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Gel Electrophoretic Determination of Atrial Natriuretic Peptide and its Truncated Peptide

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ABSTRACT: Atrial natriuretic peptide (ANP) is released from the heart and induces the elicitation of guanylate cyclase enzyme activity. It has vasodilatory and natriuretic properties. In this study we elucidated the determination of ANP deoxyribonucleotide (DNA) and its truncated peptide ANP (4-28) by agarose gel electrophoresis. The techniques combined the use of polymerase chain reaction and gel electrophoresis for simple estimation of DNA content from biological samples.

Key words: Atrial natriuretic peptide, DNA, guanylate cyclase, gel electrophoresis

Summary of the paper

Atrial natriuretic peptide (ANP) is a 28-amino acid (AA) peptide that consists of a 17-AA ring formed by a disulfide bond together with a 6-AA N-terminus and a 5-AA C-terminus (1). Studies in animal models of altered ANP production or receptor function as well as studies in humans with ANP infusion have demonstrated that ANP plays an important role in integrated cardiorenal function; ANP possesses natriuretic, vasodilatory, lusitropic, renal enhancing, and renin-angiotensin-aldosterone system (RAAS) inhibiting properties through activation of the natriuretic peptide receptor A (NPR-A) and generation of the second messenger 3',5'-cyclic guanosine monophosphate (cGMP) (2–7). By activating NPR-A, ANP also is antihypertrophic and antifibrotic, and genetic deletion of either the ANP gene *(Nppa)* or NPR-A results in hypertension, cardiac hypertrophy, and fibrosis (8).

The inhibition of specific signaling pathways has represented a classical means by which to define the function of such pathways. Gene disruption is one method by which to interrupt signaling pathways, and this has disrupted the genes for ANP, B-type natriuretic peptide (BNP), or GC-A . Disruption of the GC-A gene results in mice that display a salt-resistant elevation of blood pressure, and cardiac fibrosis and hypertrophy (9). The cardiac hypertrophy is greater than that seen in other mouse models of hypertension, suggesting that GC-A could be directly involved in the regulation of myocyte size. It has been shown, in fact, that ANP inhibits cardiomyocyte hypertrophy under *in vitro* culture conditions (10). ANP gene is expressed in the major organs of the body i.e heart, kidney, brain and testis. Keeping these important aspects in mind regarding the ANP gene, we estimated the content of ANP cDNA extracted from the rat pituitary and its synthetic oligonucleotide ANP DNA (4-28) (11) by agarose gel electrophoresis. The results of our study as depicted in the Figure-1 show that the yield of ANP DNA was 0.14ng and that of the oligonucleotide was 0.11 ng. This study thus highlights the importance of PCR and gel electrophoresis in the molecular biology studies on this important peptide.

RT-PCR and Gel Electrophoresis

ANP cDNA for hybridization was obtained by the purification and sequence of RT-PCR product of rat pituitary gland. A truncated ANP (4-28) DNA was also run alongside for comparison. 800 mg of agarose was added to 10xTAE solution. It was stirred for some time. The volume was made to 100 ml and agarose was dissolved by heating in microwave. After pouring 50 ml in to the tank a suitable comb was fixed. The gel was allowed to solidify for 20 minutes. 4 and 8 microliter of ANP DNA sample in TE buffer was added to each well and the gel run for 90 minutes. It was stained with ethidium bromide and observed on a transilluminator. The yield of DNA estimated was approximately 0.14 ng for ANP cDNA and 0.11 ng for ANP (4-28).

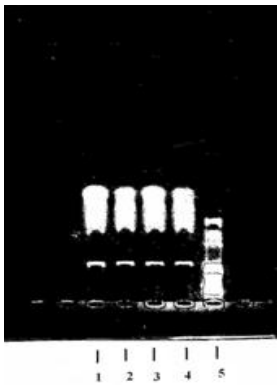

Fig.1 shows the agarose gel electrophoresis done for Atrial natriuretic peptide DNA and seen under a densitometric transilluminator. Lane 1- ANP 8 µl, Lane 2-ANP 4 µl, Lane 3-ANP (4-28) 8 µl, Lane 4-ANP (4-28) 4 µl, Lane 5- Standard DNA ladder

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