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Abstract: 300

Evaluation of the expression of transcripts coding for CNP and for its specific receptor, NPR-B, by Real Time PCR in cardiac tissue of normal and heart failure animals

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Purpose: Higher plasma levels and a cardiac production of C-type natriuretic peptide (CNP) were recently observed in patients with chronic heart failure (HF), but its cellular source and possible difference between atrium and ventricle expression are so far lacking. Aim of this study was to evaluate the expression of transcripts coding for CNP and for its specific receptor, NPR-B, in cardiac tissue (right and left atrium and ventricle) of normal and CHF animals. CNP tissue levels were also determined in cardiac extracts.

Methods: Adult male minipigs (n=5) were chronically instrumented with a unipolar pacemaker connected to the anterior left ventricular (LV) wall. HF was induced by rapid pacing (180 beats/min) for 4 weeks. End-stage HF occurred at 24±2 days of pacing when the LV end-diastolic pressure was ≥25 mmHg. As control, we studied 5 adult male minipigs. At 4 weeks, myocardial samples were collected. Both CNP mRNA and proteins were extracted from a same sample with the method of phenol/guanidine-thiocyanate/chloroform. Tissue CNP levels were determined by a radioimmunoassay after a preliminary extraction on Sep-Pak C18, while the expression of mRNA coding for CNP and NPR-B in myocardial tissue (n=40) by Real Time reverse transcriptase-polymerase chain reaction (PCR) with DDCt method. As overall control, a parallel Real Time-PCR assay for BNP mRNA expression was carried out in the same samples. Real Time-PCR analysis was performed using an automated sequence instrument (7900HT Fast, Applied Biosystems) for the real-time monitoring of nucleic acid green dye fluorescence (SYBR Green I).

Results: As to myocardial extracts, CNP was found in all cardiac chambers of controls and its content was ten fold higher in atria than in ventricles (RA: 13.7±1.9 pg/mg; LA: 8.7±3.8 pg/mg; RV: 1.07±0.33 pg/mg; LV: 0.93±0.17 pg/mg). At 4 weeks of pacing stress, myocardial levels of CNP in LV were higher than in controls (15.8±9.9 pg/mg vs.0.9±0.17 pg/mg, p=0.01). The expression of mRNA coding for CNP was higher at 4 weeks of pacing althought CNP gene expression appears to be noticeable lower than that of BNP. The NPR-B resulted to be expressed in all cardiac regions analyzed, and a down-regulation was observed in ventricles after HF. Althought further investigations are necessary, the high tissue levels of CNP found after pacing stress as well as the myocardial CNP and NPR-B expression suggest an important role of this peptide in a so complex pathology as HF.