Progress in Biophysics and Molecular Biology 115 (2014) 93–102

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

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

Original research

Mechanical control of cell biology. Effects of cyclic mechanical stretch on cardiomyocyte cellular organization

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ARTICLE INFO

Article history:
Available online 28 June 2014

Keywords:
Stretch
Golgi apparatus
Tubulin
Gap junction
Kinesin
Dynein

ABSTRACT

Objectives: The aim of our study was to elucidate how cyclic mechanical stretch is sensed by cardiomyocytes and in which way it affects cytoskeletal organization.
Methods: Neonatal rat cardiomyocytes, cultured on flexible membranes, were subjected to cyclic mechanical stretch (1 Hz, 10% elongation) for 24 h using either round or rectangular loading posts for equibi-axial or uni-axial stretch, respectively, using the FlexCell stretch system. Cells were treated either with vehicle, the focal adhesion kinase (FAK) inhibitor PF-573,228 (200 nM), or the stretch-activated ion channel blocker gadolinium (Gd^{3+}; 100 μM).
Results: Cyclic mechanical stretch (36 mm diameter silicone membrane, equibi-axial stretch, 10% elongation, 1 Hz) induced elongation of the cardiomyocytes together with accentuation of Cx43 at the cell poles, and with an orientation of the cell axis between the radial axis and the circumferential axis (mean deviation: 11° from the circumference). Moreover, stretch resulted in ca. 1.4 fold increased Cx43 expression. FAK was found to be phosphorylated at the edges of the cells. In order to find out, how cardiomyocytes might sense stretch, we investigated possible effects of $Gd^{3+}$and PF-573,228. Gd^{3+} had no effect on elongation or polarization and did not affect stretch-induced Cx43 expression. Interestingly, the FAK inhibitor completely antagonized the stretch-induced elongation, orientation and Cx43-polarization. However, the stretch-induced Cx43 expression was insensitive to this treatment. In order to clarify our result that the cells in equibi-axial stretch did not exactly organize to the circumference or to the radial axis, we decided to use a uni-axial stretch protocol. In uni-axially stretched cells, we found that the cardiomyocytes also showed elongation, Cx43 polarization, and orientation near to the stretch axis, but not exactly in the stretch axis but ca. 25° oblique to it. Furthermore, we investigated the tubular system, the Golgi apparatus, the SR and the nucleus. After 24 h stretch the microtubules were localized nearly (but not completely) parallel to the stretch axis (i.e. in longitudinal cell axis). Moreover, the localization of nucleus and the Golgi was also changed: while under static conditions, the Golgi was distributed more or less around the nucleus, after stretch the Golgi was accentuated at one site of the nucleus facing a cell pole with the nucleus facing the opposite cell pole. The plus motor protein kinesin accentuated at the cell poles and at the cell periphery, while the minus motor protein dynein was found near to the Golgi apparatus.
Conclusions: The stretch signal sensing is mediated via FAK and leads to intracellular re-organization and orientation. The oblique orientation of the cell with regard to the direction of stretch may define a directed force vector which could allow the cell to orientate.

1. Introduction

Much is known about acute effects of mechanical stretch on cells and in particular on cardiomyocytes. Thus, acute stretch can activate stretch-activated ion channels (Sachs, 2005) which allow the influx of Na^{+} or Ca^{++} and thereby can activate intracellular signal cascades or—in particular in the heart – may elicit action potentials. These

