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FUNCTIONAL CHANGES OF CARDIAC MYOCYTES DURING DEVELOPMENT: IONIC MECHANISMS, MODULATING FACTORS AND RELEVANCE TO CELL TRANSPLANTATION THERAPY

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A novel therapeutic strategy to regenerate damaged cardiac tissue is to increase the number of functional myocytes within the injured myocardium by implantation of myogenic cells. Fetal and immature cardiomyocytes can be grafted successfully into myocardial infarcts[1-5] and recent evidence has shown that pluripotent stem cells are a source of precursors of cardiomyoblasts able to differentiate into new mature cardiac cells [6,7]. In this process precursors are committed to differentiate into functionally mature cardiomyocytes through several developmental steps. From this point of view, it is of key interest the knowledge of the different functional developmental stages from precursors to mature cells and the identification of the factors responsible for them.
Animal models during growth may be helpful for understanding the functional changes leading an immature cell to differentiate in a mature cardiac myocyte. We and others[8-10] have studied the developmental changes in neonatal rat ventricular cells and have identified some electrophysiological properties, which are remodelled during physiological growth. We focused our studies on action potential characterization[11] and on the associated currents: hyperpolarization activated cation current, I_f,[11] transient outward potassium current (Ito)[11] and L-type calcium current (IcaL). The study provide a helpful background to evaluate changes of myocytes electrophysiological characteristics associated with different stages of differentiation.
Hormonal and environmental factors[10,12,13] are known to affect cardiac electrophysiological remodelling during development. Tissue oxygen availability is a critical factor for highly oxygen-demanding organ like heart. Moreover, mild/strong hypoxic state characterizes injured myocardium where myogenic cells are implanted. We have used a rat model of exposure to low level of carbon monoxide during fetal life, to produce a mild hypoxic state in the fetus. After the birth to 2 months of age, cardiac cellular electrophysiological properties of the offspring were studied. Results[11] demonstrated a delay in the maturation of some of these properties, which produce a lag of the changes of action potential profile and underlying currents during development. The finding suggests that mild oxygen deprivation alters electrophysiological maturation of neonatal cardiomyocytes and might have similar consequences on functional maturation of implanted myogenic cells.
Ex-vivo models allow to investigate developmental remodelling as a result of the complex interplay of physiological factors. In this context, *in vitro* models of cultured cells might better help to focus on the specific action of modulators. In cellular cardiology an ideal model should consist of a stable cardiac cell line, retaining a constant cardiac phenotype while proliferating in culture. HL-1 cells[14] is a cell line that shows most of these characteristics. It is derived from the atria of a transgenic mouse expressing the SV40 big T antigen under the control of the atrial natriuretic factor promoter. The oncoprotein is responsible for the unique feature of these cells to proliferate indefinitely *in vitro*[15], in contrast to other cardiac cell lines[14]. Under suitable culture conditions, HL-1 cells show spontaneous contractile activity and express many of the cardiac-specific genes[14]. In the last two years we have been working on the characterization of the basal electrophysiological properties of HL-1 cells (action potential and underlying ionic currents[16,17]), in order to study selectively the effect of modulators. Recent experimental evidence led to an increasing appreciation of the role of locally released growing and hypertrophying factors on myocytic life cycle (growth, maturation, hypertrophy, and death)[18]. Endothelin-1 is a well-known hypertrophic factor which also seems to exert a critical role in the fetal and neonatal stages of cardiac development[19]. Moreover, synthesis and release of endothelin-1 is increased in the diseased heart[20]; such an environment likely affects the outcome of both pre-existing myocytes and engrafted cells. We performed a chronic exposure of HL-1 cells to endothelin-1 and then analysed the effect on their functional properties. Preliminary results demonstrated that endothelin-1 causes a prolongation of action potential profile, which is a typical modification towards a less differentiated state[21].
In conclusion, these results allowed a deeper understanding of mechanisms and factors involved in remodelling during cardiac and cellular development and growth. Moreover, new experimental models have been tuned up to address these topics, and may be used in ongoing research to compare cell functional commitment during *in vitro* culturing and after *in vivo* implantation.

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