Review

Mechanosensitive connective tissue: potential influence on heart rhythm

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1. Introduction

Connective tissue is an essential component of cardiac histo-architecture. In particular the sinus node region of the healthy heart is rich in fibrous connective tissue [1]. The distinctive division of sino-atrial node cells in multiple strands of different size reported in the original first description of the node by Keith and Flack [2] appears to be brought about by cardiac fibroblasts forming sheet-like extensions that enwrap groups of pacemaker cells [3]. According to different quantitative studies, connective tissue occupies between 45% [4] and 73% [5] of the volume of the sinus node in man.

A number of pathological states is associated with excessive growth of fibrous tissue in other parts of the heart. These pathologies include focal scar development in regions of myocardial ischaemia and infarction, or more scattered fibrosis induced by inflammation during rheumatic heart disease and other processes. The predominant contribution of fibroblasts to post-traumatic atrial or ventricular restoration is based on their sustained proliferative potential in the adult organism, while myocyte proliferation is confined mainly to fetal periods of development [6,7].

Cardiac fibroblasts are known to be mechanosensitive. They respond to mechanical stimulation by changes in gene expression and collagen synthesis in vivo [8,9] and in vitro [10]. This generative response to changes in the mechanical environment is assumed to find clinical expression in fibrosis induced by pressure overload [11] and tissue dilatation [12].

Recently, cardiac fibroblasts have been implicated as a possible alternative substrate for some forms of cardiac mechano-electric feedback [13] observed in the sino-atrial node region and in cardiac scar tissue [14]. This short review will try to summarize the evidence available, examine potential functional consequences, and indicate directions for further investigation into the contribution of cardiac mechano-sensitive connective tissue to modulation or disturbance of heart rate. It is not aimed as a synopsis of an extensive and well established field of research but as a brief introduction into a possibly underestimated and potentially important mechanism of cardiac mechano-electric feedback.

2. Properties of cardiac fibroblasts

Cardiac fibroblasts share multiple functional properties. They play an essential role in the spatial organization of cardiac tissue during embryonic development, endorse synthesis and regulation of extracellular connective tissue components, contribute to wound healing and scar formation, and are involved in the pathogenesis of fibrotic processes. Like cells from the cardiac conduction system [15], cardiac connective tissue cells in the mammalian heart have been suggested to derive partly during ontogenesis from the cardiac neural crest [16,17].

In contrast to cells from the conduction system, fibroblasts are electrically non excitable. Their resting membrane contains background conductances for potassium, sodium, and chloride with a permeability ratio of 1.0:0.67:8.1 respectively [18]. No fast sodium channel has yet been detected in these cells. Fibroblasts are generally rather depolarized with resting membrane potentials between $-15$ and $-25$ mV and membrane resistances in the range of $10^{7}$ to $10^{9}$ $\Omega$. Morphologically, these cells are usually characterized by a clearly visible elliptically shaped nucleus, a well-developed granular endoplasmic reticulum and Golgi apparatus, and the lack of a basal membrane.

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The degree of expression of individual morphologic and biologic properties of fibroblasts has been reported to display significant variability under heterogeneous pathophysiological conditions like wound healing and fibrosis [19]. An influence of the mechanical environment in particular on fibroblast function has first been suggested almost thirty years ago in the context of hypertension-induced ventricular hypertrophy and fibrosis [6,20]. Moreover, normal cardiac contraction itself has been suggested to influence levels of protein synthesis in fibrous tissue [21]. This phenomenon could be caused by local release of fibroblast growth factors from cardiomyocytes via contraction-induced micro lesions [22]. The time course of these processes, however, does not predestine them to play a major role in the incessant adaptation of cardiac performance to mechanical changes.

