



Endothelin receptor mediated Ca^{2+} signaling in coronary arteries after experimentally induced ischemia/reperfusion injury in rat



Sarah Brøgger Kristiansen ^{a,b}, Kristian A. Haanes ^{a,*}, Majid Sheykhzade ^b, Lars Edvinsson ^a

^a Department of Clinical Experimental Research, Glostrup Research Institute, Copenhagen University Hospital, Rigshospitalet-Glostrup, Denmark

^b Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

ARTICLE INFO

Article history:

Received 7 March 2017

Received in revised form 13 July 2017

Accepted 21 July 2017

Available online 27 July 2017

Keywords:

Calcium signaling

Ischemia/reperfusion injury

Fura2-AM

Endothelin signal transduction

Stretch-induced tone

Phenotypical shift

ABSTRACT

Background: Acute myocardial infarction is one of the leading causes of death. It is caused by a blockage of a coronary artery leading to reduced blood flow to the myocardium and hence ischemic damage. In addition, a second wave of damage after the flow has been restored, named reperfusion injury greatly exacerbate the damage. For the latter, no medical treatment exist. In this study the aim was to characterize Ca^{2+} sensitivity in coronary arteries following experimental ischemia/reperfusion injury.

Methods: Arteries were isolated from hearts exposed to a well-established rat ischemia/reperfusion model. Wire myograph combined with FURA2-AM measurements was applied to study the Ca^{2+} dependency of the vasoconstriction.

Results: The results presented herein show that ET_B receptors (R) have much weaker Ca^{2+} -sensitizing effect than ET_A -R and that ET_B -R appear to be more dependent on Ca^{2+} influx presumably through voltage-gated Ca^{2+} channels (VGCC). In addition, we show that there is an increase in the stretch-induced tone after ischemia/reperfusion, and that this increase in tone is independent of the ET_B -R upregulation.

Conclusion: Our data support the theory that ischemia/reperfusion may induce a phenotypical shift, which includes increased evoked ET_B induced contraction in the smooth muscle cell, and also a higher basal tone development which both are dependent on Ca^{2+} influx through VGCCs. This is combined with alterations in the ET_A calcium handling, which has a stronger dependence on Ca^{2+} release from the sarcoplasmic reticulum after I/R injury.

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1. Introduction

Acute myocardial infarct (AMI) is usually caused by a blockage of the coronary arteries due to atherosclerosis and associated with rupture of a plaque. The only effective treatment for AMI is a rapid recanalization of

the occluded vessel directed toward restoration of the cardiac blood supply. Until now, there has been vast focus on protecting the cardiac myocytes from damage, particularly from the reperfusion injury that occurs after blood flow is restored [1]. However, we have recently shown that AMI with reperfusion should be considered a vascular disorder affecting not only cardiac myocytes but in addition the vascular smooth muscle cells (VSMC) of the coronary artery [2]. Despite improved early survival after AMI, the one-year mortality of heart failure is still very high due to complications following the first acute ischemic event [3]. One alteration after I/R is the change in vascular tone, which is defined as contractions in the absence of a stimulating ligand. Interestingly, it has been shown that there is an increased myogenic tone following ischemia and reperfusion (I/R) in other vascular beds [4]. This has, however, never been investigated in coronary artery resistance arteries following I/R in an experimental model of rodent AMI and reperfusion injury.

One of the further underlying causes of the long term complications following an AMI is believed to be pathophysiological alterations that lead to an increase in density of contractile G-protein coupled receptors (GPCRs), which in our study is hypothesized to augment ligand dependent vasoconstriction of isolated arteries, and hence potentially lead to

