European Journal of Pharmacology 791 (2016) 718–725

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

Helium postconditioning regulates expression of caveolin-1 and -3 and induces RISK pathway activation after ischaemia/reperfusion in cardiac tissue of rats

Moritz Flick[a,b,1], Martin Albrecht[b,1], Gezina T.M.L. Oei[a], Renske Steenstra[a], Raphaela P. Kerindongo[a], Coert J. Zuurbier[a], Hemal H. Patel[c], Markus W. Hollmann[a], Benedikt Preckel[a], Nina C. Weber[a,*]

[a] Department of Anaesthesiology, Laboratory of Experimental Intensive Care and Anaesthesiology (L.E.I.C.A.), Academic Medical Centre (AMC), Meibergdreef 9, 1100 DD Amsterdam, The Netherlands

[b] Department of Anaesthesiology and Intensive Care Medicine, University Hospital Schleswig-Holstein, Campus Kiel, Arnold-Heller-Straße 3, 24105 Kiel, Germany

[c] Veterans Affairs San Diego Healthcare System and Department of Anaesthesiology, University of California, San Diego, 9500 Gilman Drive, 92093 La Jolla, California, USA

ARTICLE INFO

Keywords:
Cardiac postconditioning
Helium
Caveolin
RISK pathway
Ischaemia reperfusion
Noble gases

ABSTRACT

Caveolae, lipid enriched invaginations of the plasma membrane, are epicentres of cellular signal transduction. The structural proteins of caveolae, caveolins, regulate effector pathways in anaesthetic-induced cardioprotection, including the RISK pathway. Helium (He) postconditioning (HePoc) is known to mimic anaesthetic conditioning and to prevent damage from myocardial infarction. We hypothesize that HePoc regulates caveolin-1 and caveolin-3 (Cav-1 and Cav-3) expression in the rat heart and activates the RISK pathway.

Male Wistar rats (n=8, each group) were subjected to 25 min of cardiac ischaemia followed by reperfusion (I/R) for 5, 15 or 30 min (I/R 5/15/30). The HePoc groups underwent I/R with 70% helium ventilation during reperfusion (IR+He 5/15/30 min). Sham animals received surgical treatment without I/R. After each protocol blood and hearts were retrieved. Tissue was obtained from the area-at-risk (AAR) and non-area-at-risk (NAAR) and processed for western blot analyses and reverse-transcription-real-time-polymerase-chain-reaction (RT-qPCR).

Protein analyses revealed increased amounts of Cav-1 and Cav-3 in the membrane of I/R+He15 (AAR: Cav-1, $P < 0.05$; Cav-3, $P < 0.05$; both vs. I/R15). In serum, Cav-3 was found to be elevated in I/R+He15 ($P < 0.05$ vs. I/R15). RT-qPCR showed increased expression of Cav-1 in IR+He15 in AAR tissue ($P < 0.05$ vs. I/R15). Phosphorylation of RISK pathway proteins pERK1/2 (AAR: $P < 0.05$ vs. I/R15) and pAKT (AAR: $P < 0.05$; NAAR $P < 0.05$; both vs. I/R15) was elevated in the cytosolic fraction of I/R+He15.

These results suggest that 15 min of HePoc regulates Cav-1 and Cav-3 and activates RISK pathway kinases ERK1/2 and AKT. These processes might be crucially involved in HePoc mediated cardioprotection.

1. Introduction

Coronary artery disease and successive myocardial infarction are leading causes of mortality and major medical care costs (Kramarow et al., 2013). Several experimental studies have shown that short episodes of ischaemia prior to an ischaemic event (ischaemic preconditioning) produce cardioprotection and reduce infarct size and damage (DeFily and Chilian, 1993). Conditioning cycles at the onset of reperfusion, referred to as postconditioning, are equally effective, making it a promising clinical approach to treating ischaemia/reperfusion injury (I/R) (Lupi Herrera et al., 2006). Volatile anaesthetics, e.g. sevoflurane (Toller et al., 1999) or isoflurane (Cason et al., 1997), are known to mimic cardiac protection similar to ischaemic conditioning and are mediated by identical pathways (Tsutsumi et al., 2006). However, these agents induce anaesthesia and have side effects on haemodynamic and cardiovascular parameters restricting their clinical

* Correspondence to: Academic Medical Centre (AMC), Department of Anaesthesiology University of Amsterdam, Laboratory of Experimental and Clinical Experimental Anaesthesiology (L.E.I.C.A.), Academic Medical Centre (AMC), Meibergdreef 9, 1100 DD Amsterdam, The Netherlands. .