Evidence gained in vitro suggests that fibroblast-like cells can be mechanically activated by a potentially less convoluted pathway which is independent from paracrine activation through neighboring cardiomyocytes. In myocyte-free cell lines of mouse-fibroblast origin (L-cells) direct mechanical stimulation has been shown to induce release of calcium from internal stores [23]. This increase in intracellular free calcium concentration could potentially modulate a calcium-dependent potassium conductance described for cultured fibroblasts [24] and contribute to oscillations in membrane potential observed in experiments with direct mechanical stimulation of L-cells in vitro [25]. It is worth mentioning though that the calcium-dependent potassium conductance itself is not directly stretch-activated [26].

A more direct pathway for mechanical stimulation would be based on opening of mechanosensitive ion channels in the sarcolemma of fibroblasts. Such an ion channel has been described for cultured human fibroblasts [27]. This channel is stretch-activated, permeable to sodium and potassium ions, has a single channel conductance of ~60 pS in 140 mM potassium, and a reversal potential of about 0 mV (see Fig. 1).

Physiological correlates for stretch-activation of a cation non-selective channel have been discovered on the cellular level in situ. In 1988 ‘electrophysiologically atypical’ cells were first described in spontaneously beating preparations of isolated frog atrium [28]. These cells had a low resting membrane potential of around -25 mV and displayed membrane depolarizations with a reversal potential of about 0 mV. Depolarizations occurred in these cells in the rhythm of contraction of the surrounding tissue (see also Fig. 2c). Using artificial stretch, it was demonstrated that the membrane depolarizations observed were caused mechanically [29]. In subsequent studies similar mechanosensitive cells were discovered in the hearts of rat and guinea pig and histologically identified as cardiac fibroblasts [30].

A likely molecular substrate for the stretch-induced depolarization observed in fibroblasts in situ is the cation non-selective stretch-activated channel described above.

This view is in line with results obtained in experiments where gadolinium, a blocker of stretch-activated cation channels [31], was applied to the perfusate of isolated heart preparations. Addition of 10 μM gadolinium resulted in reversible termination of depolarizations in mechanosensitive fibroblasts induced by spontaneous cardiac contraction or artificial stretch of the tissue [32]. Gadolinium is not a specific blocker of stretch-activated cation channels. Its blocking action on the L-type calcium [33] and delayed rectifier [34] channels is, however, unlikely to contribute to...
the observed termination of stretch-induced depolarizations in mechanosensitive fibroblasts. Both L-type calcium and delayed rectifier channels have not been identified in fibroblasts; even if present they would hardly determine the cellular response at the membrane potential levels typical for these cells. Thus, cardiac fibroblasts appear to be able to respond to stretch with changes in their electrophysiological characteristics via opening of stretch-activated non-selective cation channels.

The mere existence of mechanosensitive fibroblasts in the mammalian heart does not necessarily indicate their participation in cardiac mechano-electric feedback. A prerequisite for such a role would obviously be the presence of forms of information exchange between fibroblasts and cardiac myocytes.

3. Electrical interaction of cardiac fibroblast and cardiomyocytes

Although connective tissue cells constitute one of the two largest general populations of cells in the mammalian heart [7] they are not usually considered to be of importance for the electrophysiological behaviour of myocytes under normal circumstances. Only as a part of scar tissue is the impact of connective tissue on the spread of excitation generally appreciated. There, fibroblasts are considered to act as electric insulators obstructing the regular spread of excitation.

Early indications that the situation might be more complex came from studies performed in tissue culture [35] where fibroblasts and cardiac muscle cells readily form nexus connections. In neonatal rat heart cultures at room temperature the single channel conductivity in such a heterologous nexus is 29 pS; the total conductivity of one fibroblast-myocyte nexus is usually in the region of several nS [36]. This conductivity is sufficient to synchronize electrically two distant cardiomyocytes interconnected by just a fibroblast [35]. Thus, at least in cell culture, fibroblasts are capable of passively transmitting impulses between pairs of cardiac myocytes, acting as a conductor rather than an insulator.

