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Molecular Basis of Funny Current (If) in Normal and Failing Human Heart

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New insight has been achieved regarding the molecular determinants of abnormal impulse formation in human failing hearts, said Giuseppe Lonardo, PhD, here at the annual meeting of the American Heart Association.

Despite the enormous efforts made by medicine and bio-medical research to counteract cardiovascular disorders, these pathologies are still the first cause of death worldwide. Heart failure (HF) affects millions of people and hundreds of thousands die annually. Half of those deaths are sudden and unexpected, most likely due to ventricular arrhythmias. Moreover, HF is an important risk factor for the occurrence of atrial arrhythmias, such as atrial fibrillation, which is a not lethal arrhythmia, but significantly decreases life expectancy of patients. Therefore, it is of crucial importance to better elucidate cellular mechanisms, and their molecular basis, that underlie the occurrence of ventricular and atrial arrhythmias in HF patients.

During hypertrophy and heart failure, cardiac myocytes modify their electrophysiologic properties. In particular, an ionic current (the so-called "funny current" [If]), essential for electrical impulse formation in pacemaker centres of heart, is functionally over-expressed in human ventricular cells from HF patients, whilst is very small in normal conditions. This result strongly suggested the hypothesis that HF modulates If, somehow depending on the etiology, so that this current might become an important risk factor for arrhythmogenesis. HCN (standing for Hyperpolarization-activated Cyclic Nucleotide-gated channels) are the molecular components of If, and so far four isoforms of HCN have been identified: HCN1, HCN2, HCN3, and HCN4.

The present study was born from the need to better investigate the molecular basis of this dangerous functional alteration, and it is the fruit of a collaboration between the University of Florence and the Montreal Heart Institute. To this goal, HCN subunits have been investigated in ventricles and atria from human normal and failing hearts, by molecular and biochemical techniques. Present data demonstrate that behind the functional over-expression of If there is an over-expression of HCN2 and HCN4, in ventricles and atria from HF patients. Molecular data confirmed that a different etiology of HF might result in a different mechanism of If up-regulation.

The goal of this study, explained Dr. Lonardo, who is with the laboratory of D. Nattel at the Montreal Heart Institute, Montreal, Canada, and with the research unit of Dr. Mugelli at the Dept. of Pharmacology, Florence, Italy, was to evaluate the functional presence of If and to quantify the expression of HCN subunits in the ventricles and atria of both normal and failing human hearts, in order to better understand the electrophysiological abnormality.

Methods

Human ventricular myocytes were isolated from normal and failing hearts of patients with dilated (DCM) or ischemic (ICM) cardiomyopathy. Whole-cell patch-clamp configuration was used to record If in isolate human ventricular myocytes. Competitive RT-PCR and Western Blot tests were used to determine absolute mRNA expression and protein amount in the ventricles and atria from normal (n=11 and 23, respectively) and failing (n=10 and 10 [DCM, 6; ICM, 4], respectively).

Results

If density was increased in patients with heart failure, compared with those with normal hearts (17.0 pA/pF at –90 mV vs. 7.6, respectively).

HCN-4 mRNA was expressed approximately 10-fold more strongly than HCN2 ($p<0.05$) (Figures 1 and 2). If activation time constant at –90 mV averaged 1,674 ms in normal hearts, similar to reported values for HCN4 (1,271 ms), but slower than HCN2 values (278 ms), a finding that is consistent with existing molecular data.

