 0.4% fatty acid free bovine serum albumin and 20 mM Hepes, pH 7.4) at 37°C for 30 minutes. Cells were first exposed to ANP or intracellular cGMP-raising agents for 10 minutes and then to ET-1 or IL-1β for another 15 minutes. The reactions were terminated by a rapid aspiration of medium and washing twice with ice-cold phosphate buffered saline (PBS) on ice.

Preparation of GST-c-Jun

The GST fusion protein expression vector, pGEX2T-c-Jun, was introduced into E. coli. The bacterial lysates containing the fusion protein were incubated with glutathione Sepharose 4B and GST-c-Jun (1-79) fusion protein bound to glutathione sepharose beads was collected by centrifugation. The purity and amounts of purified proteins were estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent staining with Coomassie blue.

Measurement of the activities of JNK

The cells were lysed in a buffer containing 25 mM Hepes, pH 7.5, 0.3 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM dithiothreitol (DTT), 20 mM β-glycerophosphate, 1 mM vanadate, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 20 μg/ml aprotinin, and 20 μg/ml leupeptin. The cell lysates were centrifuged at 12,000 g for 30 minutes and the supernatants were used for JNK assay. The concentrations of cellular protein were determined using protein assay kit (BioRad Laboratories, CA, USA).

The activities of JNK were measured by the solid-phase kinase assay [27] because we confirmed that the kinases bound to GST-c-Jun (1-79) in this assay were 46 and 54 kDa JNK [19]. For the solid-phase kinase assay, cell lysates were incubated with GST-c-Jun (1-79) fusion protein bound to glutathione-sepharose
beads at 4°C for three hours. The beads were recovered by centrifugation at 10,000 g for 10 seconds and then washed three times with a buffer containing 20 mM Hepes, pH 7.7, 50 mM NaCl, 2.5 mM MgCl₂, 0.1 mM EDTA and 0.05% Triton X-100, and once with a kinase buffer (20 mM Hepes, pH 7.6, 20 mM MgCl₂, 20 mM β-glycerophosphate, 20 mM p-nitrophenyl phosphate, 0.1 mM vanadate, 2 mM DTT). The beads were then incubated with 30 μl of a kinase buffer containing 20 μM unlabeled ATP and 5 μCi [γ-32P]ATP at 30°C for 20 minutes. The reaction was terminated by the addition of 30 μl of 3 × Laemmli sample buffer and boiling at 100°C for five minutes. Phosphorylated proteins were resolved on 12% SDS-PAGE, followed by an autoradiography. The relative kinase activities were quantified by the densitometolic analysis of autoradiographs.

**Immunoblot analysis**

The cells were lysed in 100 μl of SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 50 mM DTT, 0.1% bromophenol blue) and sonicated for five seconds. After boiling for five minutes, the samples were subjected to 12% SDS-PAGE, followed by an autoradiography. The relative kinase activities were quantified by the densitometric analysis of autoradiographs.

**Measurement of intracellular calcium concentration**

The concentrations of intracellular calcium ([Ca^{2+}]_{i}) were measured in a single cultured mesangial cell using Ca^{2+}-sensitive fluorescent dye, fura-2 [28]. Krebs-Henseleit Hepes (KHH) buffer (130 mM NaCl, 5 mM KCl, 1.25 mM CaCl₂, 0.8 mM MgSO₄, 20 mM Hepes pH 7.4, 5.5 mM glucose, 0.1% FFA-free BSA) was used as a standard solution. Mesangial cells plated on a glass cover slip were loaded with 5 μM fura-2/AM for 40 minutes at 37°C in serum-free RPMI 1640. After rinsing twice, cells were incubated for 20 minutes at 37°C in serum-free RPMI 1640. Then, the cover slip was transferred into a chamber placed on the stage of an inverted microscope (Nikon, Diaphot-TMD, Tokyo, Japan). The solution and the chamber were kept at 37°C in a water bath. Measurements were performed using a microspectrofluorometer (Nihon Bunko, CAM-200, Tokyo, Japan). The excitation and emission wavelength were 340/380 nm and 510 nm, respectively. The ratio of the intensity of fluorescence excited at 340 nm to that at 380 nm (ratio F_{340}/F_{380}) was used as an indicator of [Ca^{2+}]_{i}. The following equation by Grynkiewicz, Poenie and Tsien [28] was
adopted for conversion to the fluorescence ratio into intracellular calcium: 
$$[Ca^{2+}]_i = (R - R_{\text{min}})/(R_{\text{max}} - R) \times K_d \times (F_{380\text{ min}}/F_{380\text{ max}})$$
when the calcium concentration is minimum (incubated with the calcium- and magnesium-free KHH buffer containing 5 mM EGTA and 5 mM ionomycin) and maximum (incubated with the KHH buffer containing 5 mM ionomycin), respectively. $K_d$ for the fura-2 and calcium complex is 224 nM at 37°C [28].