The presence of heterologous nexi in cell culture demonstrates the possibility of bio-electrical interaction between fibroblasts and cardiomyocytes. The picture in situ, however, is more complicated. In a painstaking electron-microscopical study into fibroblast-cardiomyocyte junctions of the rabbit cardiac pacemaker region, only one heterologous nexus was detected while non-nexus-like contacts dominated the picture [3]. These non-nexus-like contacts consist of regions where the fibroblast's cell membrane anchors directly into the basal membrane of adjacent cardiomyocytes without forming a histologically detectable nexus; a connection not previously discovered in cell culture. It has been suggested that these membrane contacts might promote capacitive interaction between the heterologous cells [3], which, independently, had been demonstrated in experimental studies (Fig. 2a) [37]. Whether these membrane contacts additionally accommodate modest numbers of dispersed gap-junctional channels, not detectable by the standard electron-microscopic approach, remains to be seen. Similar connections have already been found to cause electrical coupling of smooth muscle cells in coronary arteries where, likewise, nexus contacts could not be detected [38].

Electrophysiological evidence in favour of modest electrotonic coupling between cardiomyocytes and cardiac fibroblasts has been gained in intact cardiac tissue. So far, three forms of bio-electric interaction of mechanosensitive fibroblasts with cardiomyocytes have been demonstrated in situ. Apart from capacitive interaction, fibroblasts may infrequently be coupled to cardiomyocytes by nexus connections or, more often, by very small conductances, possibly constituted by a few heterologous gap-junctional channels interconnecting the cells (Fig. 7b) [14].

Given an electrotonic interaction between fibroblasts and cardiomyocytes, it is plausible that the mechanosensitivity of cardiac fibroblasts may influence adjacent cardiac cells. Since the spontaneous membrane potential of resting fibroblasts is less electro-negative than the potential recorded inside cardiac muscle cells, cardiac fibroblasts will generally have a moderate depolarizing influence on cardiomyocytes. Further stretch-induced depolarization of fibroblasts could be capable of depolarizing cardiomyocytes to an extent that would alter their electrophysiological behavior.

Unfortunately, detailed experimental investigation into this potentially crucial intercellular interaction is extremely difficult. Cardiac myocytes are usually electrically very well coupled to each other. This makes it difficult, if not impossible, to control electrophysiologically the behaviour of individual cells in situ. Such control is, however, required in order to obtain results which can be interpreted with confidence. Isolated cells do not provide a useful substitute model since they are subjected to isolation trauma and lack intercellular connections. Standard tissue co-cultures display a deceptive coupling pattern of the two cell types and, consequently, do not provide satisfactory models for experimental investigation of fibroblast-myocyte interaction either.

An alternative but highly useful approach to this problem is therefore the mathematical modelling of the interaction of these cells. Such interaction would be expected to be facilitated in regions with an exceptionally high content of fibroblasts, like the sino-atrial pacemaker and cardiac scar tissue. Both the sino-atrial node and scar tissue have been suggested to be mechanosensitive. It is, therefore, worthwhile to investigate more closely the potential interaction of mechanosensitive fibroblasts and cardiomyocytes in these regions to gain an insight into the possible participation of fibroblasts in the electro-physiological response of the heart to stretch.
4. A role for fibroblasts in sinus rhythm modulation?

The fact that sinus rhythm is sensitive to the mechanical environment has been known for some time. In 1915 Francis Bainbridge [39] found that injection of fluids into the jugular vein of anaesthetized dogs leads to a temporary increase in heart rate. He concluded that an increase in venous filling of the heart produces a reflex acceleration of the heart rate, “caused by impulses arising within the heart”. This was regarded as a potentially important reflex mechanism adjusting heart rate to changes in venous return. Later it was demonstrated that stretch-induced increase in rate may likewise be observed in the isolated heart [40], isolated sino-atrial node preparations [41], and the denervated heart in situ [42]. This evidence proves that the positive chronotropic response of the heart to stretch is at least partly caused and accomplished by an entirely intra-cardiac mechanism. Thus, stretch of the cardiac pacemaker region must be directly capable of influencing the rate of cardiac excitation.

