Volume 283, number 2, 289–290 FEBS 09778 June 1991

ADONIS 001457939100487H

Differential synthesis by cultured atrial and ventricular rat cardiac myocytes of myosin light chain isoforms

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Received 21 March 1991

Dissociated cells from neonatal rat atria and ventricles were cultured in monolayers for 3 days. Newly synthesized ^{35}S-methionine labeled myosin light chain isoforms ALC-1, ALC-2 (atrial) and VLC-1, VLC-2 (ventricular) were identified on 2D gels, and their pattern of synthesis was compared to that of myocard fragments immediately after explantation. ALCs were synthesized in 5- to 10-fold excess over VLCs by atrial cultures, whereas the converse was true for ventricular cultures, with two exceptions: one third of the LC-1 synthesized by ventricular fragments was ALC-1, and dissociated atrial cells synthesized very little LC-2 of either isoform. The former finding corresponds to the relatively high proportion of ALC-1 in neonatal ventricular tissue. We conclude that the regional programme of LC isoform expression is basically retained after tissue explantation and even after dissociation and culturing of cardiac myocytes.

Cell culture; 2D electrophoresis; Neonatal rat; Protein synthesis

1. INTRODUCTION

The myosin of cardiac muscle cells is composed of two 200 kDa heavy chains (MHCs) and two of each of alkaline (LC-1) and phosphorylatable (LC-2) light chains, all of which occur in isoforms [1–3]. In the rat, the atrium contains nearly exclusively myosin of the composition (α-MHC, ALC-1, ALC-2)$_2$, whereas in the ventricle light chains VLC-1 and VLC-2 are predominant at all ages, and two types of heavy chains, α-MHC and β-MHC, coexist at birth, their relative abundance varying with postnatal development and thyroid state [1–3]. Ventricular MHC isoform expression has been extensively studied in a variety of experimental situations, both in the animal and in culture [1–6], but less is known about atrial myocytes and LC synthesis. In order to find out whether LC isoform expression is regionally programmed in cardiomyocytes, we studied LC synthesis by comparing atrial and ventricular monolayer cultures to the myosin components present in the tissue and to those synthesized by freshly explanted tissue fragments.

2. MATERIALS AND METHODS

Cardiac tissue was obtained from newborn (1–2 days postnatal) Sprague Dawley rats. Ten to 15 hearts were collected in a HEPES buffered salt glucose solution ('HBS-glucose': 154 mM NaCl, 5.6 mM KCl, 5.0 mM HEPES, 2.2 mM $CaCl_2$, 0.12 mM $MgCl_2$, 2.5 mM glucose, pH 7.5 [7]), and were dissected into atrial and ventricular (apical two thirds) fragments under the stereomicroscope. At this stage, tissues were used for immediate labeling for control purposes.

Isolation and monolayer culture of cardiac myocytes were performed as described in [8]. After 3 days of culture, the cells were labeled for 16 h with growth medium containing 50 μCi ^{35}S-methionine per 0.2 ml. Control tissue fragments were labeled in the same medium from 1 to 21 h after preparation with periodic tilting (frequency 0.5 Hz, angle ± 15°C) of the multiwell plates.

After removal of the medium, tissue fragments or cells were harvested in 100 μl lysis solution, and myosin light chain isoforms were analyzed by 2D-electrophoresis [9]. After staining with Coomassie blue, the gels were processed for fluorography by immersing in Amplify (Amersham). Fluorographs were scanned, and the amounts of each MLC isoform were quantified by determining the peak areas. Monoclonal antibodies specific for α- and β-MHC isoforms were kindly provided by Drs J.J. and J. Léger, Montpellier [10,11].

