

PHARMACOKINETICS OF BRAIN NATRIURETIC PEPTIDE IN RATS

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The pharmacokinetics of rat brain natriuretic peptide (rBNP) was compared with that of α -rat atrial natriuretic peptide (α -rANP) in rats. After intravenous infusion in rats (600 pmol min⁻¹kg⁻¹ for 2 min), the disappearance of plasma rBNP was 4-fold slower than that of α -rANP. The estimated mean plasma clearance rates for rBNP and α -rANP were 45.9 ml min⁻¹kg⁻¹ and 74.4 ml min⁻¹kg⁻¹, respectively. The affinity of rBNP for the clearance receptor or degradation enzyme was considered to be lower than that of α -rANP.

KEYWORDS atrial natriuretic peptide; brain natriuretic peptide; clearance, pharmacokinetics

INTRODUCTION The structures of the mammalian atrial natriuretic peptides (ANP) are highly conserved: the 28-amino acid α -rat ANP (α -rANP) differs from α -human ANP by only one residue, Ile¹² for Met¹². The ring structure, which is constructed with a disulfide bond Cys⁷-Cys²³, is indispensable for the biological actions of ANP. On the other hand, the very rapid turnover of ANP has been attributed to biologically silent clearance receptors and to neutral endopeptidase (EC 3.4.24.11). Maack *et al.*¹⁾ demonstrated that a ring-deleted ANP analogue, des(Gln¹⁸-Gly²²)-ANP(4-23), binds with high affinity to the clearance receptor, thus inhibiting the clearance of ANP. Vanneste *et al.*²⁾ reported that purified endopeptidase hydrolyzes several sites, including the bonds Cys⁷-Phe⁸ and Ser²⁵-Phe²⁶. Brain natriuretic peptide (BNP) is also synthesized in and secreted from the hearts of pigs³⁾ and rats.⁴⁾ It shows a diversity in structure among species: each BNP has striking homology to ANP in the amino acid sequence of a ring structure and in the central and peripheral actions, whereas its sequences in the N- and C-terminal parts differ considerably from those of ANP.^{5,6,7)} In this study, we determined to what extent the differences in structure could lead to the differences in the pharmacokinetics of ANP and BNP.

METHODS Male Wistar rats, each weighing 300-350 g, were anesthetized with 60 mg kg⁻¹ sodium pentobarbital. Rat BNP with 45 amino acids (rBNP) and α -rANP (Peptide Institute, Osaka, Japan) was diluted with phosphate-buffered saline (pH 7.4) containing 0.1% bovine serum albumin, and 600 pmol min⁻¹kg⁻¹ was administered for 2 min *via* a jugular vein. This dose was carefully determined so that the administered peptide did not cause severe hypotension and that the plasma α -rANP and rBNP were measurable in the experimental period. Blood samples were periodically withdrawn *via* a catheter in the femoral artery. The plasma concentrations (C_t) of α -rANP and rBNP were determined by the radioimmunoassay.^{4,8)} The disposition of α -rANP and rBNP following intravenous (*i.v.*) infusion was analyzed by a two-compartment open model as described previously⁸⁾:

$C_t = C_1 (e^{-\lambda_1 t'} - e^{-\lambda_1 t}) + C_2 (e^{-\lambda_2 t'} - e^{-\lambda_2 t}) + C_3$, where t is the time from the infusion, t' is 0 for t less than the infusion time (T), and t' is $t-T$ for t equal to and larger than T . C_1 and λ_1 are the fast phase variables, and C_2 and λ_2 are the slow phase variables. C_3 is the baseline concentration before administration of the peptide. The non-linear least squares regression was analyzed by use of Nelder-Mead's algorithm⁹) on a FACOM M780 computer. In addition, total clearance (CL) was calculated as follows: $CL = \text{infusion rate} / (C_1 + C_2)$. Values are expressed as mean \pm s.e. Significance of difference between mean values was calculated by a non-paired t -test.

RESULTS Fig.1 shows the plasma concentrations of α -rANP and rBNP before and after *i.v.* infusion of peptides. TABLE I summarizes the pharmacokinetic parameters estimated by two-compartment model analysis. Significant levels of endogenous α -rANP were detected before administration of the peptide, but rBNP was not detected. Significant differences were observed in slow phase variables (C_2 , λ_2) and CL.

