Progress in Biophysics and Molecular Biology xxx (2014) 1–8

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

journal homepage: www.elsevier.com/locate/pbiomolbio

Studying dynamic events in the developing myocardium

Frantisek Vostarek [a, b], Barbora Sankova [a, c], David Sedmera [a, c, *]

[a] Institute of Physiology, Academy of Sciences of the Czech Republic, Czech Republic
[b] Faculty of Science, Charles University, Prague, Czech Republic
[c] Institute of Anatomy, First Medical Faculty, Charles University, Prague, Czech Republic

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Optical mapping
Calcium imaging
High-speed cameras
Embryo
Heart
Cardiac conduction system

ABSTRACT

Differentiation and conduction properties of the cardiomyocytes are critically dependent on physical conditioning both *in vitro* and *in vivo*. Historically, various techniques were introduced to study dynamic events such as electrical currents and changes in ionic concentrations in live cells, multicellular preparations, or entire hearts. Here we review this technological progress demonstrating how each improvement in spatial or temporal resolution provided answers to old and provoked new questions. We further demonstrate how high-speed optical mapping of voltage and calcium can uncover pacemaking potential within the outflow tract myocardium, providing a developmental explanation of ectopic beats originating from this region in the clinical settings.

1. Biomechanics at cell and organ level

1.1. From whole hearts down to cellular level

To study dynamic electrical and mechanical events occurring in the heart, simplification of the complex, three-dimensional *in vivo* system is often advantageous. This can go down to the single cell level, since isolated cardiomyocytes can spontaneously beat *in vitro* and cell culture setup is useful for studying subcellular and molecular events or drug screening purposes. If myocytes are grown *in vitro* for a prolonged period of time, they form a sheet – a functional syncytium connected by gap junctions, simplifying thus the three-dimensional geometry of myocardium into a single plane.

Historically, cells were cultivated just on plain plastic, or surfaces coated with various extracellular matrix molecules (collagen – isotropic or aligned, fibronectin) to promote attachment and influence cellular properties (Atance et al., 2004). A strong stimulus for muscle growth is work load, or stretch; so subjecting cell cultures grown on elastic membrane to mechanical loading using a pump-powered cell stretcher (Kofidis et al., 2004; Miller et al., 2000) is a way to model differentiation normally occurring during development. The next step in bringing the cell culture more closely to *in vivo* situation is growing the cells on more sophisticated 3D scaffolding (Atance et al., 2004; Evans et al., 2003) to form tissue constructs of various complexity (Tobita et al., 2006). Each of these new technological advancements, enabled by parallel development of chemistry (new polymers), mechanical engineering (stretching apparatuses) and biology (cell isolation protocols, differentiation of cardiomyocytes from stem cells) allowed answering new sets of questions. Similar to the situation *in vivo*, cell culture conditions markedly influence the functional parameters of cells; here we will focus on their conduction properties, and extend it back to the whole organ level. This area has so far received comparatively little interest in tissue engineering aimed at construction of implantable artificial myocardium (Yildirim et al., 2007), but in addition to perfusion and mechanical properties of tissue-engineered constructs, it is of vital importance for integration with the host myocardium and electrical stability.

1.2. Tissue geometry and conduction properties

Mechanical loading has profound effects on growth, behavior, differentiation and conduction properties in isolated myocytes (cell cultures). Similarly, for *in vivo* studies, people tend to view genes at the root of everything, and sometimes forget that muscles in particular are critically dependent on mechanical stimuli, and organisms in general on epigenetic influences (Pesevski and Sedmera, 2013). What is less clear is the importance of tissue geometry, also referred to as myocardial architecture, which can be elegantly simplified *in vitro*. Patterned cardiomyocyte cultures, enabled by combination of printed circuit technology and cell culture by the Rohr lab in Bern, Switzerland (Kucera et al., 1998; Rohr et al., 1999) are just an example how availability of new technology helped to

* Corresponding author. Academy of Sciences of the Czech Republic, Institute of Physiology, Videnska 1083, 14220, Prague 4, Czech Republic.
E-mail address: dsedmera@biomed.cas.cz (D. Sedmera).

http://dx.doi.org/10.1016/j.pbiomolbio.2014.06.002
0079-6107/

document the importance of tissue geometry for cardiac electrical conduction. Strands of myocytes, mimicking bundles of conduction fibers, carry electrical impulses at much higher speed than a large expansion of planar myocardial sheet, which acts as a sink. In such system, effects of various pharmacological agents or gap junctional uncoupling can be studied with ease. It was long believed that the role of cardiac fibroblasts is mostly structural support of the heart, and electrically they function as a mere isolator. Importance of fibroblast–myocyte interactions was revealed in well-defined co-culture experiments (Rohr, 2012) and showed that gap junctional, and possibly also electrical, communication exists between these two cell types.