As indicated above, the sino-atrial node is rich in cardiac fibroblasts. Their possible contribution to the positive chronotropic response of the heart to stretch has been studied in the 'Oxsoft Heart v.4.4' mathematical model of single cardiac cells [43]. This program is a biophysically detailed description of ion movements through channels and transport mechanisms and is based on actual measurements [44]. The sinus node pacemaker model [45,46] and the ventricular cell model [47] have been described in detail elsewhere.

Connective tissue cell parameters were modeled on the basis of experimental findings reported for fibroblasts in vitro [36] and in situ [14] and integrated into the Heart software. In short, fibroblasts were simulated as electrically passive cells described by a capacitance (C = 600 pF) and a resistance (R = 0.1 to 1.0 GΩ). The resting membrane conductance consisted of two modules: a background conductance (G₀ = 1 nS, E rev = -20 mV) and a stretch-dependent component (Gₛ = 1 nS, E rev = 0 mV). To simulate changes in resting tension that occur during normal cardiac activity (e.g. variations in diastolic filling), diastolic Gₛ was varied. Stretch of the tissue was simulated by temporarily increasing Gₛ.

In the simulations a mechanosensitive fibroblast connected to one sino-atrial node pacemaker cell by a time-independent coupling conductance Gₛ (simulating 10 to 30 single gap-junctional channels) was stretched (for equations please refer to [14]). This led to membrane depolarization in the fibroblast, subsequent depolarization of the adjacent pacemaker cell and an increase in the spontaneous depolarization rate (Fig. 3). These simulations showed that stretch applied to a mechanosensitive fibroblast would be sufficient to promote an increase in the depolarization rate of an adjacent sino-atrial node pacemaker cell on a beat-to-beat basis by up to 22%.

Quantitatively, the changes found in mathematical simulations are close to those reported in experimental studies where, like in the simulations, apart from an increase in heart rate a reduction in the maximum diastolic and systolic pacemaker potentials were observed during stretch [41]. This does not, of course, imply an exclusive role for connective tissue in the stretch-dependent frequency modulation. Isolated sino-atrial node pacemaker cells themselves respond to mechanical stimulation (intracellular volume increase) by activation of a chloride conductance [48] and it has been suggested that their L-type calcium channels are stretch modulated [49]. Both blocking the stretch-activated anion current and suppression of calcium handling may reduce, but not abolish the positive chronotropic response to stretch of sino-atrial tissue strips [50]. Further investigation is required to verify the relative contribution of the different mechanisms involved in this reaction.

5. Potential arrhythmogenicity of fibroblasts in cardiac scar tissue

Cardiac scar tissue is considered to be a substrate for formation of reentrant cardiac arrhythmias. Essential requirements for the formation of reentrant arrhythmias are the occurrence of premature ectopic excitation (triggering arrhythmic behavior) and unidirectional block of conduction of excitation (sustaining reentrant excitation). The three major mechanisms causing unidirectional block are associated with (i) spatial dispersion of excitability of cardiomyocytes [51–53], (ii) inhomoeneous refractoriness in closely adjacent tissue regions [54–56], and (iii) variations in the electrical load of strands of myocardium due to asymmetrical tissue structures [57–59] (for a review on related mechanisms see Janse [60]).
Fibroblast-Myocyte Interaction

Fig. 4. Membrane potentials calculated using the OXSOFT HEART program of a ventricular cardiomyocyte (C, dark curve) coupled to a mechanosensitive fibroblast (F, light curve). (A) Reduction in resting tension, simulated by a decrease in G, from 1 to 0.1 nS, and subsequent block of cardiomyocyte excitation. (B) Transient diastolic stretch applied for 45 ms results in depolarization of the fibroblast which is transmitted to the cardiomyocyte, triggering an action potential. The three small spikes are "stimulation artifacts" illustrating the simulated injection of trigger-current impulses.

