Journal of Molecular and Cellular Cardiology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Review article

Influence of mechanical stress on fibroblast–myocyte interactions in mammalian heart

Denis V. Abramochkin [a,b,*], Ilya T. Lozinsky [a], Andre Kamkin [a]

[a] Department of Fundamental and Applied Physiology, Russian National Research Medical University, Ostrovityanova str., 1, Moscow, Russia
[b] Department of Human and Animal Physiology, Moscow State University, Leninskiye Gory, 1, 12, Moscow, Russia

ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form 18 December 2013
Accepted 24 December 2013
Available online xxxx

Keywords:
Fibroblast
Heart
Stretch
Ionic current
Mechanosensitive channels

ABSTRACT

Cardiac fibroblasts are an essential component of cardiac tissue. These cells not only produce the extracellular matrix, but also are electrically and mechanically coupled with cardiomyocytes. In this way, fibroblasts can influence the electrical activity of cardiomyocytes. Cardiac fibroblasts cannot generate action potentials, but their membrane potential is controlled by mechanical stretch or compression of the surrounding myocardium which in turn affects their interaction with myocytes and the way myocytes respond to mechanical stress. This review discusses the electrical properties of cardiac fibroblasts, the present evidence of fibroblast–myocyte coupling and the way in which these cells respond to mechanical stress. This article is part of a Special Issue entitled "Cardiac Fibroblast Review SI".

Contents

1. Introduction

Cellular cardiology research during the past century has focused on the functioning of the cardiomyocytes that make up the working portion of the myocardium. Nevertheless, in addition to cardiomyocytes, the heart also contains autonomic neurons, endothelial cells, vascular smooth muscle cells and fibroblasts. Fibroblasts occupy about 10% of cardiac tissue volume [1] but due to their small size, they account for 45–70% of total cell number depending on the species of animal [2]. In several key structures of the heart they prevail over the cardiomyocytes. Production of the extracellular matrix (ECM[1]) – comprised mainly by fibrillar collagen (types I and III) and fibronectin – represents the most obvious function of cardiac fibroblasts [3]. Fibroblasts also comprise up to 75% of tissue volume in the mammalian sinoatrial node [4,5].

* Corresponding author at: Biological Faculty of the Moscow State University, Department of Human and Animal Physiology, Leninskije gory, 1, 12, Moscow, Russia. Tel.: +7 916 603 05 02.
E-mail address: abram340@mail.ru (D.V. Abramochkin).

[1] ECM — extracellular matrix, TRPC — transient receptors potential canonical channels, MIP — mechanically induced potential, AP — action potential, MGC — mechanically gated current, MSC — mechanosensitive channel.

0022-2828/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.yjmcc.2013.12.020

However, in addition to a structural role, fibroblasts interact with and modulate the function of cardiomyocytes — particularly the way in which they respond to mechanical stress. Fibroblast–cardiomyocyte crosstalk involves secretion of various paracrine signaling compounds, such as cytokines, chemokines and growth factors, by both cell types [for review see [3,6]]. Crosstalk also occurs via the components of ECM [3]. Direct fibroblast–cardiomyocyte interconnections via gap junctions and adherent junctions [7] allow modulation of electrical properties. Each of these interactions can be modulated by mechanical stress. Cardiac tissue is subject to both cyclic mechanical stress, produced by rhythmic contractions of myocardium, and static mechanical stress. These stressors alter fibroblast–myocyte interactions in addition to modulating the individual properties of these cell types. The present review is focused on direct electrical and mechanical coupling of fibroblasts and cardiomyocytes and its modulation by mechanical stretch.

Pathologic conditions – such as myocardial injury or continuous excessive mechanical stress associated with dilation of heart chambers – can induce the conversion of fibroblasts into myofibroblasts, which can produce smooth muscle-like contractions and excrete a larger quantity of ECM than normal fibroblasts [8]. This process, which is crucial for myocardium remodeling, strikingly affects the properties of fibroblasts and the features of fibroblast–myocyte interactions. The present article however, will focus mainly on the non-pathophysiological situation. For readers interested in myofibroblasts and their role in remodeling we direct you here [9].

2. Electrophysiological phenotype of cardiac fibroblasts

In normal cardiac tissue fibroblasts usually form strands and bundles, which follow the prevailing direction of cardiomyocytes, and also fill the space between the layers or clusters of muscular fibers. Therefore, in mammalian heart almost every myocyte lies in close proximity to one or more of fibroblasts [10]. Heart regions with large fraction of connective tissue can act as an obstacle for uniform spread of electrical excitation [11]. For example, in many species the sinoatrial node is separated from the interatrial septum by a wide sheet of connective tissue, while the thinner sheet delineates the sinoatrial tissue from the crista terminalis. Therefore, excitation goes from the center of sinoatrial node in frontal and caudal, but not lateral directions [12,13]. Moreover, interstitial fibrosis and collagen accumulation are believed to be an important source of local anisotropy in myocardial ischemia and hypertrophy, provoking the initiation of cardiac arrhythmias [14,15]. However, the connective tissue, which consists mainly of ECM, and the fibroblasts per se are functionally different.