Assessment of DNA-binding activity of AP-1

Nuclear extracts were prepared as described by Sadowski and Gilman with modifications [29]. In brief, cells were lysed by the addition of hypotonic buffer (20 mM Heps, pH 7.9, 1 mM EGTA, 1 mM EDTA, 20 mM NaF, 1 mM Na$_3$VO$_4$, 1 mM Na$_4$P$_2$O$_7$, 1 mM DTT, 0.5 mM PMSF, 1 µg/ml aprotin, 1 µg/ml leupeptin and 1 µg/ml pepstatin) with 0.6% Nonidet P-40, and lysates were centrifuged at 16,000 g for 20 seconds. The pellets were resuspended in high salt buffer (hypotonic buffer with 420 mM NaCl and 20% glycerol), rotated for 30 minutes at 4°C and centrifuged at 16,000 g for 20 minutes. The supernatants were used as nuclear proteins for a gel mobility shift assay. The nuclear proteins (5 µg) were incubated with 2 µg of poly(dI-dC)·poly(dI-dC) in a binding buffer (20 mM Heps, pH 7.9, 1.8 mM MgCl$_2$, 2 mM DTT, 0.5 mM EDTA, 0.5 mg/ml BSA) for 30 minutes and then reacted with a radiolabeled AP-1 consensus oligonucleotide at room temperature for 20 minutes. The reaction mixtures were run on 4% polyacrylamide gel and autoradiographed.

Statistical analysis

Analysis of variance (ANOVA) followed by Scheffé’s test was used to determine significant difference in multiple comparisons. Comparisons between two groups were analyzed by Student’s unpaired t-test.

RESULTS

Effect of atrial natriuretic peptide on endothelin-1-induced activation of JNK

Endothelin-1 was able to activate JNK in a dose-dependent (10$^{-8}$ M maximum) and time-dependent manner, with a peak stimulation at 15 minutes, in mesangial cells [19]. Thus, we stimulated the cells with 10$^{-8}$ M ET-1 for 15 minutes. Cells were first exposed to various concentrations of ANP for 10 minutes and then to ET-1 (10$^{-8}$ M) for an additional 15 minutes. As shown in Figure 1, ANP inhibited ET-1-induced activation of JNK in a dose-dependent manner.

Mesangial cells were shown to have two types of receptors for ANP, biological and clearance receptors [30]. In order to clarify
the receptors responsible for the inhibitory effect of ANP on the ET-1-induced activation of JNK, we examined the effect of HS-142-1, a competitive and selective antagonist for biological receptors of ANP [31, 32], and C-ANP, an analog specific to clearance receptors of ANP. The inhibitory effect of ANP on ET-1-induced activation of JNK was significantly reduced by treating the cells with HS-142-1 (1 mg/ml) for 10 minutes prior to the addition of ANP (Fig. 2). C-ANP (10^{-7} M) failed to inhibit ET-1-induced activation of JNK (Fig. 2).

**Effect of intracellular cGMP-raising agents on endothelin-1-induced activation of JNK**

Atrial natriuretic peptide is known to activate particulate guanylyl cyclase and to increase intracellular cGMP after binding to biological receptors in cultured rat mesangial cells, and cGMP has been proposed as a second messenger for ANP [32, 33]. To clarify whether the inhibitory effect of ANP on ET-1-induced activation of JNK was mediated by an increase in intracellular cGMP, we examined the effects of intracellular cGMP-raising agents on ET-1-induced activation of JNK. Both 8-Br-cGMP, a cell permeable analog of cGMP (Fig. 3 A, B), and SNP, an activator of soluble guanylyl cyclase (Fig. 3 C, D), were able to inhibit ET-1-induced activation of JNK, indicating that the inhibitory effect of ANP on ET-1-induced activation of JNK could be mediated by cGMP-dependent mechanism through the biological receptors.