DISCUSSION BNP was initially isolated from porcine brain.⁵) However, recent studies have demonstrated a high concentration of BNP in the rat⁴) and human heart.⁷) In addition, plasma BNP concentrations are markedly elevated in patients with various diseases, such as congestive heart failure, chronic renal failure, and essential hypertension.¹⁰) These reports, together with biological actions of BNP and ANP, imply that BNP is a novel cardiac hormone in the natriuretic peptide system. In the present study, we investigated the pharmacokinetic characteristics of rBNP. The clearance values of rBNP were approximately 38% less than those of α -rANP. Moreover, the half-time of plasma disappearance in the slow phase was 4-fold longer for rBNP than for α -rANP: 5.45 ± 0.69 min ($n=4$) vs. 1.35 ± 0.09 min ($n=7$). The slower turnover of rBNP suggests a lower affinity for the clearance receptor or endopeptidase EC 3.4.24.11 compared with α -rANP. Further studies are needed to clarify the underlying mechanism(s) for the disparity in the pharmacokinetics of natriuretic peptides.

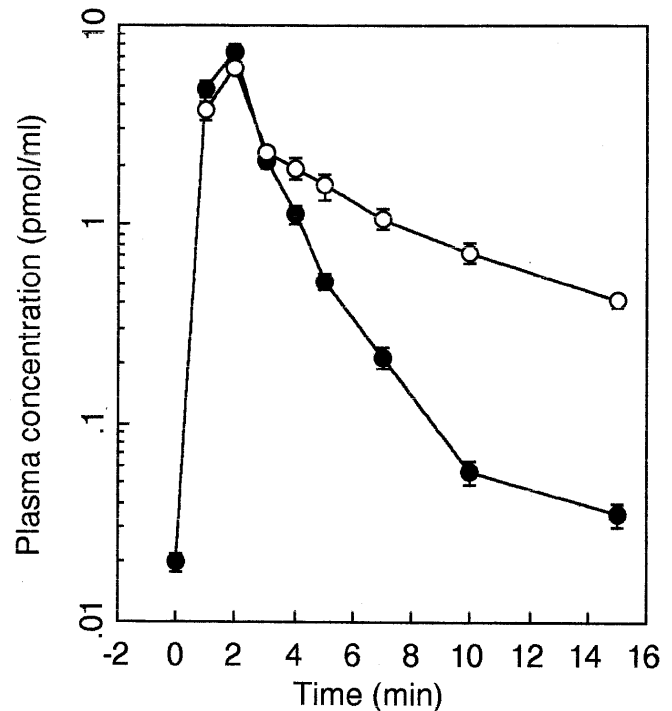


Fig. 1. Time Course of Plasma Concentration of α -rANP (\bullet , $n=7$) and rBNP (\circ , $n=4$) Following *i.v.* Infusion of the Peptide ($600 \text{ pmol min}^{-1}\text{kg}^{-1}$ from Time 0 to 2). The vertical bars represent s.e.

TABLE I. Pharmacokinetic Parameters of α -rANP and rBNP Following *i.v.* Infusion of Peptides ($600 \text{ pmol min}^{-1}\text{kg}^{-1}$ for 2 min) in Rats

	α -rANP($n=7$)	rBNP ($n=4$)
C_1 (pmol ml^{-1})	4.50 ± 0.25	3.58 ± 0.67
λ_1 (min^{-1})	1.91 ± 0.30	2.37 ± 0.45
C_2 (pmol ml^{-1})	3.78 ± 0.43	$9.58 \pm 1.07^*$
λ_2 (min^{-1})	0.536 ± 0.040	$0.132 \pm 0.013^*$
C_3 (pmol ml^{-1})	0.0223 ± 0.0018	---
CL ($\text{ml min}^{-1}\text{kg}^{-1}$)	74.9 ± 4.9	$45.9 \pm 1.9^*$

Values are expressed as mean \pm s.e. for n animals.

* $P < 0.005$.

In conclusion, we demonstrated a difference in the pharmacokinetic characteristics between BNP and ANP. This may provide new insight into the physiological role and clinical applications of BNP.

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