E-mail address: N.C.Hauck@amc.uva.nl (N.C. Weber).

[1] Both authors contributed equally

http://dx.doi.org/10.1016/j.ejphar.2016.10.012
Received 30 June 2016; Received in revised form 8 October 2016; Accepted 10 October 2016
Available online 11 October 2016

practicability.

The noble gas xenon, another volatile anaesthetic, has minimal cardiovascular side effects (Preckel et al., 2002) and also induces cardioprotection through conditioning protocols (Preckel et al., 2000; Weber et al., 2005). The noble gas helium, which has no anaesthetic potency or haemodynamic side effects, mimics cardioprotective conditioning similar to volatile anaesthetics (Oei et al., 2012b; Pagel et al., 2007). Helium postconditioning (HePoc) reduces infarct size and myocardial damage in rat and rabbit models after I/R injury (Huhn et al., 2009; Oei et al., 2012a, 2014, 2015).

Pathways involved in helium conditioning include G-protein-coupled receptors (GPCR), nitric oxide signaling, the reperfusion injury salvage kinase (RISK) pathway, and the survival activating factor enhancement (SAFE) pathway (Pagel et al., 2007). Many cardioprotective mechanisms have also been linked to the functionality of mitochondria during I/R (Fridolfsson et al., 2012). Consistently HePoc affects the mitochondrial permeability transition pore (mPTP) during cellular stress, preventing it from opening, and thereby reducing mitochondrial dysfunction (Pagel et al., 2008a).

Several of these HePoc effectors interact with and are possibly mediated through caveolae (Horikawa et al., 2008; Patel et al., 2007).

Caveolae are small flask-like invaginations of the cellular membrane (Palade, 1953). They are found in most cell types and regulate several physiological functions such as endocytosis, adrenergic receptor regulation and cellular signaling (Anderson, 1993). Besides sphingolipids, cholesterol and fatty acids as lipid components, caveolae are enriched in caveolins, their essential structural proteins. Caveolins exist in three isoforms, Cav-1, -2 and -3, and contain a scaffolding domain (CSD), that plays a key role in regulating and localizing signaling molecules (Patel et al., 2008). All three isoforms are found in cardiac myocytes (Patel et al., 2006) and cardiac myocyte-specific overexpression of Cav-3 in the heart induced upregulation of survival kinases and resulted in protection against I/R (Horikawa et al., 2008).

In our current study, we used cardiac tissue subjected to regional I/R, I/R and HePoc, or Sham operation to investigate HePoc induced changes of Cav-1 and Cav-3 protein localization and mRNA expression. HePoc induced cardioprotection was characterized further by examining the role of the caveolin-associated RISK pathway as possible underlying mechanism.

2. Materials and methods

2.1. Animal experiments

Animal experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and approved by the Academic Medical Centre's animal ethics committee (DAA102650). Data from these animal experiments, including haemodynamic measurements, were recently published (Oei et al., 2014).

2.2. Chemicals, solutions and culture media

Chemicals and solutions were purchased either from Merck (Millipore, Amsterdam, the Netherlands), Carl Roth (Karlsruhe, Germany), Roche (Almere, the Netherlands) or Sigma-Aldrich (Zwijndrecht, the Netherlands), unless stated otherwise.

