Anesthetic induced cardioprotection: from bench to bedside and retour

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Chapter 1: Main conclusions

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In this present thesis we investigated the influence of anaesthetics on myocardial ischaemia reperfusion injury, namely protection by preconditioning including the underlying mechanisms.

Preconditioning was first described by Murry et al. in 1986 (1). In 1997 Kersten et al. (2) showed that volatile anaesthetics like isoflurane also induce early preconditioning. Since then there is overwhelming evidence that several different anaesthetics induce cardioprotection via pre- or postconditioning. However, not every drug used to induce or maintain anaesthesia is capable to induce cardioprotection. Ketamine, especially the R(-)-enantiomer of Ketamine, blocks preconditioning, whereas nitrous oxide has no effect on preconditioning. The impact of anaesthesia on ischaemia-reperfusion injury is discussed in detail in the review in **chapter 2** of this thesis.

Part II of this thesis deals with the role of opioid receptors for preconditioning.

In experimental studies, subtype selective opioid receptor agonists induce early and late preconditioning. In **chapter 1 of part II** we demonstrate that morphine, a clinically widespread used unspecific opioid receptor agonist, induces late preconditioning in rat hearts *in vivo*. The treatment with morphine leads to an increase in phosphorylation of IκB and consecutive to an increase in activity of the nuclear transcription factor κB. This indicates that this important transcriptionfactor is involved in morphine induced late preconditioning. These experiments have also been performed with lipopolysaccharide of Escherichia coli (LPS) as a positive control. Interestingly, we observed that blocking of opioid receptors with the unspecific antagonist naloxone during the trigger phase abolished the LPS induced cardioprotection. Co-treatment with naloxone during the mediator phase, thus application of the substance shortly before sustained ischaemia was established, did not influence LPS induced cardioprotection. We therefore concluded that the endogenous opioid system is at least involved in LPS induced late preconditioning.

Knowing the underlying mechanisms of preconditioning is of utmost importance to understand which clinical circumstances are interfering with this phenomenon. After decades of research there is still no answer what is the end-effector of preconditioning. One of the latest candidates is the mitochondrial permeability transition pore (mPTP). In **chapter 2 of part II** we aimed to

investigate if the mPTP is involved in morphine induced early preconditioning in rat harts *in vitro*. However, performing the experiments, we were not able to induce cardioprotection through morphine induced preconditioning in our Langendorff-model at L.E.I.C.A. (**L**aboratory of **E**xperimental **I**ntensive **C**are and **A**naesthesiology) at the AMC in Amsterdam, whereas in our laboratory at the Heinrich-Heine-University in Düsseldorf we previously showed a cardioprotective effect. After intensive discussions we found that there was a small, but important, difference in the mixture of the Krebs-Henseleit buffer used in the two laboratories. The Amsterdam buffer contained glutamine, whereas the Düsseldorf buffer did not. Glutamine, added to the Krebs-Henseleit buffer completely blocked morphine induced preconditioning.

For quite a long time, the ATP-sensitive potassium channel was thought to be THE endeffector of preconditioning. However, nowadays we know that this is only one of the channels involved in signal transduction of preconditioning. Another important channel that is involved in preconditioning initiated cardioprotection is the mitochondrial calcium sensitive potassium (BKCa) channel. In **chapter 3 of part II** we investigated whether these channels are involved in morphine induced early preconditioning in rat hearts *in vitro*. Here, in the absence of glutamine, we could demonstrate that BKCa channels are involved in morphine induced early preconditioning.

Taken together, the clinically widespread used opioid morphine is capable to induce early and late preconditioning in rat hearts. Opioid receptors are involved not only through direct stimulation by morphine, but also (in case of LPS induced late preconditioning), endogenous ligands of these receptors are involved in the trigger phase. Downstream in the signaling pathway, BKCa channels are involved in early preconditioning *in vitro.* However, experimental conditions have to be taken into account while interpreting and transfering experimental results, as is shown in **chapter 2 of part II**.

In **part III** of this thesis some mechanisms of anaesthetic induced preconditioning are discussed.

Radical oxygen specimens (ROS) are thought to be responsible for part of the damage induced by reperfusion of ischaemic myocardium. However, in **chapter**

1 of part III, we could demonstrate that at least small amounts of ROS are pivotal in signaling of isoflurane induced early preconditioning in rabbits *in vivo*.

