Non-hypertrophic and hypertrophic human cardiomyocytes secrete unprocessed ANP and BNP precursors (proANP/proBNP) and exhibit a similar efficiency in their processing

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Introduction

A- and B-type natriuretic peptides (ANP and BNP) are key cardiac hormones of cardiorenal homeostasis. ANP and BNP are produced as prohormones, proANP and proBNP, which are enzymatically converted to biologically active peptides ANP and BNP, and N-terminal fragments, NT-proANP and NT-proBNP with unknown physiological function.

It's commonly considered that a diseased heart (e.g. under hypertrophy in heart failure (HF)) secretes high amounts of unprocessed precursors resulting in the lack of physiological natriuretic response in HF patients – the so called "natriuretic paradox". The appearance of intact precursors in the circulation can be attributed to their inefficient processing in the diseased heart. However, the mechanism of proANP and proBNP secretion and processing in normal heart remains elusive, since the levels of ANP and BNP under normal conditions are very low and often beyond the detection limits of available assays. One may suggest that a robust and physiologically relevant cell-based model can be used to address these issues.

The purpose of the study: to explore the secretion and processing efficiency of proANP and proBNP in human cardiomyocytes, in both normal and hypertrophic conditions.

Materials and Methods

Human cardiomyocytes: iCell Cardiomyocytes2 were from Cellular Dynamics International. These cells are highly purified human cardiomyocytes derived from induced pluripotent stem (iPS) cells, representing a mixture of atrial-, nodal-, and ventricular-like myocytes with typical biochemical electrophysiological and mechanical characteristics.

Inducing a hypertrophic response: cardiomyocytes were plated in fibronectin-coated 96-well plates. A hypertrophic response was induced on day 4 post plating with 0.004-1000 pM of endothelin 1 (ET-1). On day 5 post thaw and following induction with ET-1 for 20 h, supernatants from the cardiomyocyte cell culture plate wells were collected for analysis.

Immunoassays: concentrations of precursors as well as the total levels of the corresponding peptides (i.e. [N-terminal fragment + precursor]) were measured by means of sandwich-type immunoassays in conditioned media.

Assay for proANP: mouse monoclonal antibody (mAb) (epitope within the ring structure of ANP, Bio-Rad) was used as a capture antibody and rabbit polyclonal antibody (pAb) specific to NT-proANP (in-house, unknown epitope within the central region) labeled with stable europium chelate was used as a detection antibody.

Assay for total ANP: a rabbit pAb specific to NT-proANP (in-house, unknown epitope within the central region) was used as a capture antibody. Detection antibody was the same pAb as used in proANP assay (see above).

Assay for proBNP: mouse mAb 24C5 (HyTest, epitope 11-17 within the BNP sequence) was used as a capture antibody and mouse mAb 13G12 (HyTest, epitope 15-20 within the NT-proBNP sequence) labeled with stable europium chelate was used as a detection antibody.

Assay for total BNP: mouse mAb 29D12 (HyTest, epitope 5-12 within the NT-proBNP sequence) was used as a capture antibody and mouse mAb NT34 (HyTest, epitope 25-32 within the NT-proBNP sequence) labeled with stable europium chelate was used as a capture antibody.

Schematic representations of ANP and BNP immunoassays are shown in **Figure 1**.

Calibrators: recombinant proANP (in-house) and proBNP (HyTest) expressed in E. coli were used as calibrators.

Calculation of the processing rate: the rate of processing was calculated using the formula: (1 - [precursor]/[N-terminal fragment + precursor])*100%.

Results

ET-1 treated iPS cell-derived cardiomyocytes exhibited dose-dependent increases in total ANP and BNP production: up to 4-fold for ANP and 7-fold for BNP at highest concentration of ET-1 compared to untreated cells (**Figure 2**).

To estimate the rate of processing of ANP and BNP precursors, we measured the level of intact precursors in conditioned media. Notably, secretion of intact proANP and proBNP was observed in both non-induced and ET-1-induced cardiomyocytes, suggesting that secretion of unprocessed precursors occurs also in normal (nonhypertrophic) conditions.

