Atrial Natriuretic Peptide Induces the Expression of MKP-1, a Mitogen-activated Protein Kinase Phosphatase, in Glomerular Mesangial Cells*

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Atrial natriuretic peptide (ANP) has been shown to inhibit the proliferation of various types of cells including glomerular mesangial cells. The activation of mitogen-activated protein kinase (MAPK) is one of the main signal transduction systems leading to cell proliferation. MAPK is tightly regulated by the activating kinase, MEK, and specific phosphatase MKP-1. Constitutive expression of MKP-1 has been shown to inhibit cell proliferation by suppressing MAPK activity. In order to understand the mechanism of the anti-proliferative effect of ANP, we examined whether ANP could inhibit MAPK by inducing MKP-1 in cultured rat glomerular mesangial cells. ANP increased the expression of MKP-1 mRNA in a dose-dependent (10 nM maximum) and time-dependent, with a peak stimulation at 30 min, manner. Receptor for ANP is a transmembrane guanylyl cyclase. Activation of guanylyl cyclase of ANP receptor by ligand plays an essential role in ANP signal transduction. 8-Bromo-cGMP, a cell permeable analogue of cyclic GMP, and sodium nitroprusside, an activator of soluble guanylyl cyclase, could mimic the effects of ANP and were able to induce the expression of MKP-1 in a similar time course as ANP. The protein expression of MKP-1 was maximally stimulated by ANP at 120 min. Treatment of the cells with ANP for 120 min resulted in an inhibition of phorbol ester-induced activation of MAPK, while the activation of MEK was not affected by ANP. These results indicate that ANP might inhibit the proliferation of mesangial cells by inactivating MAPK through the induction of MKP-1.

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1 The abbreviations used are: ANP, atrial natriuretic peptide; MAPK, mitogen-activated protein kinase; ERK, extracellular regulated protein kinase; FBS, fetal bovine serum; PDBu, phorbol 12,13-dibutyrate; MBP, myelin basic protein; C-ANP, des-[(Gln18-Ser19-Gly20-Leu21-Gly22)ANP 4–23]NH2; SNP, sodium nitroprusside; GST, glutathione S-transferase.

EXPERIMENTAL PROCEDURES

Materials—Rat ANP1–28 was obtained from Peptide Institute (Suita, Japan). Rat des-[(Gln18-Ser19-Gly20-Leu21-Gly22)ANP 4–23]NH2 (C-ANP), phorbol 12-13-dibutyrate (PDBu), bovine myelin basic protein (MBP), 8-bromo-cGMP (8-Br-cGMP), and sodium nitroprusside (SNP) were purchased from Sigma. [γ-32P]ATP (6000 Ci/mmol) and [γ-32P]dCTP (3000 Ci/mmol) were purchased from DuPont NEN. Anti-ERK2 and anti-MKP-1 antibodies were from Santa Cruz Biotechnology.
Recombinant GST-MAPK (p44mapk), GST-HVH-1, and anti-MEK antisera were prepared as described previously (18, 26–28). Human glyceraldehyde-3-phosphate dehydrogenase cDNA (29) was kindly provided by Dr. Paul Killen, University of Michigan. 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 100–150 g by sieving method, in RPMI 1640 medium containing 20% fetal bovine serum (FBS) and antibiotics as described previously (30). Cells at passage 2 to 9 were cultured mesangial cells. As shown in Fig. 2, ANP (100 nM) increased the expression of MKP-1 mRNA rapidly and the maximal induction was obtained at 30 min. ANP-induced expression of MKP-1 mRNA was observed in a concentration-dependent manner of ANP with a maximal response at 10–100 nM (Fig. 3), concentrations enough to inhibit the proliferation of mesangial cells (5).

ANP has been reported to activate receptor guanylyl cyclase and to increase intracellular cyclic GMP (cGMP) in cultured mesangial cells (4, 5). In order to know the mechanism of ANP-induced expression of MKP-1 mRNA, we examined the effect of cGMP on MKP-1 mRNA expression in mesangial cells. When the mesangial cells were incubated with 8-Br-cGMP, a cell permeable analogue of cGMP, MKP-1 mRNA expression was increased in a similar time course as ANP (Fig. 4A). However, C-ANP, an analogue specific to clearance receptors for ANP (5), failed to induce MKP-1 mRNA expression (data not shown). SNP, an activator of soluble guanylyl cyclase, was also able to induce the expression of MKP-1 mRNA (Fig. 4B).

