Natriuretic Peptide Family — Physiological Significance and Clinical Implication

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Summary

Natriuretic peptide family, which possesses potent diuretic, natriuretic and vasorelaxing properties, is now recognized to be composed of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). We have elucidated that ANP and BNP are the cardiac hormone mainly secreted from the atrium, and the ventricle, respectively. We have also discovered that CNP first recognized as the neuropeptide is produced in endothelial cells and considered to be an autocrine/paracrine regulator in the vascular wall. The aim of this study is to elucidate clinical significance of natriuretic peptide family in salt handling mechanism using molecular biology technique, and seek for the clinical application of natriuretic peptide family in salt homeostasis. To elucidate the significance of natriuretic peptide family in chronic regulation of salt handling and blood pressure, we isolated mouse BNP gene and cDNA and tried to generate BNP gene over-expressing transgenic mice. Mouse BNP gene contains three exons and two introns. Typical TATAAA sequence exists about 100 base pairs upstream of the translation initiation site. By microinjecting the expression vector which contained cloned mouse BNP gene linked to human serum amyloid P promoter into mouse male pronucleus of fertilized egg, we succeed in obtaining several F1 transgenic mice with various copy numbers of BNP gene (15 ~ 50 copies). Northern blot analysis revealed that mouse BNP mRNA was abundantly expressed in the liver in the BNP transgenic mice. The BNP mRNA concentration in the liver was ten-times higher than that in the ventricle. We established the radioimmunoassay specific to mouse BNP and examined the plasma level of BNP in the transgenic mice. The plasma BNP concentration in the transgenic mice was 2-15 pmol/ml, which was at least 10 to 100 times higher than the control mice (less than 0.16 pmol/ml). Blood pressure of the transgenic mice determined by the direct measurement was 106 $\pm$ 1mmHg, which was significantly lower than that of the control mice (126 $\pm$ 2mmHg). These findings indicate that BNP can chronically exert biological action to modulate blood pressure and body fluid homeostasis, and support the long term effectiveness of natriuretic peptide family for clinical application. The significance of natriuretic peptide family in salt handling is now under investigation, using the transgenic mice. We also succeed in cloning human BNP and CNP genes. In 1.8 kb 5'-flanking sequences of the cloned human BNP gene were linked upstream to the bacterial chloramphenicol acetyltransferase (CAT) gene, and their promoter activities were assayed in cultured neonatal rat ventricular cardiocytes. The 1.8 kb promoter region showed a high-level CAT activity. When the CT-rich sequences (-1288 to -1196) were deleted, the high-level activity was reduced to approximately 30%. The results revealed that the 1.8 kb human BNP promoter region contains DNA sequences important for ventricular expression of the BNP gene. Further study is on-going to elucidate the molecular mechanism responsible for the up-regulation of gene expression of natriuretic peptide family in sodium loading.