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Changes in vasodilation following myocardial ischemia/reperfusion in rats

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ABSTRACT

Background: Blockage of a coronary artery, usually caused by arteriosclerosis, can lead to life threatening acute myocardial infarction. Opening with PCI (percutaneous coronary intervention), may be lifesaving, but reperfusion might exacerbate the cellular damage, and changes in the endothelium are believed to be involved in this worsened outcome.
Aim: The aim of the present study was to compare endothelial dependent and independent vasodilatory effect after experimental myocardial ischemia/reperfusion (I/R).
Methods: A well-established rat model of myocardial ischemia with 24 h of reperfusion was applied, followed by a study in a wire myograph.
Results: Endothelial NO dependent relaxation in response to carbachol, was sensitive to arterial depolarization, and was unaffected by I/R. In contrast, endothelial NO dependent ADPβS signalling, which was not sensitive to arterial depolarization, was significantly reduced after I/R. Following I/R, an H_2O_2 dependent EDH induced dilation appears in response to both of the above agonists. In addition, calcitonin gene-related peptide (CGRP) induced vasodilation was reduced.
Conclusion: These data show that NO dependent ADPβS induced dilation is reduced after I/R. However, there is some compensation by released H_2O_2 causing an EDH. Combined with a loss of maximal dilation in response to CGRP, the reduced vasodilation could be an important factor in understanding the exacerbated damage after I/R.

1. Introduction

Blockage of a coronary artery often following the rupture of a plaque, caused by arteriosclerosis can lead to life threatening acute myocardial infarction (AMI). Current treatment includes PCI (percutaneous coronary intervention) with stenting of the artery or thrombolysis, however, often a secondary damage occurs after the reperfusion of the coronary vasculature [1]. This injury is named ischemia/reperfusion (I/R) injury, and the one year mortality following an AMI is still very high [2]. We have in rats previously shown that $G_{\alpha q}$ -coupled vasocontractile receptors are upregulated in the vascular smooth muscle cell (VSMC) following I/R, which could be an important factor in understanding the damage that occurs after the initial AMI [3]. Current research has been focused on the VSMC as the mediator of intrinsic changes in the artery following I/R. However, changes in the endothelium are possible important regulators of vasomotor tone, the vascular resistance, and hence the coronary blood flow.

In humans, the coronary vasodilator response is significantly attenuated following AMI [4], and the impact of endothelium-dysfunction is important in understanding myocardial I/R injury [5]. Coronary flow in rats is reduced following permanent AMI, although accompanied by increased NO (nitric oxide) production [6]. However, the effects were limited by the presence of superoxide and it is generally believed that generation of superoxides is the major limiting factor for NO accessibility or availability during acute reperfusion [7]. Indeed, overexpression of superoxides dismutase reduced infarct size in mice hearts, using in situ coronary ligation in a Langendorff setup [8]. To our knowledge there is only one study investigating the pharmacological response of isolated

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List of abbreviations

ADP	adenosine diphosphate
AMI	acute myocardial infarction
Ca^{2+}	free ionic calcium
cAMP	cyclic AMP
cGMP	cyclic GMP
CGRP	calcitonin-gene related peptide
EDH	endothelial-derived hyperpolarization
EDHF	EDH factor
ET-1	endothelin-1
GPCR	G-protein coupled receptor
I/R	ischemia and reperfusion
IP3-R	inositol triphosphate
KPSS	kalium PSS
LAD	left anterior descending
L-NAME	L-N^G-Nitroarginine methyl ester
NO	nitric oxide
NOS	nitric oxide synthase (eNOS endothelial NOS)
PCI	percutaneous coronary intervention
PDE	phosphodiesterase
PSS	physiological saline solution
ROS	reactive oxygen species
SR	sarcoplasmic reticulum
VSMC	vascular smooth muscle cell

coronary arteries following I/R by utilizing a myograph setup. Symons & Schaeffer only showed acute changes in rats, as the measurements was performed 15 or 60 min, after the ischemia was induced in vivo [9]. Their data showed a reduced vasorelaxant response to acetylcholine, and it is worth mentioning that acute changes could be caused by an increase in acetylcholinesterase activity.