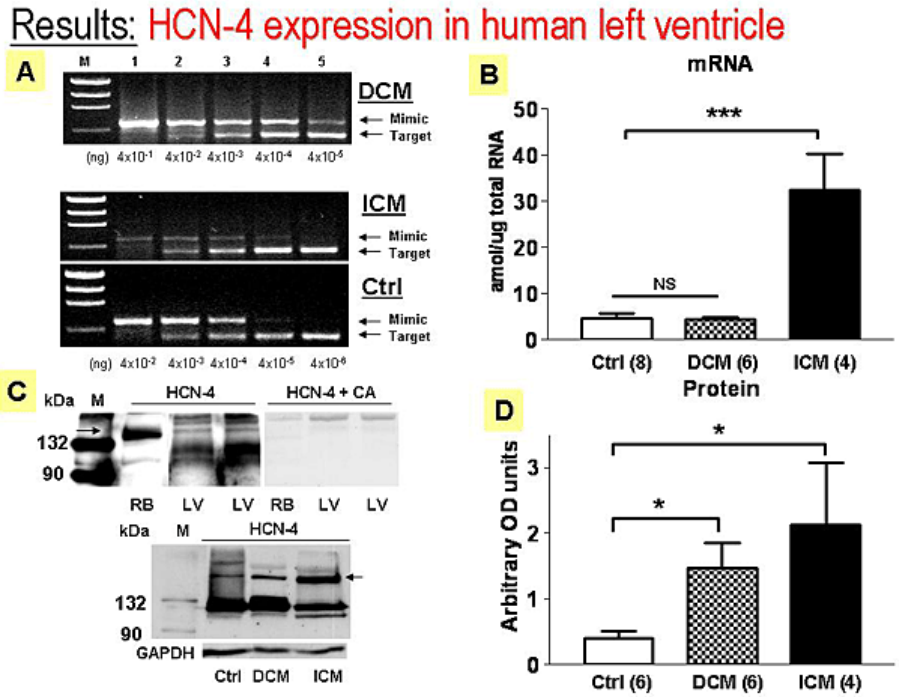

Figure 1

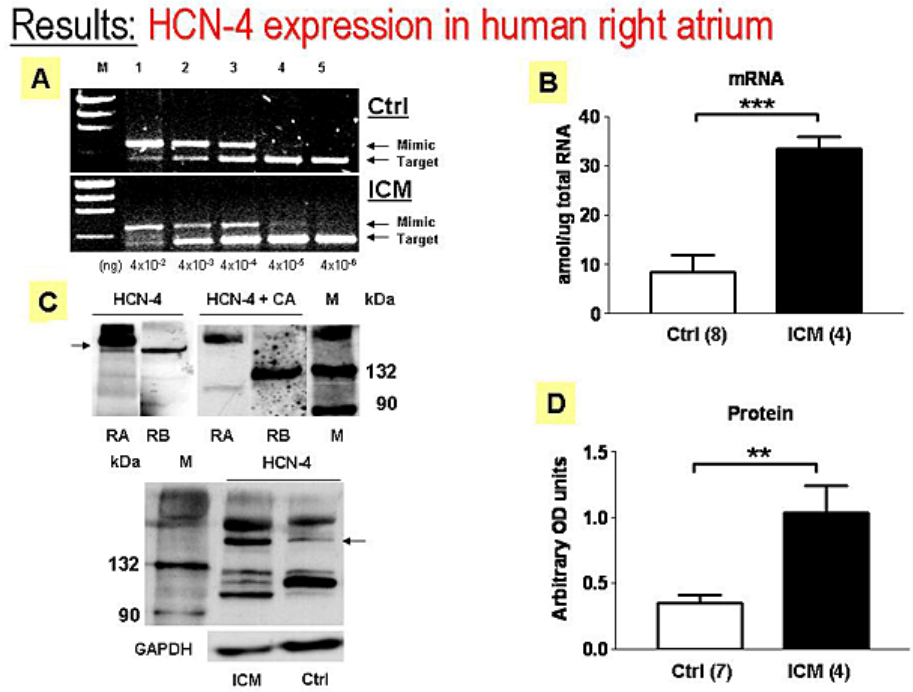

Figure 2

In normal hearts, HCN2 expression was stronger in ventricles than in atria for mRNA ($p<0.0001$) and for protein ($p=0.0041$) (Figures 3 and 4).