**Effect of atrial natriuretic peptide on interleukin 1β-induced activation of JNK**

JNK was first found to be activated in response to environmental stress such as UV irradiation and osmotic shock, and to inflammatory cytokines such as interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) in various types of cells [34–38]. Recently, IL-1β has been shown to activate JNK in rat and human mesangial cells [39, 40]. Therefore, we next examined the effect of ANP on IL-1β-induced activation of JNK in mesangial cells. As shown in Figure 4, IL-1β was able to activate JNK in a dose-dependent manner with a maximum stimulation at 5000 pg/ml. In contrast to ET-1-induced activation of JNK, ANP failed to inhibit the activation of JNK induced by either maximum (5000 pg/ml) or half-maximum (1000 pg/ml) concentrations of IL-1β (Fig. 5).

**Effects of atrial natriuretic peptide on endothelin-1- and interleukin 1β-induced phosphorylation of JNK**

The activation of JNK was shown to be induced through the phosphorylation at Thr-183 and Tyr-185 by the dual specific protein kinase, SAPK/ERK kinase (SEK1), also called MKK4 [41]. As shown in Figure 6A, an immunoblot analysis using an anti-ACTIVE JNK antibody that can recognize the phosphorylation at Thr-183 and Tyr-185 of JNK revealed that the phosphorylation of 46 kDa JNK was enhanced by the stimulation of 10^{-8} M ET-1 or 5000 pg/ml IL-1β. The phosphorylation of 54 kDa JNK was also detected, although the intensity was much weaker than 46 kDa JNK. Atrial natriuretic peptide inhibited ET-1-induced phosphorylation of JNK, while ANP failed to inhibit IL-1β-induced phosphorylation of JNK (Fig. 6A). The protein content of JNK was not affected by the addition of these substances (Fig. 6B).
Effect of atrial natriuretic peptide on an endothelin-1-induced increase in intracellular calcium

We have reported that an increase in $[\text{Ca}^{2+}]_i$ is necessary for ET-1-induced activation of JNK in mesangial cells [19]. Thus, the inhibitory effect of ANP on ET-1-induced activation of JNK could be due to the inhibition of the ET-1-induced increase in $[\text{Ca}^{2+}]_i$. Although ANP was shown to prevent an increase in $[\text{Ca}^{2+}]_i$ by angiotensin II in mesangial cells [42], it has been reported that ANP failed to inhibit ET-1-induced increase in $[\text{Ca}^{2+}]_i$ in cultured vascular smooth muscle cells [43]. Hence, we next examined the effect of ANP on the ET-1-induced increase in $[\text{Ca}^{2+}]_i$ in mesangial cells. Typical waveforms obtained from a single mesangial cell exposed to $10^{-7} \text{ M}$ ET-1 with or without pretreatment of $10^{-10}$ to $10^{-7} \text{ M}$ ANP are shown in Figure 7 A-E. The peak concentrations of calcium are calculated and shown in Figure 7F. Endothelin-1 increased $[\text{Ca}^{2+}]_i$ from 129.9 ± 19.2 to 888.5 ± 105.7 nM (mean ± SEM, $N = 16$, $P < 0.01$). Basal calcium levels were not affected by ANP, while the peak $[\text{Ca}^{2+}]_i$, induced by ET-1 was significantly attenuated by ANP in concentrations sufficient to inhibit ET-1-induced activation of JNK.

Effects of atrial natriuretic peptide on endothelin-1- and interleukin 1β-induced DNA-binding of AP-1

Since the activation of JNK was found to be linked to the activity of AP-1 [16], it is likely that ANP inhibits ET-1-induced DNA-binding activity of AP-1. Thus, we examined whether ANP could inhibit DNA-binding activity of AP-1 enhanced by ET-1 or IL-1β. Cells were first preincubated with ANP ($10^{-7} \text{ M}$) for 10 minutes and then exposed to ET-1 ($10^{-8} \text{ M}$) or IL-1β (1000 pg/ml) for another 60 minutes. As shown in Figure 8, ANP was able to inhibit ET-1-induced increase in DNA-binding activity of AP-1, while the IL-1β-induced increase in DNA-binding activity of AP-1 was not affected by ANP.

