Molecular Studies of the Atrial Natriuretic Factor Gene

CHRISTINE E. SEIDMAN, KENNETH D. BLOCH, JEROME ZISFEIN,
JOHN A. SMITH, EDGAR HABER, CHARLES HOMCY, ALLAN D. DUBY,
EDMUND CHOI, ROBERT M. GRAHAM, AND J. G. SEIDMAN

SUMMARY The molecular biology of human atrial natriuretic factor was studied. A cloned rat cDNA probe was used to analyze tissue for the synthesis of atrial natriuretic factor, and the human gene was identified and sequenced. Nucleotide sequence comparison of human and rodent atrial natriuretic factor genes suggests regions that are critical for regulated expression of this cardiac hormone. (Hypertension 7 [Suppl I]: I-31-I-34, 1985)

KEY WORDS • cDNA • preprohormone • genomic clones • nucleotide sequence

ATRIAL natriuretic factor (ANF) is a small vasoactive peptide hormone synthesized by the heart that appears to affect circulatory homeostasis through diuretic and natriuretic activities.1,2 Purification and amino acid analysis of ANF peptides have recently been accomplished,3 which permit isolation of DNA sequences complementary (cDNA) to ANF messenger RNAs (mRNA).4-7 Nucleotide sequence analysis of a rodent cDNA clone suggests that the 24 amino acid peptide is synthesized as a 129 amino acid precursor, proANF. This precursor contains a glycosylation site and two potential protease processing sites, presumably for cleavage of the biologically active ANF peptide, as is seen in other peptide hormone precursors.8 The proANF molecule is derived from a prepropeptide that contains a hydrophobic leader segment typical of secreted processed peptides.9 Such molecular data strongly support the hypothesis that ANF is synthesized as a preprohormone in atrial tissues and is a cardiac hormone. To further study the regulated expression of this hormone, we screened tissues for ANF mRNA synthesis and cloned and characterized the human ANF gene.

To assess which tissues actively synthesize ANF, total mRNA was isolated, purified on an agarose gel, and transferred to nitrocellulose for blot hybridization10 with a rat ANF cDNA probe. The following list shows the rodent tissues with specific ANF mRNA expression.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Expression</th>
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</thead>
<tbody>
<tr>
<td>Atria</td>
<td>++++</td>
</tr>
<tr>
<td>Ventricle (apex)</td>
<td>+</td>
</tr>
<tr>
<td>Aortic arch</td>
<td>+</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>+</td>
</tr>
<tr>
<td>Pituitary</td>
<td>+</td>
</tr>
<tr>
<td>Kidney</td>
<td>+</td>
</tr>
<tr>
<td>Adrenal</td>
<td>+</td>
</tr>
<tr>
<td>Pancreas</td>
<td>+</td>
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<tr>
<td>Testes</td>
<td>+</td>
</tr>
<tr>
<td>Epididymis</td>
<td>+</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
</tr>
</tbody>
</table>

The plus signs indicate that ANF mRNA is present in the tissues; the minus signs indicate its absence. Total RNA was isolated from tissue by the guanidine isothiocyanate extraction procedure and analyzed by RNA blot hybridization to 32P-labeled rat ANF probe. Quantification of the amounts of ANF mRNA was based on the intensity of autoradiographic signal and duration of exposure. The absence of mRNA for ANF is only valid within the detection limits of the assay. Rat atria make significant levels of ANF mRNA, which suggests that the ANF gene is actively transcribed. Ventricular samples were obtained from the...
We have cloned and sequenced the human ANF gene to help define controls involved in transcription, processing, and expression of this cardiac hormone. A human genomic bacteriophage library was screened for ANF sequences with the use of a cloned rat cDNA probe. Five bacteriophage clones containing a 3.3 kilobase pair BamHl fragment were subcloned into pBR322 for nucleotide sequence analysis. A rat cDNA probe or an oligonucleotide that was derived from the 3' end of the human cDNA sequence was used to identify human ANF coding sequences. This oligonucleotide was required to detect the terminal coding sequence and a polyadenylation signal at the 3' end of the human gene.

The human ANF gene contains many of the features of a typical eukaryotic gene (Figures 1 and 2). A TATAA box is present, and ANF mRNA initiation depends on the dideoxy chain termination method. The human ANF genomic sequence is shown in Figure 1.

Figure 2 schematically displays the human ANF gene. Both the human and mouse ANF genes have three coding blocks (I, II, and III) separated by two intervening sequences (IVS 1, IVS 2). The first coding block encodes the hydrophobic leader segment and the first 20 amino acid residues of proANF. The second coding block encodes the remainder of the proANF molecule less one amino acid. This carboxyl terminal amino acid is encoded in coding block III.