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http://dx.doi.org/10.1016/j.pbiomolbio.2014.06.006
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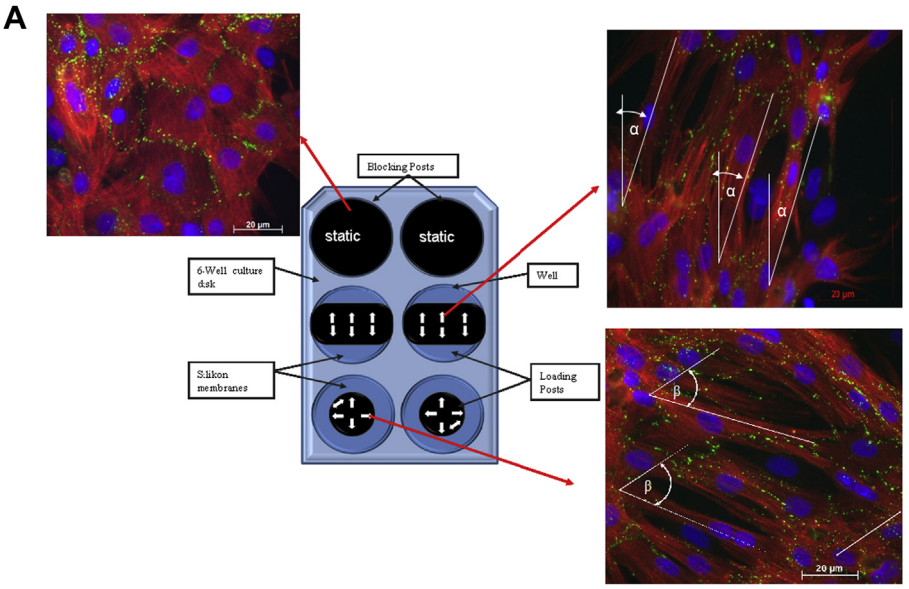
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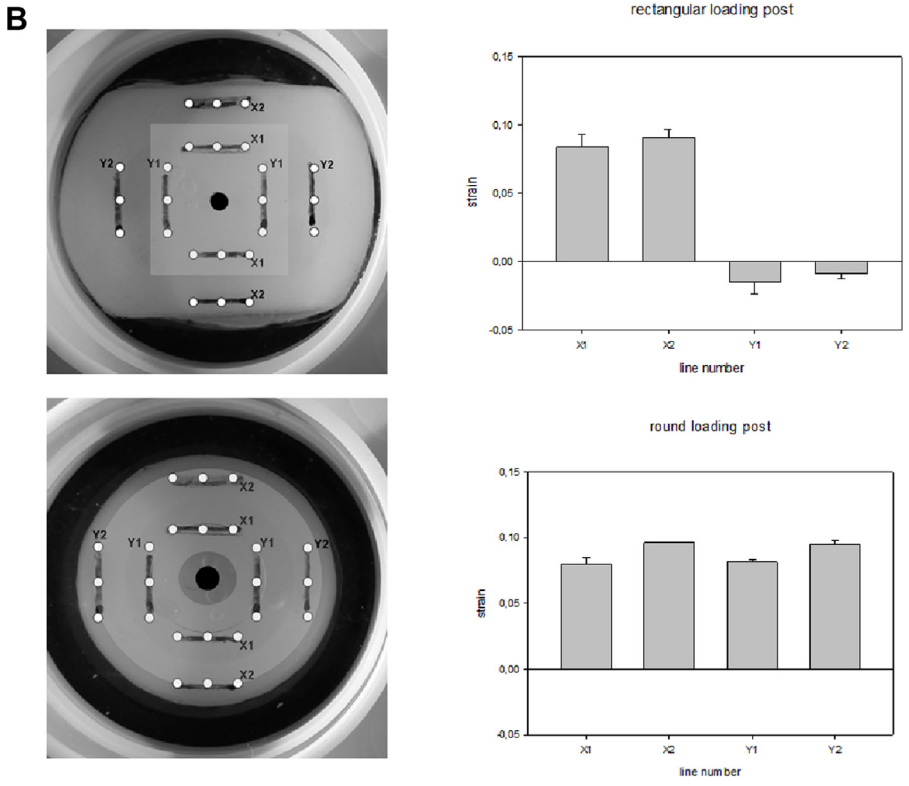
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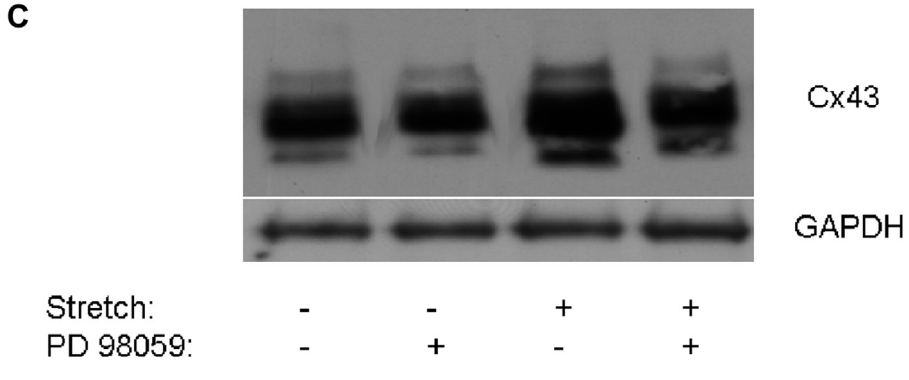
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GAPDH
Stretch: - - + +
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