Abbreviations: $[\text{Ca}^{2+}]_i$, intracellular calcium concentration; $[\text{Ca}^{2+}]_o$, extracellular calcium concentration; 2-APB, 2-Aminoethoxydiphenyl borate; AMI, acute myocardial infarction; ATP, adenosine triphosphate; BQ123, D-tryptamine-D-aspartic acid-L-proline-D-valine-L-leucine; BQ788, N-[(cis-2,6-Dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl-L-(methoxycarbonyl)-D-tryptophyl-D-norleucine; Ca^{2+} , free ionic calcium; ET-1, endothelin-1; ET_A -R, endothelin receptor subtype A; ET_B -R, endothelin receptor subtype B; GPCR, G-protein coupled receptor; I/R, ischemia and reperfusion; IP_3 -R, inositol triphosphate; KPSS, kalium PSS; LAD, left anterior descending; MLC, myosin light chain; MLCK, myosin light chain kinase; PKC, calcium dependent protein kinase; PSS, physiological saline solution; ROCK, Rho-associated protein kinase; S6c, sarafotoxon 6c; SCA, septal coronary artery; SERCA, sarco/endoplasmic reticulum ATP-ase; SOCC, store operated calcium channel; SOCE, store operated calcium entry; SR, sarcoplasmic reticulum; TRP, transient receptor potential; VGCC, voltage gated calcium channel; VSMC, vascular smooth muscle cell.

* Corresponding author at: Glostrup Research Institute, Nordre Ringvej 69, 2600 Glostrup, Denmark.

E-mail address: kristian.agmund.haanes@regionh.dk (K.A. Haanes).

reduced blood flow to the ischemic area. The endothelin B (ET_B-R) receptor has been shown to be one of the most essential receptors that are upregulated following both cerebral and coronary ischemic events [2,5,6]. The ET_B-R has also been suggested to work as ligand scavenger receptor [7]. Usually expressed on the endothelium, such a scavenger role would be beneficial; ET-1 binding to ET_B-R when expressed on VSMC could on the other hand lead to vasoconstriction, in addition to scavenging ET-1. Vasoactive agonists, such as endothelins (ET) and angiotensin II are released at enhanced levels into the circulation in the critical time-window that follow an AMI [8,9]. Their corresponding receptors are known to promote vessel contraction by a rise in intracellular Ca²⁺ concentration [Ca²⁺]_i in the vascular smooth muscle cells (VSMCs). During physiological situations, it is well established that ET-1 mediates its dual actions via two GPCR subtypes: ET_A-Rs located on the VSMC mediate vasoconstriction while ET_B-Rs are located on

vascular endothelial cells mediate vasodilatation [10]. Earlier results from our group have demonstrated that I/R change the contractile phenotype for the potent vasoconstrictor peptide endothelin-1 (ET-1), causing stronger contractions at lower ET-1 concentrations [2,11]. The increased sensitivity to ET-1 is associated with enhanced ET_B-R mediated contractility in the VSMCs.

The ET-1 induced contraction in coronary arteries involves various Ca²⁺ entry mechanisms and channels, and is traditionally viewed as a two-phase response. Firstly, a rapid Ca²⁺ release from the sarcoplasmic reticulum (SR) stimulated by inositol triphosphate (IP₃) which is responsible for the phasic contraction and depolarization of the cell. Secondly, this is followed by a sustained Ca²⁺ entry from the extracellular space by opening of voltage gated Ca²⁺ channels (VGCC), which is responsible for the tonic phase of the contraction. Force development is initiated by an increase in 4[Ca²⁺]_i-CaM complex and stimulation of