The human ANF gene contains many of the features of a typical eukaryotic gene (Figures 1 and 2). A TATAA box is present, and ANF mRNA initiation (cap site) appears to be 27 nucleotides later. There are splice signals (GT–AT) flanking each intervening sequence and a polyadenylation signal at the 3' end of the gene.

**Figure 1** Schematic representation of the human ANF gene (DNA), mRNA, and peptide hormone (proANF). Peptide coding blocks (I, II, and III) are separated by two intervening sequences (IVS 1 and IVS 2 containing reiterated ALU sequences). A putative enhancer (E), TATAA box (T), and cap site (C) are present at the 5' end of the gene. A potential glucocorticoid receptor binding site (GR) and polyadenylation site (A) are shown. The oligonucleotide GAATTCCTTTCGAGGGTGCTCACGG (O) was used to identify the 3' end of the human gene. Processing of precursor ANF mRNAs yields the mature mRNA containing 5' untranslated sequences, joined coding blocks (I, II, III), and 3' untranslated sequences. Translation of this mRNA produces proproANF. The hydrophobic leader segments and ANF peptide are indicated.
To help delineate those sequences involved in the regulated expression of this cardiac hormone, genomic nucleotide sequences from humans and mouse were determined. Those sequences that are critical to the production of a functional ANF molecule may be conserved between these species, while less important sequences may be nonhomologous because of evolutionary drift. Figure 3 shows a dot matrix analysis of these sequences. Individual nucleotide bases from the human ANF gene (abscissa) and mouse (ordinate) are compared. Sequence identity between these species is indicated by a dot. Homologous regions between these genes will linearize confluent dots.

The 5' flanking sequences of mouse and human ANF genes are highly conserved. As this region may be important in directing ANF transcription, it was screened for previously characterized regulatory sequences. There is an 11 base pair sequence (see Figures 1 and 2) in the human ANF gene that is homologous to an SV40 enhancer sequence. Although a consensus enhancer sequence has not yet been defined, portions of the viral SV40 enhancer sequence are present in other gene enhancers. These sequences may play a causative role in the high level of atrial-specific transcription of the ANF gene.

Sequences encoding proANF (coding block I and II) are highly conserved between mouse and humans (see Figure 3). The role of this precursor is poorly understood, but extensive sequence homology between divergent species suggests a specific, necessary function. Nakayama et al. suggest that this region encodes a separate cardiac hormone. Alternatively, these sequences may be involved in processing and storage of ANF through biosynthesis of atrial granules. Such granules are thought to be integrally related to ANF production. Further studies on the prohormone should help delineate its role.

The amino acid and nucleotide sequences of the murine and human mature ANF peptide are strikingly homologous, which suggests that the molecular structure of this hormone is critical to its function. The carboxyl terminus of these peptides is, however, markedly different (coding block III). A single nucleotide change terminates the human protein transcript two amino acids before that in rodents. This change removes a putative protease cleavage signal from the human mRNA and replaces it with a stop codon. These data suggest that ANF may have evolved from an an-
cestral, larger peptide that required proteolytic processing to form its carboxyl terminus. In rodents this may have been achieved by peptide cleavage signals, which evolved to the termination signal in humans. In addition, the maintenance of a solitary tyrosine residue in human coding block III, despite rapid evolution of surrounding sequences, strongly suggests a critical role for this amino acid in ANF activity.

There are two intervening sequences in the human ANF gene (see Figures 1 and 2): IVS I shows homology to the corresponding sequences in rodents but not to previously characterized regulatory sequences; IVS 2 contains two tandem reiterated (ALU) sequences that probably have little functional importance. In addition, IVS 2 contains a potential glucocorticoid receptor binding sequence (GR in Figure 1). This sequence is identical at 9 of 11 base pairs to the consensus sequence. This sequence is absent from the murine ANF gene. A similar difference is found in the growth hormone genes, in which a functional glucocorticoid receptor binding sequence is present in humans but absent in rodents. Delineation of the functional importance of these sequences in the human ANF gene and the potential role of mineralocorticoids and glucocorticoids on ANF production require further investigation.

In summary, research on the molecular biology of ANF with the use of rodent ANF cDNA probes has defined the tissue specificity of this peptide hormone and permitted characterization of the human ANF gene. Nucleotide sequence analysis reveals many classical features of eukaryotic genes and substantial sequence homology with rodent ANF genes. Further research on these conserved regions should provide insights into the mechanisms of tissue-specific expression of ANF and advance our understanding of factors that regulate intravascular pressure and volume.

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References


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