DISCUSSION

The present study was performed to clarify the mechanisms of the intracellular interaction between endothelin-1 (ET-1) and
Atrial natriuretic peptide (ANP); the results clearly indicate that ANP is able to inhibit ET-1-induced activation of JNK through a cGMP-dependent mechanism. Our data also indicate that ANP is able to inhibit ET-1-induced increase in intracellular calcium ([Ca\(^{2+}\)]\(i\)) and DNA-binding of AP-1.

Atrial natriuretic peptide has been shown to counteract ET-1-induced contraction, proliferation and extracellular matrix (ECM) production in mesangial cells [6–9]. To understand the mechanism of the interaction between these two peptides, it is important to clarify the effect of ANP on signal transduction systems of ET-1. Recent studies indicate that the mitogen-activated protein kinase (MAPK) family plays an important role in various cellular functions in response to diverse extracellular stimuli, including vasoconstrictive peptides such as ET-1 and angiotensin II [11–15, 19]. Among the MAPK family, ERK (also known as p44 and p42 MAPK) was shown to be typically activated by various growth factors, while JNK (also known as stress-activated protein kinase; SAPK) was found to be activated by inflammatory cytokines and environmental stress [34–38]. We and others have reported that ET-1 is able to activate both ERK and JNK in a similar degree [11, 12, 18, 19], suggesting that both ERK and JNK contribute to the regulation of the cellular responses to ET-1 in mesangial cells. Thus, we hypothesized that ANP might counteract the effect of ET-1 by interfering with these signal transduction systems of ET-1. The present results and our previous observations [18, 24] clearly indicate that ANP is able to inhibit ET-1-induced activation of both ERK and JNK.

The inhibitory effect of ANP on ET-1-induced activation of JNK shown in the present study is considered to be mediated by the biological receptors of ANP because of the following reasons. First, the inhibitory effect of ANP on ET-1-induced activation of JNK was prevented by HS-142-1, a competitive and selective antagonist for biological receptors for ANP [31], which could inhibit ANP-induced cGMP production in mesangial cells [32]. Secondly, C-ANP, an analog specific to clearance receptors for ANP [44], failed to inhibit ET-1-induced activation of JNK. Furthermore, the inhibitory effect of ANP on ET-1-induced activation of JNK was mimicked by 8-Br-cGMP, a cell permeable analog of cGMP, and SNP, an activator of soluble guanylyl cyclase. Therefore, the inhibitory effect of ANP on ET-1-induced activation of JNK is considered to be mediated by an increase in intracellular cGMP resulted from the activation of the biological receptor-coupled guanylyl cyclase [33].

Endothelin-1 is known to bind to G protein-coupled receptor and activate phospholipase Cβ (PLCβ), leading to an increase in [Ca\(^{2+}\)]\(i\) and the activation of protein kinase C (PKC) through the production of inositol 1,4,5-trisphosphate and diacylglycerol, respectively [1–3]. In addition, the activation of protein tyrosine kinases (PTK) was also found to be involved in the signal transduction of ET-1 [23, 45, 46]. We have reported that ANP is able to inhibit ET-1-induced activation of ERK by inhibiting the activation of PKC [18]. However, the mechanism of the inhibition of ET-1-induced activation of JNK by ANP should be different from that of the inhibition of ERK because the activation of PKC was not involved in ET-1-induced activation of JNK in mesangial cells [19]. Instead, an increase in [Ca\(^{2+}\)]\(i\) and the activation of PTK were found to be responsible for the activation of JNK by ET-1 [15, 19]. In the present study, we demonstrated that ANP was able to attenuate the ET-1-induced increase in [Ca\(^{2+}\)]\(i\), in sufficient concentrations to inhibit ET-1-induced activation of JNK, suggesting that the inhibition of an increase in [Ca\(^{2+}\)]\(i\) could be a potent mechanism of the inhibitory effect of ANP on the ET-1-induced activation of JNK. In addition, a novel calcium-dependent protein-tyrosine kinase (CADTK) has been shown to transmit Ca\(^{2+}\)-signals from G protein-coupled receptors to JNK in rat liver epithelial cells [47]. Thus, ANP may inhibit this calcium-dependent tyrosine kinase through its Ca\(^{2+}\)-lowering effect, although this possibility needs to be clarified further.