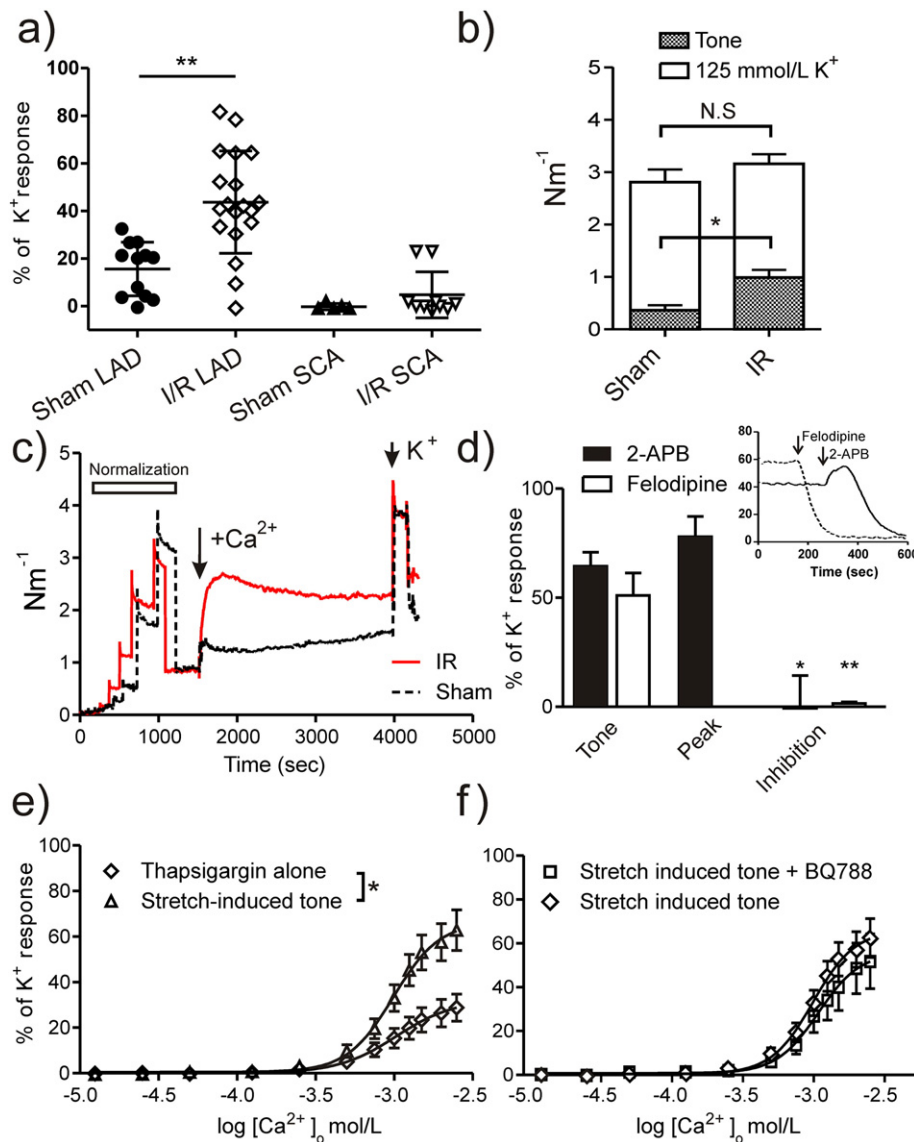


Fig. 1. Stretch-induced tone is a part of the depolarized contraction, and is not dependent on the ET_B receptor. a) Following the normalization of the arteries in Ca²⁺ free solution, the buffer was changed to PSS containing 2 mmol/L Ca²⁺. The tone that then developed was significantly higher following I/R compared to sham. There was no significant stretch-induced tone in the SCA from sham animals. b) Following the stabilization of the tone, the arteries were depolarized with 125 mmol/L K⁺, the contraction is not elevated in I/R arteries compared sham arteries. c) Raw-trace illustrating the normalization process in Ca²⁺ free PSS, the addition of PSS containing Ca²⁺ and the first K⁺ induced contraction. d) 2-APB and felodipine both relaxed the stretch-induced tone in arteries from I/R animals. 2-APB also triggered a small increase in the contraction, before the artery relaxed, see inserted raw-trace. e) The development of the stretch-induced tone in the presence of thapsigargin (compared to paired control) is significantly inhibited. f) The development of the stretch induced tone in the presence of the specific ET_B antagonist BQ788 (0.9 μmol/L) did not have a significant effect. Unpaired, students t-test, *p < 0.05, ** p < 0.01 (Fig. a-d). *p < 0.05, two-way ANOVA repeated measures with Bonferroni's Multiple Comparison Post-hoc Test (Fig. e/f).

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