

Euro Neuropharmacology 2018 - Regulation of guanylyl cyclase/natriuretic peptide receptor-A gene expression and signaling: Interactive roles of histone modifications and transcription factors - Kailash N Pandey - Tulane University

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Atrial Natriuretic Peptide is synthesized as an inactive preprohormone, encoded by the human NPPA gene located on the short arm of chromosome. The NPPA gene is expressed in atrial myocytes and consists of 2 introns and three exons, with translation of the gene yielding a high molecular mass 151 amino acid polypeptide known as preproANP. The preprohormone is activated and involves cleavage of the 25 amino acid signal sequence to produce proANP, a 126 amino acid peptide is the major form of ANP stored in intracellular granules of the atria. Stimulation of atrial cells, proANP is released and converts to the 28-amino-acid C-terminal mature ANP on the cell surface by the cardiac transmembrane serine protease corin. ANP discovered as O-glycosylated. ANP is secreted in response to stretching of the atrial wall, via Atrial volume receptors or increased Sympathetic stimulation of β -adrenoceptors or Increased sodium concentration (hypernatremia), though sodium concentration is indirect stimulus for increased ANP secretion. All cell surface receptors guanylyl cyclase-A (GC-A) also known as natriuretic peptide receptor-A (NPRA/ANPA) or NPR1, guanylyl cyclase-B (GC-B) also known as natriuretic peptide receptor-B (NPRB/ANPB) or NPR2, natriuretic peptide clearance receptor (NPRC/ANPC) or NPR3 are identified as atrial natriuretic peptide receptors. NPR-A and NPR-B have an extracellular domain that binds the ligand. The intracellular domain has two catalytic domains for guanylyl cyclase activity. Binding of a natriuretic peptide induces a conformational change in the receptor that leads to receptor dimerization and activation. The binding of ANP to receptor causes the conversion of GTP to cGMP and raises intracellular cGMP. As cGMP activates a cGMP-dependent kinase (PKG or cGK) that phosphorylates proteins at specific serine and threonine residues. In the medullary collecting duct, the cGMP generated in response to ANP may act not only through PKG but also via direct modulation of ion channels. Mouse mesangial cells (MMCs) were isolated and cultured in Dulbecco's modified Eagle's medium (DMEM) added with 10% fetal calf serum. The MMCs were seeded in 24-well plates. Cloned mouse Leydig tumor (MA-10) cells were cultured in modified Waymouth's medium added with 15% horse serum. The cell cultures were maintained at 37°C in an atmosphere of 5% CO₂ and 95% O₂. The cells were transfected using Lipofectamine-2000 reagent with 1 μ g of promoter-reporter construct and 300 ng of pRL-TK carrying the Renilla luciferase gene downstream of thymidine kinase promoter, which was used as an internal transfection control. For co-transfection experiments, expression plasmids of varying concentrations were transfected along with the promoter-reporter construct. Cells were lysed after 48 h and the lysate was used to measure firefly and Renilla luciferase activities

with Promega dual luciferase assay kit using a TD 20/20 luminometer (Turner Designs). In Ets-1 overexpression experiments, cells were transfected with expression vectors for Ets-1 (pEVRFO-Ets-1). The total DNA content was equalized by inclusion of pEVRFO plasmid. To examine the transfection efficiency, cells were transfected with pCMV β -galactosidase control plasmid and transfection efficiency was assessed by using in situ β -galactosidase staining kit from Stratagene. In MMCs and MA-10 cells, the transfection efficiency was found to be 85% and 90%, respectively using Lipofectamine-2000.

One microgram of total RNA was reverse-transcribed using the Superscript one-step RT-PCR with platinum Taq system. The amplified PCR product increased linearly up to 40 cycles. Controlled experiments were performed with RNA samples but without reverse transcriptase. The specific primers for β -actin gene were included in the PCR reaction as an internal control. The expected sizes of the amplified NPRA and β -actin PCR products are 456 and 256 bp. After amplification, 15 μ l of each PCR reaction mixture were electrophoresed through a 1.5% agarose gel with ethidium bromide (0.5 μ g/ml). The gel was digitized and signal intensities of the corresponding bands were quantified using an Alpha Imaging System. NPR-C functions mainly as a clearance receptor by binding and sequestering ANP from the circulation. All natriuretic peptides are bound by the NPR-C. Fragments derived from the ANP precursor, including the signal peptide, N-terminal pro-ANP and ANP, have been detected in human blood. ANP and related peptides are used as biomarkers for cardiovascular diseases such as stroke, coronary artery disease, myocardial infarction and heart failure. A particular ANP precursor called mid-regional pro-atrial natriuretic peptide (MRproANP) is a highly sensitive biomarker in heart failure. MRproANP levels below 120 pmol/L can be used to effectively rule out acute heart failure. Large amounts of ANP secretion has been noted to cause electrolyte disturbances (hyponatremia) and polyuria. These indications can be a marker of a large atrial myxoma. Npr1 promoter activity is regulated by Ets-1 and show that the endogenously expressed Ets-1 protein physically associates with Npr1 promoter in vivo and binds to its consensus motifs under in vitro conditions. Overexpression of Ets-1 greatly increased NPRA mRNA and protein levels and stimulated GC activity of the receptor in both MMCs and MA-10 cells. On the other hand, gene silencing of Ets-1 significantly reduced the basal promoter activity, indicating the critical role of Ets-1 in Npr1 gene transcription. Deletion of Ets motifs along with the TSS present in the region -46 to +55 showed significant decrease in luciferase activity in the construct -356/-46. The decrease in luciferase activity may also be attributed to removal of initiation sequence CATACTCC present

at the TSS in the region -46 to $+55$. Therefore, to confirm the involvement of Ets sites in Npr1 gene regulation we performed in vitro site-directed mutagenesis experiments. The individual contribution of each Ets-1 motif appears to be equivalent because mutation of either element reduced Ets-1-induced promoter activity by almost 50% as compared with wild type construct thus emphasizing the importance of Ets sites in Npr1 gene transcription. Ets-1 is essential for Npr1 gene transcription and mediates its effect by binding to its consensus sites present in the Npr1 promoter. The findings of the present study should prove important in elucidating the molecular mechanisms for the expression and regulation of members of the GC receptor family. The results of the present study showed the transcriptional regulation of Npr1 gene, an important locus in the control of hypertension and cardiovascular homeostasis.