Increased production of Heat Shock Proteins (HSP) is observed in the cells, including cardiomyocytes, to protect themselves against stress stimuli. These proteins, that appear to be involved in repair processes and have a number of cellular functions such as protein assembly and folding and transport across intracellular membranes, are also involved in cardiovascular diseases. Increased circulating levels of Hsp 65 as well as increased titers of antibodies against mycobacterial Hsp 65 have been found to be associated with different cardiovascular diseases. Chronic heart failure (CHF) is a situation of chronic stress, characterized by the presence of factors, such as cytokines, able to increase the HSP. The concentrations of HSP in the failing hearts are increased with respect to nonfailing hearts, both in humans and in animal models. However, data about the presence of HSP or their antibodies in peripheral circulation in this situation are so far lacking. For this, circulating anti-mycobacterial antibodies to heat shock protein 65 in patients with heart failure were assayed. Scalar dilution of a negative sample and of 2 different positive samples were also evaluated in each run for quality control purposes. Antibodies to Hsp 65 were found in the 70% of the patients with CHF (titer ranged from 1:100 to 1:1600) and in the 68% of controls (titer ranged from 1:100 to 1:400). Mean ± SEM antibody titres resulted: 222 ± 32 in controls, 304 ± 43 and 408 ± 55 in CHF patients in I-II and III-IV NYHA class respectively (p=0.035 III-IV class vs. controls by Fisher test after ANOVA). No significant association between antibody titre and degree of myocardial dysfunction, as assessed by LVEF, was found.

The positive correlation of the anti-Hsp 65 titres with clinical severity (NYHA class) suggests a role for HSP in this disease, however the clinical relevance of this determination has to be assessed by the comparison with the distribution of anti-Hsp 65 antibodies in larger age- and sex-matched healthy population ( whose recruitment is now in progress) as well as by the comparison with hemodynamic and biohumoral markers of CHF.

In the heart, Heat Shock Proteins (HSP) constitute an endogenous stress response that protect myocytes from damage. Although HSP are intracellular proteins, these can be found also in peripheral circulation where their increase is associated with early cardiovascular disease. Among the HSP, Hsp 72, the inducible isoform of the 70-kDa family, is considered as the most responsive to a variety of stresses and experimental evidences indicate that the Hsp 72 has an important function in cardioprotection. Heart failure (CHF) represents a condition of chronic sustained stress characterized by the overexpression of factors able to increase HSP, such as cytokines. In humans, the heart expression of the main HSP is increased in CHF with respect to nonfailing hearts. Since no data are available on the circulating levels of Hsp 72 in patients with CHF, we deemed it interesting to assess the serum levels of Hsp 72 in patients with diagnosis of heart failure (CHF) and to investigate its association with the severity of disease. Serum levels of Hsp 72 were measured in 127 patients with CHF due to dilated cardiomyopathy (82 in I-II NYHA class and 45 in III-IV class) by using an ELISA method (Stressgen, Canada) sensitivity: 0.47 ± 0.21 ng/ml, mean ± SD; between-assay variability: 10.8 ± 0.89 ng/ml, CV%: 8.2 and within-assay variability: 10.6 ± 0.41 ng/ml, CV%: 3.9).

Measurable amounts of circulating Hsp 72 were observed in the 90% of the patients studied. The range of the Hsp 72 concentrations was 0.4 - 21.6 ng/ml. A tendency to increase of the serum levels of Hsp 72 as a function of the NYHA class was observed (3.6 ± 0.43 ng/ml in I-II NYHA class vs. 4.3 ± 0.46 in III-IV class, mean ± SEM, p<ns). A weak negative relationship with the values of ejection fraction (LVEF%) was also found.

To evaluate the involvement of this stress protein in the progression of CHF, its association with functional parameters that reflect more accurately the severity of disease than LVEF, as well as the comparison with an age- and sex-matched control group, now in progress, are of pivotal importance. Moreover, the possibility to reliably measuring HSP in the peripheral circulation substantially increases the possibility of studying the role of these proteins in various pathophysiological processes.

Objective: Murine P19 embryonal carcinoma (EC) cells can differentiate into spontaneously beating cardiomyocytes in vitro and have revealed important insight into the early molecular processes of cardiomyocyte differentiation. We assessed the suitability of the P19 cell model for studying cardiac ion channel regulation at the molecular and functional level.

Methods: P19 cells were induced to differentiate towards cardiomyocytes. mRNAs for cardiac markers and ion channels were determined by RT-PCR at 6 timepoints during the differentiation process. Action potentials and individual ion currents were measured by whole cell patch clamp.

Results: Ion channel mRNA expression of several channels is temporally regulated during differentiation, while others show little or no regulation. L-type calcium and transient outward channels are expressed from very early on, while sodium and delayed and inward rectifier channels are upregulated at somewhat later stages during differentiation, which mirrors the in vivo murine cardiomyocyte differentiation during embryogenesis. Spontaneous cardiomyocyte action potentials exhibit a low upstroke velocity, which often can be enhanced by hyperpolarizing the cells, hence activating thusfar dormant ion channels to contribute to the action potential upstroke. Action potential duration decreases considerably during the differentiation of spontaneously beating cells. In late stages, non-beating myocytes can be found which only generate action potentials upon electrical stimulation. Their shape is comparable to neonatal/juvenile ventricular mouse myocytes in culture. Finally, we show that P19-derived cardiomyocytes display a very complete set of functional ion channels.

Conclusion: P19 cells represent a powerful model to study the regulation of myocardial electrophysiological differentiation at the molecular and functional level.

Role of calcineurin in chronic hypoxia-induced hypertrophic response of right ventricle

Chronic exposure to hypoxia leads to pulmonary hypertension and then to a significant right ventricle (RV) hypertrophy. It is well known that this hypertrophic process is associated with an increased proportion of...