Cardiac fibroblasts are electrically inexcitable cells [16], but express various ion channels including voltage-gated channels. A number of ionic currents have been registered using patch-clamp technique from cultured or freshly isolated fibroblasts. Voltage-gated K^+ channels are abundant in cardiac fibroblasts, since the delayed rectifier currents were found in cells from adult [17] and newborn [18] rat hearts as well as in cultured human fibroblasts [19] and transient potassium current (I_{to}) was confirmed in the two latter types of cells [18,19]. Other types of potassium currents: inward rectifier and Ca^{2+}-activated currents are both present in cultured human cardiac fibroblasts [19], though the former current was also detected in 70% of studied fibroblasts from adult rat hearts [20].

As cardiac fibroblasts are not excitable, it is surprising that inward currents are also present in cultured fibroblasts from human heart. Two distinct sodium currents have recently been described in these cells [19] though their functional role remains unknown. While the first current can be abolished by nanomolar concentrations of tetrodotoxin, the second one is tetrodotoxin-resistant. The threshold potentials were −40 and −50 mV, respectively. It is also unclear if these sodium currents are present in freshly isolated cells. Finally, the presence of non-selective cation currents, conducted via transient receptor potential canonical (TRPC) and vanilloid channels was demonstrated in freshly isolated [21] and cultured [22] rat cardiac fibroblasts. At present, these channels represent the only confirmed way for extracellular Ca^{2+} to enter the cardiac fibroblasts and therefore might play an important, currently unknown, physiological role.

Despite the considerable diversity of ionic channels expressed in cardiac fibroblasts, we are far from understanding how membrane potential and its oscillations are controlled. According to early data obtained using cultured fibroblast lines, the resting membrane potential in these cells oscillates, varying from −10 to −40 mV [23,24]. The membrane potential of isolated fibroblast is quite stable; no periodic oscillations are detected. For example fibroblasts isolated from rat atrial tissue exhibit a stable resting potential with mean value of −37 ± 3 mV [25]. It should be noted that this value obtained by patch-clamp experiments with cardiac fibroblasts may be more positive than the real membrane potential. It was demonstrated in cultured non-cardiac embryonic murine fibroblasts that the resting potential measured by patch-clamp technique (whole-cell mode) is usually more positive than the real membrane potential by 30–40 mV due to the decrease of the seal resistance after rupturing the cell membrane [26]. However, in cardiac fibroblasts such extensive analysis of possible patch-clamp artifacts has never been done.

However, in situ sharp microelectrode recordings obtained from fibroblasts in rat atrial tissue indicate that the resting potential varies in a wide range between −5 and −70 mV [27–29]. The mean resting potential registered with sharp microelectrodes was about −22 mV, although it could be underestimated due to the lower glass-membrane seal resistance of sharp microelectrodes in comparison with patch pipettes. This disadvantage of sharp microelectrode technique can be particularly limiting in the studying of small cells, like fibroblasts. However, sharp microelectrodes also represent the only method currently employed to record membrane potential in intact, non-isolated cardiac fibroblasts in situ. Moreover, rhythmic depolarizations coinciding with spontaneous contractions of right atrial preparations can be observed in the majority of fibroblast cells using sharp electrodes [27,30]. These oscillations of fibroblast membrane potential were called mechanically induced potentials (MIPs). Amplitude of MIPs depends on the resting membrane potential of the fibroblast; it increases after membrane hyperpolarization (Fig. 1). Also, MIPs differ from myocardial action potentials (APs) as they lack a fast upstroke velocity and commence approximately 10 ms after the initial rise of the action potential. Therefore, in their normal tissue environment cardiac fibroblasts demonstrate electrophysiological properties that are different from completely quiescent cells enzymatically isolated from intact myocardium. It is unlikely that these differences can be completely attributed to artifacts of the sharp microelectrode technique. Thus, it is acceptable to conclude that the electrophysiological properties of fibroblasts strongly depend on their surroundings in situ, particularly on the functionality of cardiomyocytes. Perhaps optical techniques which are now common place for recording in situ membrane potential in excitable cells [31] may be applied to understanding fibroblast membrane potential and its control by cardiomyocytes in the future.

3. Electrical and mechanical coupling between cardiac fibroblasts and myocytes

It is well known that all cardiomyocytes are connected in a functional syncitium via gap junctions providing the basis for impulse propagation across the myocardium [for review see [32]]. More than 40 years ago Goshima demonstrated the possibility of functional gap junction formation between a cardiomyocyte and inexcitable cell [33]. Later, Rook and colleagues first characterized the gap junctions between cultured cardiomyocytes and cardiac fibroblasts [34]. It is now generally accepted that cardiomyocytes and fibroblasts form functional gap junctions in vitro [for review see [35]]. For example, it was shown using optical recording of membrane potential that strands of fibroblast as thick as 300 μm can successfully maintain the propagation of excitation between

Please cite this article as: Abramochkin DV, et al, Influence of mechanical stress on fibroblast–myocyte interactions in mammalian heart, J Mol Cell Cardiol (2014), http://dx.doi.org/10.1016/j.yjmcc.2013.12.020