1.3. Importance of physical conditioning for myocardial growth and differentiation

Effect of altered mechanical loading – an epigenetic stimulus – on developing heart and its conduction system could be studied also at the organ level, *in vivo* as well as *in vitro*. Elegant studies by Thompson and associates (Thompson et al., 2003) showed that at the early developmental stages, the fate of cardiomyocytes in the tubular heart (proliferation vs. differentiation) is plastic, and could be reversed by simply inverting the slice of the cardiac tube inside out. This gradient persists also in the trabeculated heart, and could be modeled mathematically (Damon et al., 2009). Cardiovascular development from biomechanical perspective was reviewed by Larry Taber (Taber, 2001). Growth and remodeling are two important processes occurring during both development and adaptations of the cardiovascular system to changing functional requirements. Most cardiac growth during prenatal development is based on hyperplasia (Clark et al., 1989; Sedmera and Thompson, 2011). At the tissue level, an important biomechanical parameter is the residual strain, changes in which are a sensitive indicator of active remodeling (Taber and Chabert, 2002). This regional growth can be easily measured as an opening angle obtained by cutting open a circular section of the vessel or heart. A decrease in opening angle following creation of pressure overload by conotruncal banding correlated with induced growth 12 h after the procedure. These events can be also modeled mathematically. Schroder and colleagues (Schroder et al., 2002) found that the material properties of the developing heart are regulated by mechanical loading and that microtubules play an important role in this adaptive response during cardiac morphogenesis. Specifically, there was an increased amount of both total and polymerized beta tubulin the hypoplastic left ventricle. This smaller ventricle was also stiffer (analyzed by increased hysteresis loop); both parameters were normalized by the treatment with colchicine, which induced microtubule depolymerization.

During the transition from the trabeculated to compact myocardium, spiraling of myofibers within the left ventricular compact layer is the major factor of fetal myocardial differentiation (Jouk et al., 1995; Sedmera et al., 2000). Tobita and associates analyzed the angle of myocyte inclination during normal and abnormal hemodynamic loading (Tobita et al., 2005); they found that increased pressure loading accelerated this normal morphogenetic process, while there was a delay in the settings of hemodynamically-induced left ventricular hypoplasia. Therefore, hemodynamically induced changes in myocardial architecture in these models (Sedmera et al., 1999) that are based on changes in cell proliferation (deAlmeida et al., 2007; Sedmera et al., 2002a) could be the morphological substrate of altered electrical pathways. These were investigated as well using optical mapping on isolated hearts (Hall et al., 2004; Reckova et al., 2003). We found that increased pressure loading accelerated maturation of ventricular conduction system, while there was a dysfunction of the (morphologically normal) left bundle branch in left ventricular hypoplasia. At the molecular level, these changes were paralleled by up/down regulation of conduction system differentiation marker connexin40.

The hemodynamic unloading of the developing heart could be easily taken to extreme by culturing the spontaneously beating, but not pumping heart *in vitro*. In such settings, we noticed not only an arrest of normal differentiation of the ventricular conduction system, but actually a regression towards even more immature conduction patterns (Sankova et al., 2010). To test whether these profound changes were not simply an artefact of organ culture, we performed re-loading of the ventricle by a droplet of viscous silicone oil, which stretches the ventricle and was shown previously to considerably increase myocardial oxygen and glucose consumption (Romano et al., 2001). Remarkably, this led to a complete rescue of conduction phenotype to *in vivo* values, showing that simple myocyte stretch, rather than hemodynamic shear stress transmitted through the endothelium, is the governing factor in early conduction system differentiation. It agreed well with our older data testing the importance of hemodynamically induced signaling via endothelin receptors, which was found to be important during the later (bundle branches differentiation), but not the early stages of conduction system formation in the chick embryonic heart *in vivo* (Sedmera et al., 2008).

2. Functional imaging of the developing heart

2.1. History of electrophysiological recordings in impulse propagation studies

These functional studies, including those performed on cell cultures, would not be possible without adequate technology for recording of impulse propagation in the heart. The golden standard for action potential recordings are microelectrodes, including those arranged in arrays (sock, brush, or balloon electrodes). They work very well on large adult hearts (sheep, pig, human), but are of limited use in embryos (spatial issues, fragility). Nevertheless, a few carefully positioned electrodes poked into the isolated chick embryonic heart allowed determination of general direction of impulse propagation and enabled postulation of ventricular trabeculae as nascent network of the ventricular conduction system (Arguello et al., 1986; de Jong et al., 1992). Using just two electrodes, Chuck and colleagues (Chuck et al., 1997) discovered the transition in ventricular activation of the chick embryonic heart from primitive base-to-apex direction to mature apex-to-base pattern, and correlated this event with ventricular septation. These results were later confirmed using high-resolution optical mapping studies, discussed in more detail below. For certain question, and in particular in larger, late gestation avian hearts, microelectrodes are still useful, as was proved recently by the Leiden group (Kolditz et al., 2008, 2007). Two exploration electrodes, together with simultaneous recording of volume-conducted ECG, were enough to demonstrate the accessory atrio-ventricular pathways occurring normally during fetal avian development, and their increased frequency in a model of epicardial ablation that results in deficient formation of fibrous atrioventricular insulation.

2.2. Optical methods for visualization of electrical impulse spreading

However, alternative optical methods, recently reviewed (Boukens and Efimov, 2014), exist for studying spread of electrical activation in excitable tissue by means of supravital staining with voltage-sensitive dyes (Kamino et al., 1981). This approach depends on several technological platforms. First, of course, is the availability of

Please cite this article in press as: Vostarek, F., et al., Studying dynamic events in the developing myocardium, Progress in Biophysics and Molecular Biology (2014), http://dx.doi.org/10.1016/j.pbiomolbio.2014.06.002