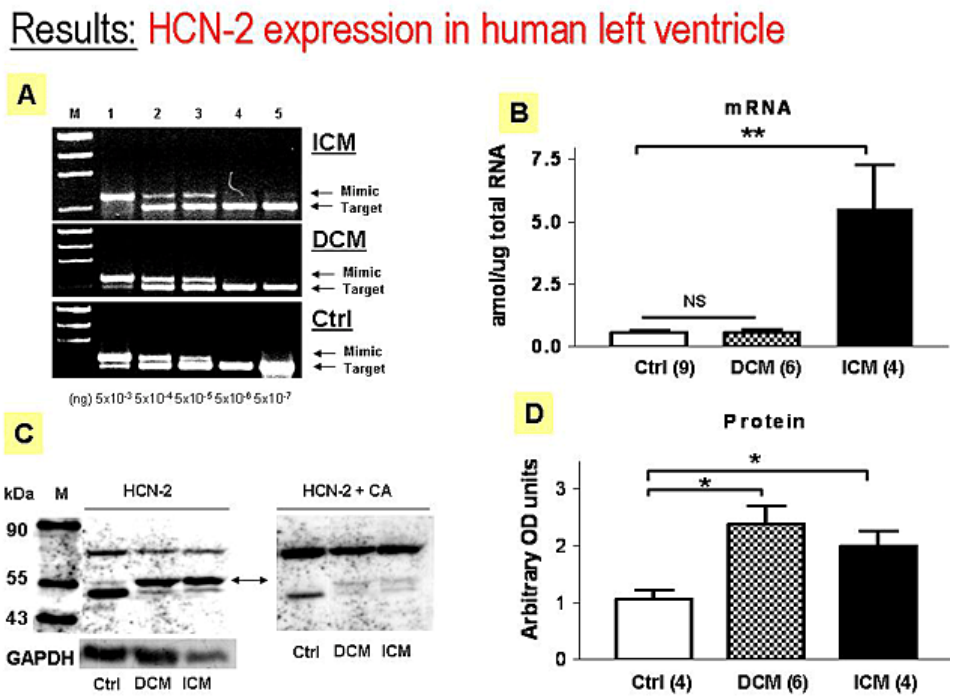

Figure 3

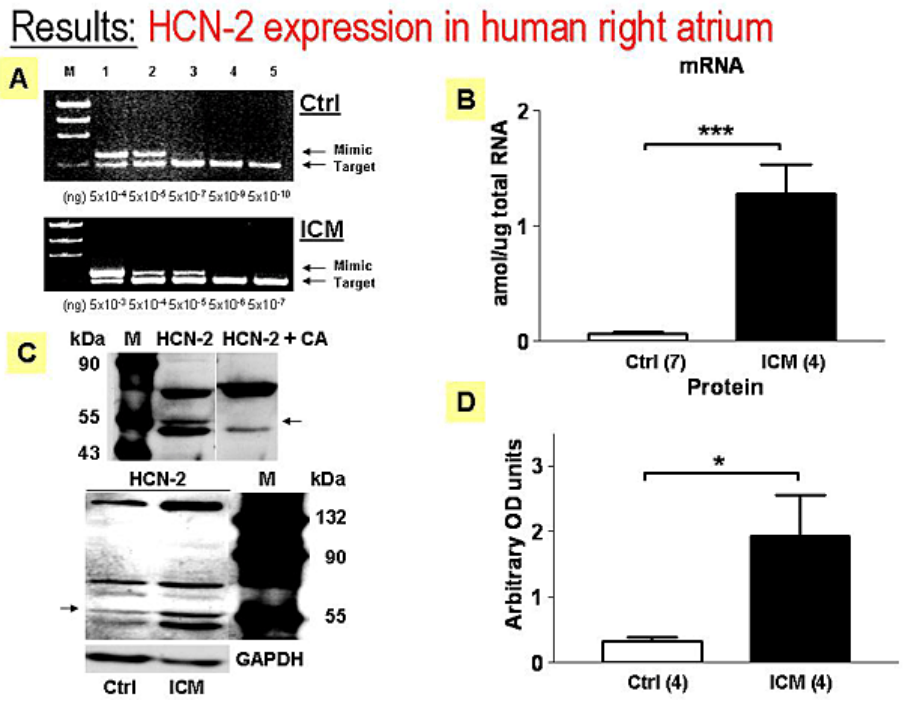

Figure 4

In failing hearts, protein expression of HCN2 and HCN4 in both the atria and ventricles was increased. Parallel changes in mRNA were seen for ischemic cardiomyopathy, but no increase in mRNA expression was seen for dilated cardiomyopathy, suggesting a post-transcriptional mechanism.