There are three main pathways that can induce endothelium dependent vasodilation; NO, EDH (endothelial derived hyperpolarization) and the prostaglandins, and these pathways are all triggered by an increase in intracellular Ca^{2+} in the endothelial cell [10] and the level of Ca^{2+} increase is important for which of the pathways are triggered [11]. Increases in local NO, causes an increase in VSMC cGMP levels that reduces the contraction [10]. There are many candidates for EDHF, nevertheless, their ability to hyperpolarize the VSMC are the common factor in which they cause a relaxation [12]. In most species, including rat coronary arteries, the endothelial dependent dilation is caused primarily by NO and EDH, as both potential relaxing prostaglandins (PGE_2 and PGI_2) are not altering coronary flow [13,14], and both PGI_2 and its stable analogue Iloprost had no effect in rat compared to in other species [15]. In general, prostaglandins have small impact on dilations in the rat circulation [16].

There are multiple endothelial receptors that when activated cause vasodilation. Acetylcholine or its stable analogue carbachol predominantly activates the muscarinic M3 receptor, which leads to an increase in NO or an EDH, dependent both on the vascular bed and arterial size [16] although there are some evidence that M1 might also be involved in the coronary vasodilator response [17]. Importantly, in Wistar-Kyoto rats, L-NAME was shown to block acetylcholine-induced relaxation in the LAD completely [18] while others have shown that EDH can contribute up to 20% [19]. A study using Long-Evans Tokushima rats recorded smooth muscle membrane potential in LAD and showed hyperpolarization to ACh combined with vasodilation, where both were not affected by L-NNA [20]. However, in these arteries there was a large constitutive active NO release. Gschwend and colleagues, also found a minor EDH (less than 20% dilation) in septal coronary arteries on Sprague-Dawley rats [21].

Purinergic signalling (signalling with nucleotides), is an important vasodilatory mechanism [22,23]. ADP, the natural ligand for the endothelial P2Y1 receptors has been shown to cause limited intracellular Ca^{2+} increases and is most often associated with the release of NO [11]. Although the endothelium is very important in inducing relaxation of the coronary arteries, also agonists binding to receptors directly on the VSMC, such as the peptide CGRP, has been shown to be an important component in understanding the responses to I/R injury [24]. Following myocardial infarction and increase in lactate concentration, plasma CGRP is increased, and a protective role is assumed [25]. Interestingly, the pharmacological profiles of CGRP induced vasodilation on VSMC after I/R has not been investigated earlier.

Since previous coronary artery flow data suggest less NO availability, it is highly relevant to investigate the pharmacological response of the endothelium following I/R, and investigate if the endothelium have lost some of its ability to produce NO dependent dilations [26]. Therefore, this study aimed to compare endothelial dependent and independent relaxation between sham animals and a rodent model of AMI with reperfusion. Here we show that the dilation in response to P2Y1 activation is reduced, while carbachol induced dilation are unaffected, most likely due to activation of different intracellular pathways. Following I/R, both the above agonists now cause an additional release of H_2O_2. In addition, it appears that CGRP loses some of its relaxing properties after I/R injury.

2. Methods

2.1. Surgery

The model applied for I/R in rats is well established, and a detailed description can be found in Skovsted et al. [3,27]. Briefly, Sprague Dawley rats (280–350 g) were anaesthetized with a mixture of Hypnorm-Dormicum by subcutaneous injection and mechanically ventilated with room air (10 ml/kg, 75 strokes/min). By the opening of the third, fourth and fifth intercostal space a thoracotomy was performed, followed by an opening of the pericardium. A proline suture was placed around the LAD coronary artery, approximately 1/3 down from the aorta. The ligature was tightened around the LAD to induce ischemia which was confirmed by an immediate ST-elevation on the ECG trace. After 30 min of ischemia, successful reperfusion was achieved by loosening the ligature suture. After the ligature around the LAD coronary artery was relieved (reperfusion), the ECG pattern returned to normal within one to 2 h. Sham surgery is similar with the exception that the ligature around the LAD was not tightened. The animals were given a post-operative injection of buprenofin (0.03 mg/kg) allowed to recover (with the access to 0.4 mg/kg buprenofin in Nutella). After 24 h of reperfusion the animals were sacrificed, similar to previous studies on I/R [3,27]. All procedures performed were approved by the Danish animal inspectorate (Dyreforsøgstilsynet) approval nr 2013-15-2934-00940, and therefore also according to the guidelines of Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.