2.3. Study design for ischaemia/reperfusion injury

Male Wistar rats (354–426 g, age range of 12–16 weeks) were acclimatized for 7 days under conditions of 12 h light and dark cycles and ad libitum access to food and water. All animals received anaesthesia and surgical procedures as described previously (Oei et al., 2012a). Rats were mechanically ventilated and the carotid artery was cannulated to measure the mean arterial pressure and draw blood

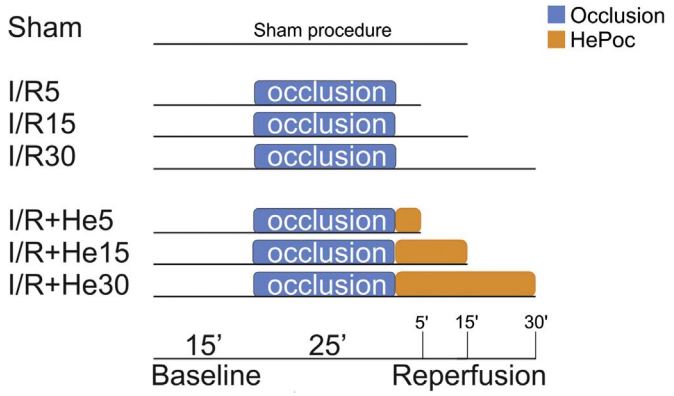

Fig. 1. : Experimental setting. After 15 min of baseline-stabilisation the left anterior descended artery (LAD) was ligated to commence ischaemia in the left ventricle. The study groups underwent 15 min of reperfusion and 15 min of reperfusion with HePoc respectively. The Sham operated group did not undergo ischaemia. The experiments were performed with 5 and 30 min periods, respectively (supplementary Fig. S3 and S4)..

samples. After 15 min of stabilisation the left anterior descending coronary artery (LAD) was encircled with a single puncture 5-0 Prolene suture (Ethicon Johnson & Johnson, Amersfoort, the Netherlands) through the myocardium. A snare was formed with the ends of the suture through a propylene tube to allow reversible ligation of the LAD, thereby inducing 25 min of ischaemia followed by reperfusion. For those animals subjected to I/R only, the ischaemic period was followed by 5, 15 or 30 min of reperfusion, respectively (I/R5, 15, 30). The treatment groups receiving HePoc commenced administration of helium gas (70% helium and 30% oxygen, Linde Gas Benelux, Dieren, the Netherlands) at 24 min of ischaemia (providing sufficient helium presence in the lungs at the onset of reperfusion). HePoc was performed for 5, 15 or 30 min of reperfusion (I/R+He5, 15, 30). The Sham group received surgical treatment without undergoing I/R or HePoc (Fig. 1).

2.4. Preparation of cytosol, membrane and mitochondrial fractions

After each protocol hearts were excised and shock frozen in liquid nitrogen. Tissue was obtained from the ischaemic area at risk (AAR) and the non-ischaemic, non area at risk, (NAAR) as described previously and ground in a pre-cooled mortar (Oei et al., 2014). Subsequently a lysis buffer (Tris base, EGTA, NaF and Na_3VO_4 (as phosphatase inhibitors), protease-inhibitor mix (aprotinin, leupetin and pepsatin), DTT and okadaic acid) was added to prevent degradation. Samples were mixed with the homogenizer (Ultra-Turrax T8, IKA) and 50 µl of sample was pipetted into new vials containing 450 µl of Tripur for RNA analysis. The rest of the homogenate was centrifuged at 1000*g*, 4 °C for 10 min, the supernatant subsequently at 10,000*g*, 4 °C for 15 min. The resulting supernatant was pipetted into new vials, while the remaining pellet was resuspended with lysis buffer (1% Triton X-100) to be used as the mitochondrial fraction. Samples were centrifuged again at 5000*g*, 4 °C for 15 min and the supernatant was used as the cytosolic fraction. The pellet was mixed with lysis buffer, vortexed and after 60 min of incubation on ice centrifuged at 5000*g*, 4 °C for 15 min. The supernatant was obtained as the membrane fraction. All fractions were stored at −80 °C.

2.5. RNA isolation and cDNA preparation

A total of 450 µl homogenate-Tripur mix was diluted 1:10 with BCP (1-Bromo-3-chloropropane) (Sigma-Aldrich, Zwijndrecht, the Netherlands), and shaken vigorously for 15 s. After 10 min of incubation at room temperature, the samples were centrifuged at 12000*g*, 4 °C for 15 min. The upper layer containing RNA was carefully pipetted into clean vials and precipitated by adding 250 µl isopropanol followed