Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular and Cellular Cardiology 45 (2008) 88–92
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

Compartmentalisation of cAMP-dependent signalling by caveolae in the adult cardiac myocyte

Sarah Calaghan *, Lukasz Kozera, Ed White

Institute of Membrane and Systems Biology, University of Leeds, Leeds, LS2 9JT, UK

Received 19 March 2008; accepted 16 April 2008
Available online 24 April 2008

Abstract

Cyclic AMP exhibits local (sarcolemmal) and global (cytosolic) patterns of signalling, allowing receptor-specific signals to be generated by a single second messenger. Here we determine whether caveolae, invaginated lipid rafts, are responsible for confining the β_2 adrenoceptor (AR) cAMP signal to the sarcolemmal compartment. Myocytes were treated with the cholesterol-depleting agent methyl-β-cyclodextrin (MβC) to disrupt caveolae. Caveolae-containing membrane fractions were isolated by detergent-free sucrose gradient fractionation. Cell shortening and phosphorylation of the sarcoplasmic reticular protein phospholamban (PLB) and the myofilament protein troponin I (TnI) were measured in response to β_2 AR stimulation (with salbutamol in the presence of 1 μM atenolol). Ser^{16} phosphorylation of PLB (pPLB), $Ser^{22,23}$ phosphorylation of TnI (pTnI), and positive lusitropy were used as indices of global cAMP signals. The ability of MβC to disrupt caveolae was confirmed by selective depletion of the buoyant membrane fractions of cholesterol and caveolin 3, the 2 essential components of caveolae. In control cells, no change in pPLB, pTnI or time to half relaxation was recorded with β_2 AR stimulation ($P>0.05$), but following caveolar disruption a 60–70% increase in phosphorylation of both proteins was seen, accompanied by positive lusitropy ($P<0.05$). These data show for the first time that disrupting caveolae converts the sarcolemmal-confined cAMP signal associated with β_2 AR stimulation to a global signal that targets proteins of the sarcoplasmic reticulum and myofilaments, with functional sequelae. The role of caveolae in spatial control of cAMP may be relevant to perturbation of β AR signalling in cardiovascular disease.

Keywords: Cyclic AMP; β-adrenoceptor; Compartmentation; Caveolae; Sarcoplasmic reticulum; Phospholamban; Troponin I

1. Introduction

Compartmentation of signalling allows a diversity of cellular responses to be achieved with a finite pool of second messengers. One such second messenger is cAMP, which exhibits local (sarcolemmal-restricted) and global (diffuse cytosolic) patterns of signalling [1]. Our recent data suggest that caveolae, small invaginations of the cell membrane, play a key role in generating β_2 adrenoceptor (AR)-specific cAMP signals in the adult cardiac myocyte [2].

Caveolae are a type of lipid raft distinguished by the presence of caveolin, a ≈ 20kDa cholesterol-binding protein, which lines the internal surface of caveolae and promotes their typical flask-like shape (see [3,4]). A variety of signalling molecules are targeted to caveolae. Enrichment in the caveolar microdomain can increase the efficiency and fidelity of signal transduction, however caveolin oligomers add another level of regulatory control by assembling signalling complexes via 20 residue scaffolding domains which bind to a range of molecules generally stabilising them in an inactive conformation [3].

Treatment of adult cardiac myocytes with the cholesterol-depleting agent methyl-β-cyclodextrin (MβC), which is commonly used to disrupt caveolae [5-8], markedly enhances the inotropic response to β_2 AR stimulation, without affecting the β_1 response [2]. β_1 AR couple to $G_{\alpha s}$ proteins giving rise to a diffuse cAMP signal which, via protein kinase A (PKA), phosphorylates a range of proteins including the L-type Ca^{2+} channel at the sarcolemma, phospholamban (PLB) of the sarcoplasmic reticulum (SR) and the myofilament regulatory protein troponin I (TnI) [9,10]. This global cAMP signal results in increased contractility (through phosphorylation of the Ca^{2+} channel and PLB) and an accelerated rate of relaxation (through phosphorylation of PLB and

* Corresponding author. Tel.: +44 113 343 4309; fax: +44 113 343 1407.
E-mail address: s.c.calaghan@leeds.ac.uk (S. Calaghan).

0022-2828/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.yjmcc.2008.04.004

TnI) [11]. By contrast, β_2 AR couple to both $G_{\alpha s}$ and $G_{\alpha i}$ proteins, and the G_i pathway confines the cAMP-dependent signal to the sarcolemmal compartment (primarily the Ca^{2+} channel) [12,13]. This spatial restriction underlies the small magnitude of the inotropic response and the lack of lusitropy relative to β_1 effects. The consequence of caveolar disruption for the β_2 AR response is consistent with a role for caveolae in G_i-dependent signalling, and in support of this we have shown that we can mimic caveolar disruption by disabling G_i with pertussis toxin (PTX) [2].

The aim of this study was to test directly our hypothesis that disrupting caveolae modulates the spatial characteristics of β_2 AR signalling. We used phosphorylation of the SR protein PLB and the myofilament protein TnI and their functional correlate, positive lusitropy, as indices of global cAMP-dependent signalling.

2. Materials and methods

All animal experimentation was carried out in accordance with the Animals (Scientific Procedures) Act 1986 and conforms to the Recommendation from the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals. Myocytes were isolated enzymatically from the hearts of male Wistar rats (250–280g) according to the method described by Calaghan et al. [14]. Myocytes were treated with 1–2 mM MβC for 1h at 37 °C to disrupt caveolae.

