

## Detection of C-type natriuretic peptide compared with brain and atrial natriuretic peptide transcripts in human heart by the polymerase chain reaction

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C-type natriuretic peptide (CNP), initially isolated from porcine brain [3], is the most recently characterized member of the natriuretic peptide family. The human CNP gene consists of at least two exons and an intron [4]. Whereas synthesis of atrial and brain natriuretic peptides (ANP and BNP) has been demonstrated mainly in the heart, but also in various other organs [2, 5], CNP mRNA in man has so far been found only in the central nervous system. We have investigated human cardiac tissues for CNP expression and compared the abundance of CNP transcripts with that of ANP and BNP.

Atrial and ventricular tissues were obtained from a 76-year-old female patient with congestive heart failure at autopsy. Extraction of total RNA, assessment of RNA integrity, RNA quantification and cDNA synthesis by reverse transcriptase were performed as described in detail elsewhere [1]. The sense and antisense primers used for PCR amplification corresponded to sequences in the first and second exon of the ANP, BNP and CNP genes, respectively. Thus, amplification of the cDNA and genomic DNA templates yielded fragments of 445 bp and 576 bp for ANP, 291 bp and 522 bp for BNP, and 358 bp and 802 bp for CNP. Various amounts (60–180 ng) of cDNA and 85 ng of human genomic DNA were coamplified, allowing for quantitation of the cDNA relative to total cDNA [1]. Amplification products were separated by agarose gel electrophoresis, transferred to nylon membranes (Hybond, Amersham) and hybridized to end-labelled oligonucleotides, corresponding to sequences located between the two sequences used as PCR primers. Quantification was performed by image analysis densitometry of the hybridization bands.

CNP transcripts of similar size were detected in brain, atrial and ventricular cDNA. Omission of cDNA served as negative control (Fig. 1). As summarized in Table 1, CNP transcripts were about three orders of magnitude less abundant than ANP and BNP transcripts in both atrial and ventricular tissue. We here present evidence for CNP expression in the human heart.

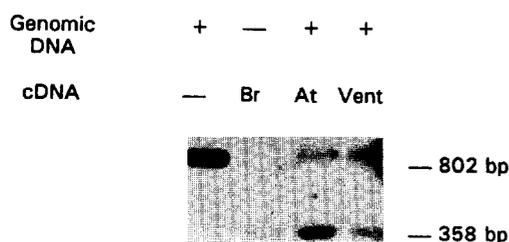


Fig. 1. CNP transcripts in cDNA of brain (*Br*), atrial (*At*) and ventricular (*Vent*) tissue with or without added human genomic DNA

**Table 1.** Natriuretic peptide cDNA abundance relative to genomic DNA

cDNA/total cDNA (% × 10 <sup>-7</sup> )	Atrial tissue	Ventricular tissue
ANP	6770	1690
BNP	2840	1072
CNP	3.4	4.8

Since there is evidence for a pharmacological profile of CNP that is different from that of ANP and BNP [3], interest should focus on the potential role of CNP in cardiovascular physiology and pathophysiology.

**Acknowledgement.** The work reported in this paper was supported by an ASCHE stipendium from the Deutsche Gesellschaft für Verdauungs- und Stoffwechselkrankheiten to A.L.G.

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Received: April 7, 1993

Accepted: April 13, 1993

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