Generation of Mice that Overexpress or Lack Natriuretic Peptide Family and Its Significance in the Regulation of Salt Metabolism

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Summary

Natriuretic peptide family consists at least three endogenous ligands, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). These peptides influence body fluid homeostasis and blood pressure control by their potent natriuretic, diuretic, vasorelaxant, and vascular growth inhibitory activities. To examine the significance of natriuretic peptide family in the regulation of salt metabolism, in the present study, we have tried to generate mice that overexpress or lack natriuretic peptide family.

The mouse BNP genomic clone was isolated from a 129Sv mouse genomic library. A targeting vector for the disruption of BNP was constructed, in which the 2nd and 3rd exons of the mouse BNP gene were replaced by the neomycin resistance gene. Several chimeric mice were obtained from one of the targeted ES cell lines, which are now tested for its capability to transmit the disrupted allele to their offsprings. The mouse CNP genomic clone was also isolated, and a targeting vector was constructed, so that the 1st exon of the mouse CNP gene was disrupted. One chimera generated from a targeted ES cell line transmitted the disrupted allele to its offsprings. We are currently mating heterozygotes to obtain mice that are homozygous for the disrupted allele.

It has been demonstrated that the ANP and BNP genes are tightly linked on mouse chromosome 4 and on human chromosome 1. In the present study, we have characterized the genomic DNA fragment containing the ANP and BNP genes in mice and humans. In mice, the BNP gene was located about 12 kb upstream of the ANP gene. By a long-range PCR analysis, a 11-kb human genomic DNA fragment was isolated, which contained the 3rd exon of the BNP gene and the 1st and 2nd exons of the ANP gene, approximately 8 kb apart. These results provide evidence that two cardiac natriuretic peptide genes (ANP and BNP) are organized in tandem in mice and humans.

A monoclonal antibody (MAb) against mouse BNP was prepared by the fusion of mouse myloma cells X63-Ag8.653 with spleen cells of the immunized mouse. RIA was performed with MAb and ^{125}I-mouseBNP. Effects of intraperitoneal (i.p.) administration of 500 μg MAb to transgenic mice that overexpress BNP on urine volume and cGMP excretion were examined. The MAb showed high affinity for mouseBNP and the RIA was highly sensitive and specific for mouseBNP. Significant amount of BNP was present in the atrium, ventricle and kidney from control non-transgenic controls. The liver and plasma BNP levels in the transgenic mice were markedly elevated as compared with non-transgenic controls. The BNP levels in the kidney were also elevated in the transgenic mice. In the transgenic mice, urinary cGMP excretion was markedly increased than that in non-transgenic controls. The enhanced cGMP production was almost completely abolished by i.p. administration of MAb. Basal urine volume in the transgenic mice was not different from that in non-transgenic controls, while tended to be decreased after MAb administration. These observations suggest the potential usefulness of the MAb to examine the significance of BNP in transgenic mice that overexpress it.