In addition to direct myocardial damage, endothelial damage occurs during ischaemia reperfusion injury. Consequently, interactions between the endothelium and constitutes of the blood may lead to attraction of leucocytes and release of pro-inflammatory cytokines. Tumor necrosis factor (TNF) α is one of these cytokines and is a known strong activator of cell adhesion molecules (CAM) as intracellular adhesion molecule 1, vascular adhesion molecule 1 and E-selectin. The expression of these CAM is regulated by NFκB. In **chapter 2 of part III** we tested the hypothesis that different anaesthetic agents as xenon, isoflurane, nitrous oxide and the analgesic morphine are able to abolish the TNF-α induced expression of these CAM in human umbilical vein endothelial cells *in vitro*. All four agents blocked the TNF-α induced expression of intracellular adhesion molecule 1. However, morphine had no influence on vascular adhesion molecule 1 expression after TNF-α stimulation, whereas the anaesthetic agents (xenon, isoflurane and nitrous oxide) did attenuate expression. None of the investigated substances affected E-selectin expression. NFκB activation was abolished by all four agents.

Xenon could be the ideal anaesthetic agent for cardiac compromised patient due to its negligible hemodynamic side effects. In **chapter 3 of part III** we show that xenon induces late preconditioning in the rat heart **in vivo**. The cardioprotective effect was comparable with that induced by ischemic late preconditioning. As one step in the signaling cascade, cyclooxgenase-2 was identified. Although functional blockade of this enzyme led to a complete blockade of xenon as well as ischaemia induced late preconditioning, only ischaemic late preconditioning induced cyclooxygenase-2 on messenger RNA and protein level. These results indicate different patterns in the signaling of ischaemic and xenon induced late preconditioning.

As mentioned in **chapter 2 of part III** nitrous oxide is able to inhibit CAM. In **chapter 4 of part III** we investigated the impact of nitrous oxide on preconditioning. Nitrous oxide itself was not able to induce preconditioning and is therefore the only inhalational anesthetic gas without the capability to induce this powerful cardioprotection. However, nitrous oxide did not interfere with

isoflurane induced preconditioning and one of the crucial signaling steps like protein kinase C activation.

With all the experimental evidence from other laboratories and our own results it was reasonable to translate the promising results from animal experiments into the clinical setting (**part IV**). In **chapter 1** of this section the results of our clinical trial are described. In patients undergoing coronary artery bypass grafting (CABG) procedures, we tested two different preconditioning protocols and demonstrated that the concept of myocardial anaesthetic preconditioning works in humans. Our results indicated that at least two stimuli are necessary to induce cardioprotec-tion by anaesthetic preconditioning.

Part V of this thesis addresses the question why most of the clinical trials failed to show cardioprotection induced by preconditioning.

Most research was done in young, healthy animals. Therefore, we broadened our research from healthy myocardium to experimental studies in animals with pathophysiologic changes suspected to interfere with the mechanisms of preconditioning.

As mentioned in part II chapter 3, BKCa channels are involved in the signal transduction of preconditioning. In **chapter 1** of part V we demonstrated that age has a significant influence on mitochondrial respiration due to changes in BKCa activity. These changes are capable to block the preconditioning effects of cardioprotective agents.

Diabetes mellitus is one of the risk factors for the development of coronary artery disease. It is known that diabetes mellitus abolishes preconditioning. The underlying mechanisms are not yet clear. In **chapter 2 of part V** we investigated how diabetes mellitus interacts with several kinases that are involved in signaling preconditioning. Although diabetes mellitus abolished ischaemic preconditioning, we could not detect an impairment of phosphorylation (= activation) of mitogen activated protein kinases or heat shock protein 27 after ischemic preconditioning. Therefore we conclude that the blockade of ischaemic preconditioning by diabetes mellitus is downstream of mitogen activated protein kinases and heat shock protein 27 in rats *in vivo*.

After reviewing the results of our clinical trial in part IV **chapter 1** we asked our self why we observed a cardioprotection in our study population, while other groups did not. We indentified two major differences with the protocols of the other studies: the preconditioning protocol and the fact that our patients were not treated with aprotinin. Going back from bed to bench we conducted an experimental study to answer two questions: which influence has the preconditioning protocol on cardioprotection and does aprotinin affect preconditioning by anaesthetics. In **chapter 3 of part V** the answers are given. Multiple cycles of anaesthetic preconditioning led to a stronger protection compared with one cycle of preconditioning. Aprotinin, formerly used in CABG procedures to prevent blood loss, completely abolished anaesthetic induced preconditioning in rat hearts *in vivo*, independently of the chosen preconditioning protocol. In addition, we demonstrated that endothelial nitric oxide synthase is involved in anaesthetic induced preconditioning and its blockade by aprotinin.

References

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