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Novel neprilysin-derived BNP fragment in the circulation: evidence from a rat model

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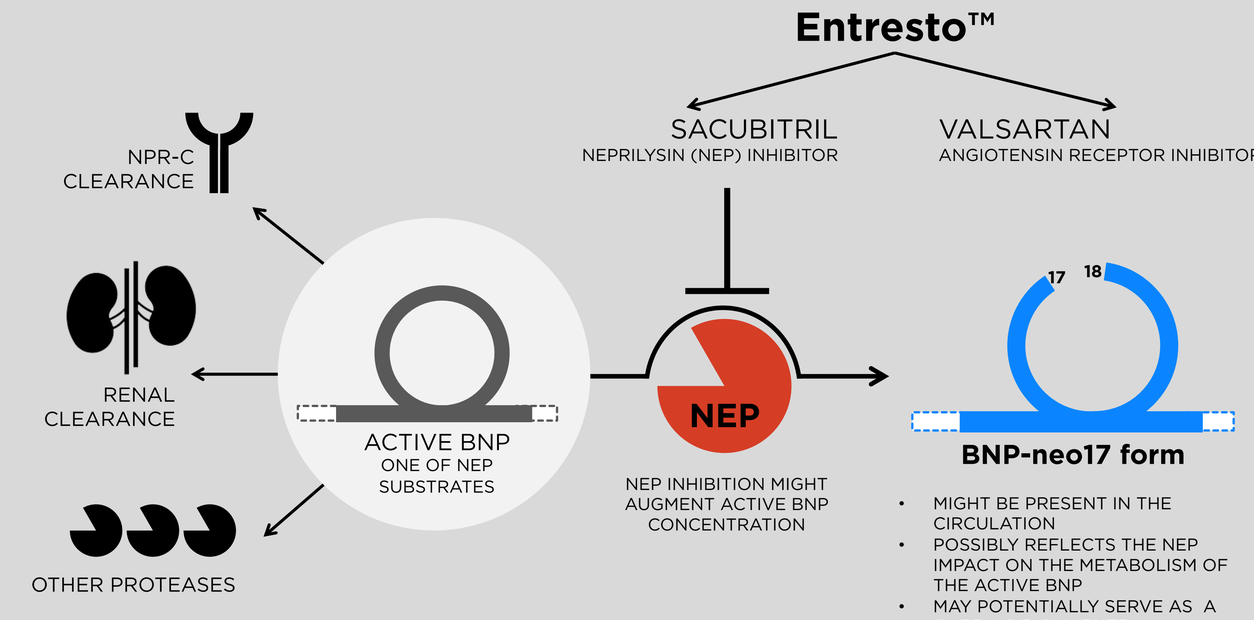
HyTest

Background



- MIGHT BE PRESENT IN THE CIRCULATION
- POSSIBLY REFLECTS THE NEP IMPACT ON THE METABOLISM OF THE ACTIVE BNP
- MAY POTENTIALLY SERVE AS A CARDIAC BIOMARKER

ENTRESTO™ IS A FIRST-IN-CLASS ARNi (ANGIOTENSIN RECEPTOR NEPRILYSIN INHIBITOR) DRUG FOR HEART FAILURE (HF) THERAPY, WHICH COMPRISES NEPRILYSIN (NEP) INHIBITOR SACUBITRIL.

ONE OF THE IMPORTANT NEP SUBSTRATES IS AN ACTIVE FORM OF B-TYPE NATRIURETIC PEPTIDE (BNP); THE AUGMENTATION OF THE ACTIVE BNP LEVEL DUE TO NEP INHIBITION IS CONSIDERED AS A POSSIBLE MECHANISM OF CARDIAC FUNCTION IMPROVEMENT BY ARNi.

WE HYPOTHESIZED THAT PRODUCTS OF BNP PROTEOLYSIS BY NEP ARE PRESENT IN THE CIRCULATION AND THAT THEIR LEVEL MIGHT REFLECT THE NEP IMPACT ON THE ACTIVE BNP METABOLISM.