In contrast to ET-1-induced activation of JNK, ANP failed to inhibit the IL-1β-induced activation of JNK in mesangial cells. Although little information is available on the mechanisms responsible for the activation of JNK by IL-1β in mesangial cells, the...
present results are consistent with the previous report demonstrating that cAMP was able to inhibit Ca\(^{2+}\)-dependent but not stress-induced activation of JNK in rat liver epithelial cells [48]. In addition, IL-1β was shown to activate JNK by both PKC- and PTK-independent mechanisms [39], and it was recently found that [Ca\(^{2+}\)]\(_i\) was not involved in the signaling of IL-1 in mesangial cells [49]. These results indicate that IL-1β activates JNK by mechanisms different from those of ET-1, and also indicate that the effect of ANP on JNK might not be general but specific at least to vasoconstrictive peptides such as ET-1.

We and others have shown that ET-1 enhances DNA-binding activity of AP-1 composed of c-Jun and c-Fos proteins [19, 23]. In the present study, we found that ANP was able to inhibit the ET-1-induced increase in DNA-binding activity of AP-1. This inhibition of AP-1 activity by ANP might be due to the inhibition of the production of c-Jun and c-Fos through the inhibition of ET-1-induced activation of both JNK and ERK. Alternatively, the inhibition of ET-1-induced activation of JNK by ANP might be more important. The latter possibility is supported by recent reports indicating that the phosphorylation of c-Jun protein is crucially important for AP-1 complex to potentiate its transcriptional activity [50], and that the inhibition of thrombin-induced cell proliferation and AP-1 activity by cAMP is correlated with a decrease in JNK activity in vascular smooth muscle cells [17]. Thus, the inhibitory effect of ANP on ET-1-induced AP-1 activity possibly through the inhibition of the activation of JNK is considered to be one of the important mechanisms of antiproliferative effect of ANP. Because ANP was shown to inhibit the proliferation of mesangial cells by inducing TGF-β through cGMP-dependent mechanism [51], ANP could be considered to have dual mechanisms for its antiproliferative effect: one is the inhibition of AP-1 activity shown in the present study and the other is the induction of TGF-β.

A number of genes of ECM proteins such as type I collagen and fibronectin were shown to have potential AP-1-binding sites, and these sites were found to be essential for the regulation of gene expression [52, 53]. Since ANP has been also reported to inhibit ECM protein production stimulated by thromboxane and ET-1 [8, 9], it can be speculated that ANP could also counteract ECM protein production by ET-1 by inhibiting the DNA-binding activity of AP-1.

Glomerular mesangial cells are exposed in vivo to various vasoactive substances and the functions of mesangial cells are regulated by a feedback mechanism as well as an interaction of these substances. For example, ET-1 was shown to down-regulate its receptors [54] in mesangial cells, while an increase in the production of ET-1 by ET-1 itself was also found in mesangial
cells [55, 56], suggesting the existence of a complex positive or negative feedback system. Although these complex regulations should be taken into consideration to understand the cellular response in physiological and pathological conditions, the crosstalk of the signal transduction system between ET-1 and ANP at the level of MAPK family shown in the present study and our previous study [18] may provide one of the important mechanisms of the interaction between these substances in mesangial cells.

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APPENDIX

Abbreviations used in this article are: ANP, atrial natriuretic peptide; 8-Br-cGMP, 8-Bromo-cGMP; [Ca2+]i, intracellular calcium; CADTK, calcium-dependent protein-tyrosine kinase; C-ANP, rat des-[Glu18-Ser19-Gly20-Leu21-Gly22]ANP4-23-NH2; DTT, dithiothreitol; ERK, extracellular signal-regulated kinase; ET-1, endothelin-1; FBS, fetal bovine serum; GST, glutathione-S-transferase; IL-1B, interleukin-1B; JNK, c-Jun NH2-terminal kinase; KHH, Krebs-Henseleit Hepes buffer; MAPK, mitogen-activated protein kinase; PBS, phosphate buffered saline; PLCB, phospholipase Cb; PMSF, phenylmethylsulfonyl fluoride; PTK, protein tyrosine kinase; SEK1, the specific protein kinase SAPK/ERK kinase; SNP, sodium nitroprusside; TNF-α, tumor necrosis factor-α.

REFERENCES


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