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MOLECULAR BASIS FOR HCN (Hyperpolarization-activated Cyclic Nucleotide gated channels) EXPRESSION IN MYOCARDIAL HYPERTROPHY: A STUDY IN ANIMAL MODELS AND CELL CULTURES

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Background Structural and functional remodeling of cardiomyocytes is a feature of myocardial hypertrophy and failure, consisting of alterations in the expression and function of membrane channels and receptors [1]. These abnormalities may lead to the deterioration of cardiac function and to arrhythmias, that are likely responsible for the majority of cases of sudden death in this setting. Animal models of cardiac hypertrophy as well as cultured rat ventricular cardiomyocytes (VCM) exposed to hypertrophic factors may be helpful for the understanding of events associated to myocardial remodeling. In VCM isolated from both hypertensive rats and patients undergoing cardiac transplantation, similar electrical abnormalities take place [1-5]: in particular, a functional over-expression of the f-current (I_f) has been demonstrated. This current is involved in cardiac pacemaking [6] and its over-expression in the diseased myocardium might contribute to the occurrence of arrhythmias, especially in the presence of an increased adrenergic activity [4,7]. Short-term culture of adult rat VCM, either in the absence or presence of serum, promotes the re-expression of the f-current [8]. Recently, four different genes encoding for the f-channel have been identified and termed as Hyperpolarization-activated Cation Channels (HCN) isoforms (HCN 1–4). Their distribution seems to vary among species and tissues, including the rat and human heart [9]. However no information is available on the influence of cardiac disease on isoform expression, either in humans or rats, and its relationship with the functional expression of the f-current.

Aims (i) To get insight into the molecular determinants of the increased functional expression of I_f in myocardial hypertrophy, by measuring HCN expression in rat ventricular tissue; (ii) to investigate the effect of hypertrophic factors on HCN isoform expression in primary cultures of adult rat VCM.

Methods and Results Ventricular samples were obtained from hearts of old (18 months) spontaneously hypertensive rats (SHR). After their rapid removal, they were weighed and frozen immediately in liquid nitrogen. VCM were isolated from the left ventricle of adult rat hearts by enzymatic digestion and were plated either in control condition and in the presence of endothelin-1 0.1 µM. This procedure allowed a comparison of the effect of agonist within the same preparation. Total mRNA was obtained by guanidinum-isothicyanate extraction method and reverse transcriptase-polymerase chain reaction (RT-PCR) technique was used to identify mRNA of HCN isoforms. We used the classic RT-PCR technique for cultured VCM, the real-time RT-PCR (TaqMan system) for ventricular tissue from SHR. In ventricular tissue from 18-month old SHR, where the presence of a large f-current was consistently documented at a cellular level [3], both HCN2 and HCN4 isoforms are present, HCN2 being expressed in larger amount with respect to HCN4. Similarly, analysis of HCN distribution in VCM at 4 days of culture demonstrated the expression both HCN2 and HCN4; again, HCN2 resulted to be the most abundant transcript. Moreover, VCM cultured in the presence of endothelin-1 during culture showed a marked increase of mRNA expression for HCN2 isoform.

Conclusions Both ventricular samples from old SHR and cultured rat VMC express HCN2 and HCN4 isoforms with a predominance of HCN2 with respect to HCN4. In cultured VMC, exposure to endothelin-1, a well-known hypertrophic factor, increases the expression of HCN2 isoform with respect to control condition. These results are in agreement with those obtained by patch-clamp measurements of functional f-current density in the same model [8]. Future experiments will address the quantification of the differential expression of HCN isoforms both in SHR and cultured VMC by means of the real time-PCR.

References

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