WE SUGGEST THAT BNP CLEAVAGE BY NEP AT 17-18 AAR RESULTS IN THE BNP RING STRUCTURE OPENING AND THE FORMATION OF THE **BNP-NEO17 FORM.**

AIM OF THE STUDY

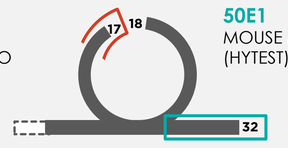
EXPLORE THE NEP-DEPENDENT **BNP-NEO17** GENERATION
IN VITRO AND *IN VIVO*

Methods

IMMUNOASSAYS

BNP-NEO17 SANDWICH

POLY-NEO17
POLYCLONAL RABBIT ANTIBODY, SPECIFIC TO NEO17-EPITOPE



50E1
MOUSE MONOCLONAL ANTIBODY (HYTEST), EPITOPE 26-32

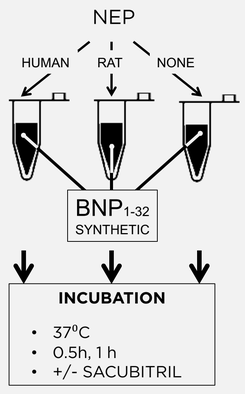
TOTAL BNP ASSAY

SES (SINGLE EPITOPE SANDWICH)
- MAB 24C5 (EPITOPE 11-17, HYTEST)
- MAB AB-BNP-2 (SPECIFIC TO THE IMMUNE COMPLEX OF BNP WITH MAB 24C5)

ANP COMPETITIVE ASSAY

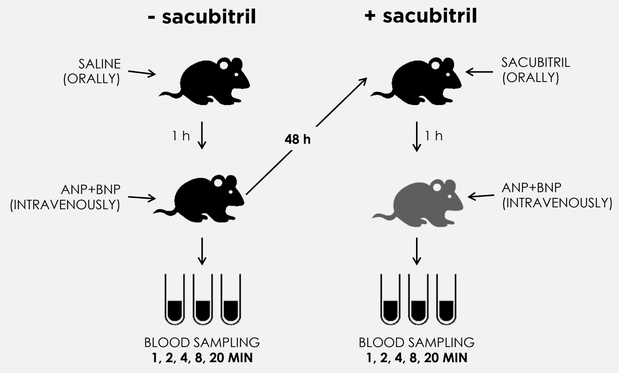
ANTI-ANP MAB 23/1
MOUSE MONOCLONAL ANTIBODY (BIORAD)

IN VITRO BNP CLEAVAGE



- SYNTHETIC BNP 1-32 WAS INCUBATED WITH HUMAN AND RAT NEP EITHER WITH OR WITHOUT SACUBITRIL.
- SAMPLES WERE ANALYZED BY THE BNP-NEO17 SANDWICH IMMUNOASSAY AT 0, 0.5 AND 1 HOUR TIME POINTS.

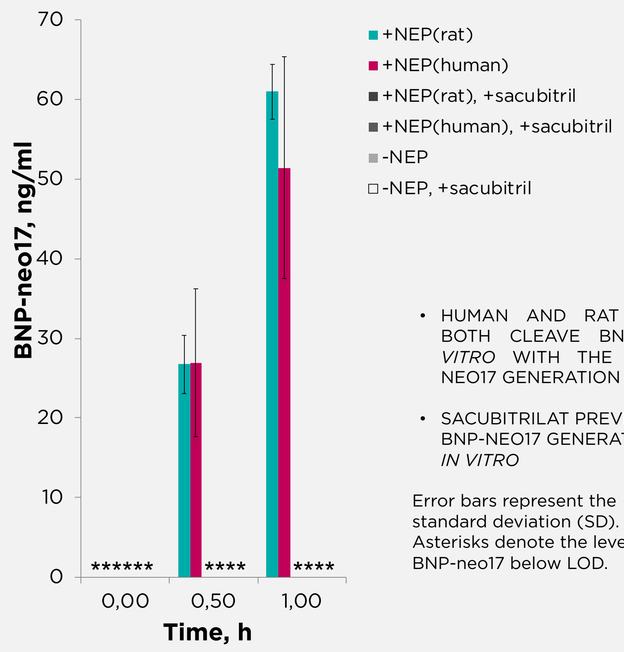
IN VIVO BNP CLEAVAGE



- 8 WISTAR RATS WERE ANESTHETIZED AND THE RIGHT FEMORAL ARTERY AND VEIN CANNULATED.
- THE SAME RATS WERE USED FOR (-) AND (+) SACUBITRIL KINETICS.
- ANP WAS INJECTED TOGETHER WITH BNP AS A CONTROL FOR NEP INHIBITION AS A PREFERABLE NEP SUBSTRATE.
- SAMPLES WERE ANALYZED BY BNP-NEO17, TOTAL BNP AND ANP IMMUNOASSAYS AT 0, 1, 2, 4, 8 AND 20 MINUTES POST INJECTION