3. RESULTS AND DISCUSSION

In monolayer culture, atrial and ventricular cells started to contract about 12 h after plating. Atrial cells contracted with a mean frequency of 2.5 ± 0.4 Hz, ventricular cells with 1.8 ± 0.6 Hz. Immunochemical staining with monoclonal antibodies [10,11] against α- and β-MHC, respectively, showed that α- and β-MHC positive myocytes were present in atrial as well as in ventricular cultures. With the electron microscope, atrial granules (the storage organelles for the cardiac hormone ANP) were seen in atrial but not in ventricular myocytes [8,12], but there were no morphological differences between these cells in the light microscope.

Myosin light chain (LC) isoforms were identified by 2D electrophoresis and Coomassie staining of tissue ex-

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Fig. 1. Comparison of atrial (upper series) and ventricular (lower series) myofibrillar proteins. Extracts from the atrium (a) and the ventricle (b) in neonatal rat heart (Coomassie blue staining). (c,d) Polypeptides synthesized by tissue fragments and (e,f) by monolayer cultures (^{35}S-methionine label, fluorographs). (g,h) Synthetic drawings combined from a,c,e and b,d,f, respectively. Only part of the gel is shown. ALC-1, ALC-2, VLC-1, VLC-2, atrial and ventricular isoforms, respectively, of myosin light chains. Arrowheads point to the positions of atrial (empty symbols) and ventricular (filled symbols) light chains (the positions were determined on parallel fluorographs from mixtures of atrial and ventricular polypeptides). In (g) and (h), fully drawn lines indicate Coomassie blue stained spots whereas dotted lines indicate the position of polypeptides not seen by staining. Horizontal hatching: products labeled in tissue fragments; vertical hatching: products labeled in monolayers.

tracts from neonatal atrium and ventricle as shown in Fig. 1a,b. Only the atrial specific light chains ALC-1 and ALC-2 were identified in atrial tissue, whereas the ventricle contained VLC-1 and VLC-2 and some ALC-1. LC synthesis was studied by 2D-electrophoresis of ^{35}S-methionine labeled extracts (Fig. 1c–f). The pattern of LC synthesis by atrial and ventricular fragments was similar to that of stained proteins from the same sources, although the radioactivity of ALC-2 was relatively low (Fig. 1c,d). In labeled atrial monolayer cultures, there was substantial radioactivity at the position of ALC-1, but only a trace at the position of ALC-2. Ventricular cells expressed VLC-1 and VLC-2 and in addition a smaller amount of ALC-1 (Fig. 1e,f). The quantified results of 2 independent experiments are summarized in Table I.

We conclude that the basic regional pattern of atrial and ventricular LC isoform expression is retained in dissociated cardiomyocytes for at least 3 days in culture, i.e. under conditions lacking any hemodynamic influence. This suggests that the programme of isomyosin expression is retained cell-autonomously by the cardiomyocytes themselves, but does not exclude the possibility that the regional information is mediated by the fibroblast population. The reduced ALC-2 expression by atrial myocytes may be a consequence of the cell isolation procedure or of the monolayer environment. These factors have been shown to cause partial reprogramming of gene expression in cardiac myocytes, e.g. the activation of the cellular oncogene c-fos [13].

Table I

Relative amounts of LC isoforms synthesized by tissue fragments and monolayer cultures of dissociated myocytes

	Tissue fragments		Cell culture	
	A	V	A	V
ALC-1	55 (51, 59)	15 (12, 19)	69 (63, 74)	8 (≤3, 13)
VLC-1	4 (2, 6)	35 (27, 43)	8 (7, 9)	40 (40, 41)
ALC-2	37 (37, 37)	4 (≤3, 6)	14 (9, 20)	5 (≤3, 8)
VLC-2	4 (2, 6)	45 (32, 58)	9 (9, 9)	46 (39, 54)

Fluorographs were scanned, the areas under the peaks of LCs were determined and expressed as percent of the sum of all 4 LC peak areas. Two independent experiments. Mean values and single values are given. A and V are polypeptides

Acknowledgements: We thank F.-W. Kluxen for technical advice and R. Klocke for typing the manuscript. Part of this work was supported by research grants of the University of Bielefeld.

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