The processing rate of proANP and proBNP in both normal and hypertrophic cells was similar: ~30% for proANP and ~60% for proBNP (Figure 3). There was no alteration of processing efficiency in the cells treated with different ET-1 concentrations (**Figure 3**).

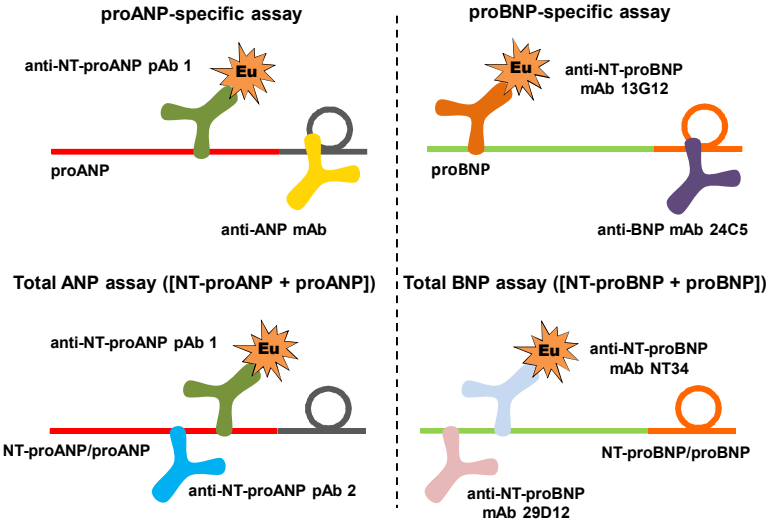

FIGURE 1. Schematic representation of ANP and BNP immunoassays used in the study.

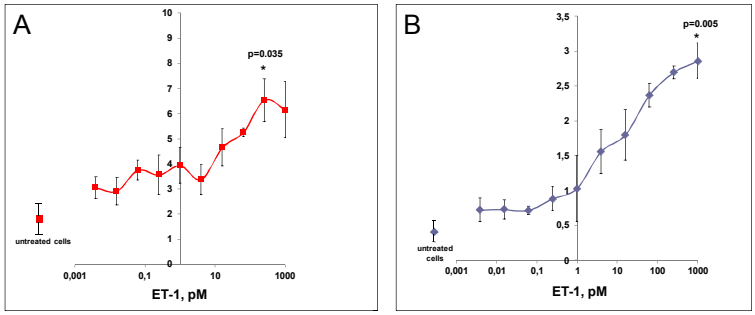

FIGURE 2. Total ANP (A) and BNP (B) levels measured by immunoassays in conditioned media after 20 h of induction with different concentrations of ET-1. Mean ± SD; n=3 wells for each point of the curve.

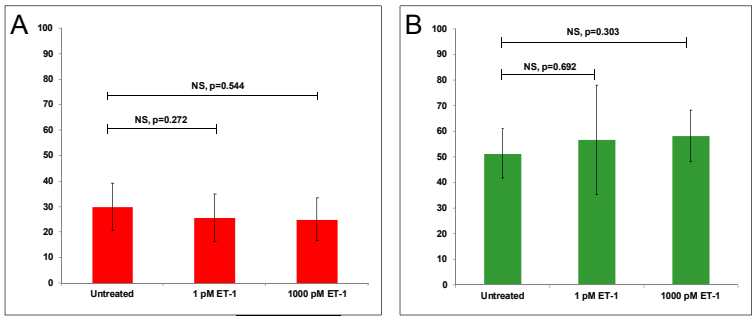

FIGURE 3. ProANP (A) and proBNP (B) processing rate measured in conditioned media after 20 h of induction with 1 and 1000 pM ET-1 compared to untreated cells. Mean ± SD; n=3 wells; NS – differences are statistically insignificant.

Conclusions

The results obtained in a cell model, human iPS-derived cardiomyocytes, show that the increase in ANP and BNP production observed in human cardiomyocytes under hypertrophy does not result in alteration of the processing efficiency of precursor molecules.

The present data suggest that intact proANP and proBNP may be secreted by normal hearts similarly to what is observed in HF patients. This might indicate that the intact precursors could have a specific role in the circulation.