Results

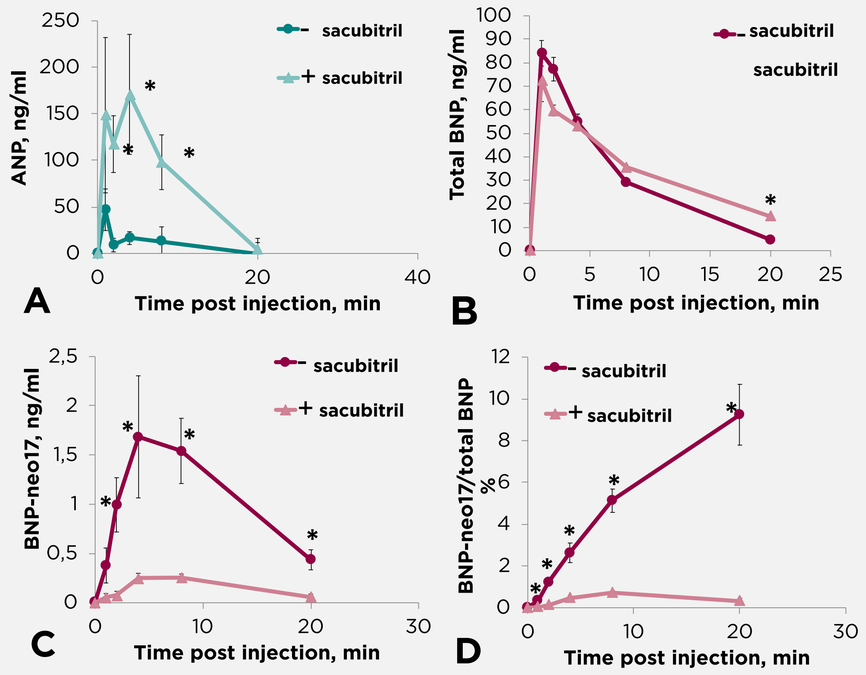
BNP CLEAVAGE BY RAT AND HUMAN NEP *IN VITRO*



- HUMAN AND RAT NEP BOTH CLEAVE BNP *IN VITRO* WITH THE BNP-NEO17 GENERATION
- SACUBITRILAT PREVENTS BNP-NEO17 GENERATION *IN VITRO*

Error bars represent the standard deviation (SD). Asterisks denote the levels of BNP-neo17 below LOD.

CHANGE IN AVERAGE BNP AND ANP CONCENTRATIONS IN RAT PLASMA WITH AND WITHOUT NEP INHIBITION



(A) ANP CONCENTRATIONS WERE SIGNIFICANTLY LOWER AT 2, 4 AND 8 MINUTES POST INJECTION IN (-) SACUBITRIL RATS

(B) THERE WAS NO SIGNIFICANT DIFFERENCE IN TOTAL BNP CONCENTRATIONS BETWEEN (-) AND (+) SACUBITRIL RATS

(C) BNP-NEO17 CONCENTRATIONS AT 2, 4, 8 AND 20 MINUTES POST INJECTION WERE SIGNIFICANTLY LOWER IN (+) SACUBITRIL RATS

(D) BNP-NEO17 WAS ACCUMULATED IN THE CIRCUATION OF (-) SACUBITRIL RATS

Error bars represent the standard error of the mean (SE). Asterisks mark the points where the parameter difference +/- sacubitril was statistically significant.

CONCLUSIONS

We have shown for the first time that **BNP-neo17** is generated *in vitro* and *in vivo,* and that its formation is NEP-dependent.
In the light of this, we suggest that **BNP-neo17** concentration might reflect the inherent NEP activity of the organism and serve as a potential cardiac biomarker.

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