2.1. Validation of the effects of MβC

Preparations were fractionated using detergent-free methods [15,16]. Peripheral membrane proteins were extracted in 500 mM Na_2CO_3 (pH 11.0) containing 0.5 mM EDTA and 1% protease inhibitor cocktail (Sigma). Samples were homogenised (Ultra-Turrax T8; Ika), then sonicated (Vibra Cell; Sonics) 3 times each for 20s at full power. Approximately 2 ml of homogenate was mixed with an equal volume of 90% sucrose in MES-buffered saline (25 mM MES, 150 mM NaCl, 2 mM EDTA, pH 6.5) to form a 45% sucrose solution. A discontinuous sucrose gradient was created by layering on to this a further 4 ml each of 35% and 5% sucrose solution (MES-buffered saline with 250 mM Na_2CO_3). Gradients were centrifuged for 17h at 280,000*g* (Beckman SW40Ti rotor) at 4 °C. A total of 12 fractions (each 1 ml) were collected following spinning. Cholesterol was measured in all fractions using the Amplex Red assay (Invitrogen). Caveolin 3 (Cav3), the muscle-specific isoform of caveolin, was measured by Western blotting (Cav3 antibody 610420; BD Biosciences) following SDS-PAGE [14]. Equal volumes of fractions were loaded onto the gel and the band density normalised to the sum of the band density in all fractions.

2.2. Determination of the spatial characteristics of the β_2 AR signal

Shortening was measured using video-edge detection as described previously [14]. Myocytes were perfused with HEPES-based physiological solution containing 1 μM atenolol (to block β_1 AR) at 22–24 °C and field-stimulated at 0.5 Hz. β_2 AR stimulation was achieved with 5 and 50 μM salbutamol [2]. The effect of β_2 AR stimulation on shortening was assessed when the inotropic response attained maximal effect (at around 5 min). In a parallel set of experiments, populations of cells were fixed at 5 min after exposure to 50 μM salbutamol in Laemmli sample buffer containing phosphatase inhibitors (50 mM NaF, 5 mM Na pyrophosphate) [17]. Following SDS-PAGE and Western blotting [14], protein phosphorylation was assessed using antibodies specific for the Ser^{16} site of PLB (A010-12; Badrilla) and the $Ser^{22,23}$ sites of TnI (4004; Cell Signaling Technology) which are sites recognized by PKA [11]. The effect of MβC on total levels of PLB (A1; A010-14; Badrilla) and TnI (4002; Cell signalling technology) was also determined.

3. Results

3.1. Disruption of caveolae with MβC

MβC is used routinely to disrupt caveolae in a variety of cell types, but its effects on cholesterol and Cav3, the main components

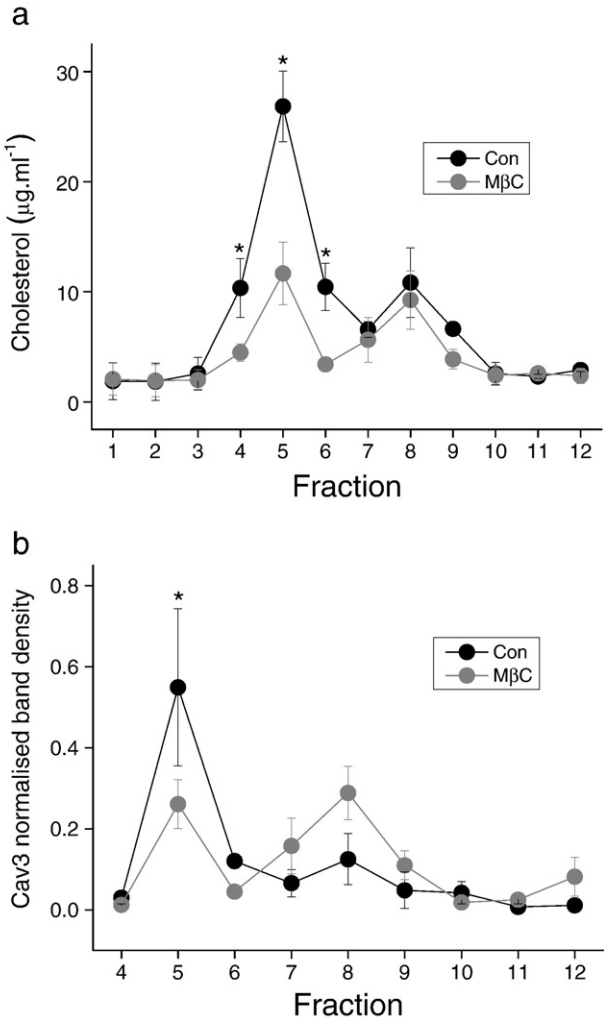

Fig. 1. MβC reduces cholesterol and Cav3, the 2 main components of caveolae, in the buoyant fractions (BF) of the rat cardiac myocyte. a. MβC treatment selectively decreased cholesterol in the BF (fractions 4–6). b. MβC caused translocation of Cav3 from BF to heavier membrane fractions. Data are mean±S.E.; * $P<0.05$ vs. MβC, repeated measures 2 way ANOVA with post-hoc analysis via Holm Sidak; $n=3$ pairs of hearts. No protein was detected in fractions 1–3 of the gradient.