The formation of fibrous tissue in the heart increases tissue inhomogeneity which is involved in all three key mechanisms described above. It could therefore contribute passively to the development of sustained arrhythmias. Given the mechanosensitivity of cardiac fibroblasts they could, furthermore, be involved actively in mechanical induction or maintenance of cardiac arrhythmias.

This hypothesis was examined in a theoretical study. Using the approach described earlier, interaction of one mechanosensitive fibroblast with one ventricular myocyte was studied. In the model, the cells were electrically coupled by a conductance of 40 nS simulating increased likelihood of heterologous cell-coupling under pathological accumulation of connective tissue. Under these conditions, cardiomyocytes are capable of driving the membrane potential in electrically coupled fibroblasts, inducing a myocyte-like potential form as seen experimentally [14]. In turn, fibroblasts slightly depolarize resting cardiomyocytes. This could contribute to the dispersion of both excitability and refractoriness in cardiac scar tissue.

Variations in the fibroblast's resting stretch-activated conductance were found to significantly modulate the minimal effective electric stimulus (current injection) required to trigger action potentials in the coupled cardiomyocyte. Critical reduction in fibroblast resting tension (as observed during hardening of cardiac scar tissue due to collagen-remodelling) [10,61,62] was shown to induce block of myocyte excitation (Fig. 4A) [63]. This is obviously caused by an increase in the electrical inertia of the system. Due to the reduction in a current (i_e) oriented inwardly during diastole the cells hyperpolarize. Thus, the threshold for excitation rises. In the case illustrated, excitation of the cardiomyocyte is halted (Fig. 4A) despite the myocyte being fully excitable in terms of availability of fast sodium channels (Fig. 4B). This fibroblast-inactivation of cardiac excitation is modulated by resting tension.

Fig. 5. Schematic diagram of the possible contribution of cardiac mechanosensitive connective tissue to mechanically caused variations/disturbances in heart rhythm. Slow structural changes are illustrated on the left hand side, fast responses with possible consequences in terms of cardiac mechano-electric feedback on the right.
Simulation of transient stretch of the fibroblast-enriched tissue (as observed, for example, under temporary increase in ventricular filling or during stretch of ischaemic regions by surrounding healthy myocardium) modeled by a transitory increase in the fibroblast’s stretch-activated membrane conductance resulted in a pronounced depolarization of the tissue (as observed, for example, under temporary increase in the fibroblast's stretch-activated membrane conductance). Stretch-activation of cardiomyocytes in a scar could, therefore, occur as a result of interaction with mechanosensitive fibroblasts. These simulations support the hypothesis of potentially mechanically induced arrhythmogenic properties of cardiac fibroblasts. The mechanisms involved could go beyond a mere insulating effect of connective tissue that produces permanent obstacles to the spread of excitation. Arrhythmogenesis in fibroblast-enriched regions could be based both on stretch-dependent functional block of conduction and/or on generation of stretch-induced ectopic beats.

6. Closing remarks

Cellular elements of cardiac connective tissue are mechanosensitive. There is electrophysiological evidence that these cells may interact electrotonically with cardiac myocytes via low conductances interconnecting the heterologous cells. The histological substrate for low-conductivity connection of fibroblasts and cardiac myocytes still needs to be verified. This form of coupling could provide a pathway for mechanical modulation by the connective tissue of cardiac rhythm. For more detailed investigations it will be necessary to develop simplified experimental models which allow cells to be controlled both mechanically and electrically, and which furthermore comprise realistic intercellular electrical connections as found in situ. Possible candidates for such models could be mechanically controlled isolated myocytes or fibroblasts connected electrically to heterologous real or model cells, or improved tissue cultures, perhaps exposed to cyclic mechanical stimulation during the culturing process.

Connective tissue is a population of cells whose contribution to cardiac mechano-electric feedback may have been underestimated. Its effect on heart rhythm could be based on a number of mechanisms (summarized in Fig. 5) and include the positive chronotropic response to stretch, stretch-dependent block of conduction and stretch-induced ectopic beats.

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