The expression of MKP-1 protein was next examined by an immunoblot analysis. The 39-kDa protein, the same molecular mass protein as MKP-1 reported in PC12 cells (23), was detected from rat mesangial cell lysate. This protein could not be detected when the antibody was preincubated with recombinant MKP-1, GST-HVH-1 (data not shown). As shown Fig. 5, ANP (100 nM) induced the expression of MKP-1 protein with a maximal stimulation at 120 min. ANP also induced the expression of the protein with 42–44 kDa molecular mass. This protein might be MKP-2 reported in PC12 cells (23).
Furthermore, PDBu-induced activation of MAPK cascade was blocked by ANP at the level of MAPK. MKP-1 (also called as 3CH134, CL100, erp, or HVH1) is a dual specificity phosphatase that selectively dephosphorylates MAPK in vitro (15, 18) or in vivo (16, 31). In mesangial cells, MKP-1 mRNA was induced by fetal bovine serum or phorbol ester, the agents known to activate MAPK (10, 11). The induction of MKP-1 by these growth-promoting agents may be responsible for the down-regulation of MAPK after growth stimuli as suggested by Sun et al. (16). In the present study, we demonstrated that ANP, which could not activate MAPK (10), rapidly increased the expression of MKP-1 mRNA and MKP-1 protein in cultured mesangial cells. This is the first report that the anti-proliferative agent could induce MKP-1 gene expression.

ANP-induced expression of MKP-1 might be mediated by cGMP-dependent pathway, because 8-Br-cGMP and SNP were also able to induce the expression of MKP-1, while C-ANP, an analogue specific to clearance receptors of ANP, was without effect. It has been reported that MKP-1 mRNA expression is induced by the activation of protein kinase C or cAMP-dependent kinase (protein kinase A) cascade (19). The activation of protein kinase C or cAMP-dependent kinase (protein kinase A) has been shown to activate transcription factor(s) which bind to the 12-O-tetradecanoylphorbol-13-acetate responsive element or to cAMP responsive element. However, little information is available on how the cGMP-dependent signal transduction may influence gene expression. Recently, nitric oxide-releasing agents and the membrane permeable analogue of cGMP have been reported to activate transcription from AP-1 responsive promoters in rodent fibroblast and epithelial cell line (32). Since the human MKP-1 gene (CL100) contains one AP-1 site in the region upstream of the transcription start site (33), we hypothesize that ANP may induce the expression of the MKP-1 gene through the activation of this AP-1 site.

Constitutive expression of MKP-1 has been shown to attenuate serum- or oncogenic ras-induced MAPK activation (16, 22), to block MAPK-dependent gene expression (23, 24), and to inhibit cell proliferation (19, 22), suggesting that the inactivation of MAPK in vivo by MKP-1 has a negative effect on cell proliferation. In the present study, when MAPK-1 protein was maximally induced by exposing the cells to ANP for 120 min, phorbol ester-induced activation of MAPK was inhibited, while the activation of MEK was not affected, indicating that MAPK cascade was blocked by ANP at the level of MAPK. These data indicate that ANP may negatively regulate MAPK through the induction of MKP-1, leading to the inhibition of the proliferation of mesangial cells. We have previously reported that phorbol ester-induced activation of MAPK was inhibited by ANP in cultured mesangial cells when the cells were treated with ANP.

**DISCUSSION**

ANP, a potent vasorelaxing peptide, is able to inhibit the proliferation of glomerular mesangial cells by cGMP-dependent mechanism (5). The present study was performed to clarify the mechanism of anti-proliferative action of ANP and the results indicate that ANP is able to induce the expression of MAPK phosphatase, MKP-1, by cGMP-dependent mechanism in concentrations enough to inhibit the proliferation of mesangial cells. Furthermore, PDBu-induced activation of MAPK cascade was inhibited by ANP at the level of MAPK.

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for only 10 min (10). Ten-min incubation periods might not be enough for ANP to induce the expression of MKP-1 protein and ANP has been shown to directly suppress phorbol ester-induced activation of protein kinase C in mesangial cells (34). Therefore, ANP is able to attenuate phorbol ester-induced activation of MAPK through two independent mechanisms; one might be the inhibition at a step proximal to MAPK as a short term effect and the other is due to the induction of MKP-1 expression as a relatively long term effect shown in the present study. Thus, MKP-1 induction by ANP shown in the present study might provide a new mechanism of anti-proliferative action of ANP in glomerular mesangial cells.

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