Levels of HCN1 mRNA was similar in the atria of both normal and failing hearts. HCN-1 mRNA was not found in the ventricles, and HCN1 protein was absent in both the atria and ventricles. (Figure 5).

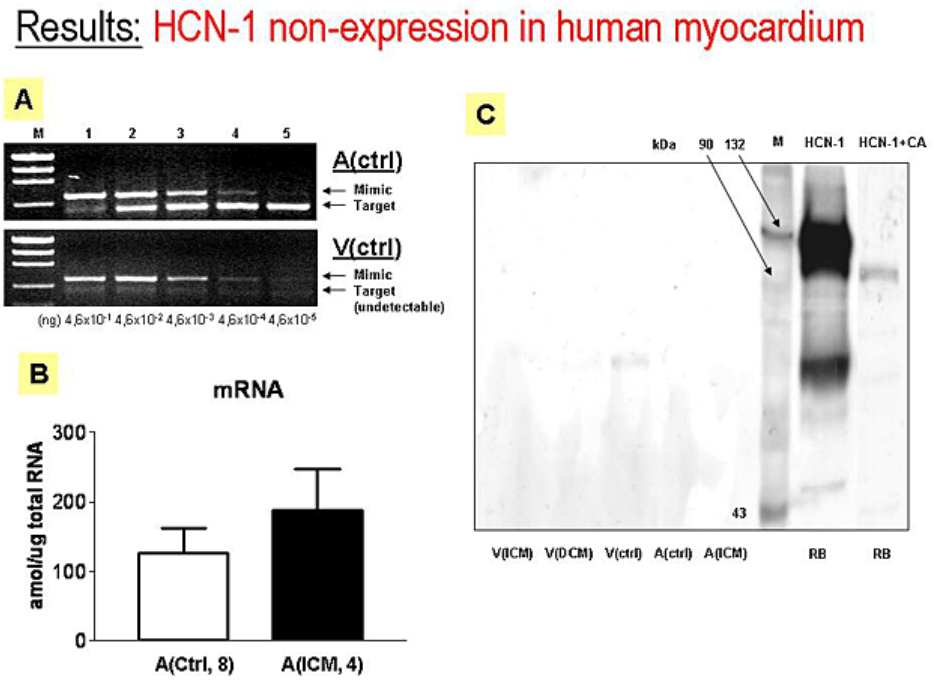

Figure 5

Since MiRP1 has been recently proposed as ß-subunit which can modify If for a given level of HCN expression and is subject to remodeling, MiRP1 protein level was quantified, and found to be unaffected by HF.

Conclusions

HCN4 is the predominant subunit underlying If in the human myocardium. Differences in atrial and ventricular If may be due, in part, to differential expression of HCN2. HCN up-regulation surely contributes to increased If and possibly to ectopic rhythm formation in failing human hearts. This study provides new insights into the functional and molecular determinants of altered impulse formation in the failing human heart.
These results provide a correlation between functional (If current) and molecular (HCN subunits) data in normal and failing human hearts, and elucidate thus an important arrhythmogenic mechanism in HF. Moreover they add important piece of information regarding the occurrence of atrial arrhythmias in HF. Finally, the knowledge of HCN subunits composition and regulation in human normal and failing heart might be helpful to design new therapeutic approaches for HF: 1) gene therapy suppressing over-expressed If; 2) drugs that selectively inhibit this current, that might be a new pharmacological target for HF-related arrhythmias.

References

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