UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Anesthetic induced cardioprotection: from bench to bedside and retour

Frässdorf, J.

Publication date
2012

Link to publication

Citation for published version (APA):
Frässdorf, J. (2012). *Anesthetic induced cardioprotection: from bench to bedside and retour*. [Thesis, fully internal, Universiteit van Amsterdam]. Boxpress.

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date:20 Jan 2025

Chapter 2: General discussion

Jan Fräßdorf

Cardiovascular disease is one of the major causes of death in Western nations. In 2004 more than 50 million individuals suffered from angina pectoris all over the world (WHO website), leading to approximately 8 million myocardial infarctions per year. Clinically the main therapeutic goal is to reestablish myocardial perfusion to salvage myocardium that would be irreversible damaged by ischaemia. The more myocardium is irreversible lost, the more myocardial function is limited. This may lead to congestive heart failure with its consequences as invalidity or death. However, reperfusion of ischaemic myocardium is leading to additional myocardial damage. This paradoxical phenomenon is called reperfusion injury. Therefore, finding a therapy that renders myocardium resistant to ischaemia and/ or limits the extent of reperfusion injury has potential to save millions of life.

Anesthesiologists are involved in the treatment of myocardial ischaemia in the emergency room, the intensive care, the cardiology suite or in the operating theatre, and cardiovascular research is from the beginning of modern anaesthesia one of the main topics of anaesthesiology related research. In 1976, Bland and Lowenstein reported that the volatile anaesthetic halothane reduces ST-segment changes after myocardial ischemia in dogs (1). Tarnow et al. demonstrated that isoflurane increases the ischaemic threshold after pacing induced ischaemia in men, indicating that volatile anaesthetics could have cardioprotective properties in humans (2). However, these results were not used to intensify research in this area. In the meantime, Murry et al. discovered an endogenous cardioprotective mechanism: preconditioning. In 1986 they published their observation that short term ischaemia, itself not lethal, prior to a prolonged and infarct inducing ischaemia reduces infarct size by 75% compared with untreated controls (3). Subsequently, this strong cardioprotection could be reproduced by several groups in every investigated species including human myocardium (for review see (4). As preconditioning is such a powerful cardioprotective mechanism, further research focused to unravel the signal transduction cascade. The past 25 years with intensive research has shown that signal transduction of myocardial preconditioning is more complex than thought in the early beginnings.

This complexity was even increased by the discovery that preconditioning has two different phases. The first one, called early preconditioning (EPC) occurs

immediately after the preconditioning stimulus and lasts for two to three hours. Then, after a protection free interval of 24 hours, cardioprotection reoccurs and lasts for up to 72 hours. This phase is called late preconditioning.

Liu et al. reported in 1991 that EPC is triggered by activation of adenosine A_1-receptors (5). This was the first evidence that EPC is receptor mediated. Further research showed that the signaling pathway can be divided into two different phases: the trigger and the mediator phase. During the trigger phase, different G_i protein coupled receptors are activated. Their endogenous ligands are released in the myocardium during the preconditioning stimulus. Besides adenosine, bradykinin and endogenous opioids are these ligands (6). Blockade of one of these receptors completely abolished the cardioprotection observed after EPC with one single cycle of preconditioning. By repeating the precondition stimulus the signal transduction becomes more robust and blockade of one of the receptors does not abolish the cardioprotection (7), indicating that there is a threshold to induce EPC mediated cardioprotection. After one cycle of preconditioning, only a small amount of adenosine, bradykinin and endogenous opioids are released and all three pathway are necessary to activate the following step in the signal cascade. After multiple cycles preconditioning, more of these ligands are released and the blockade of one of the pathways is not sufficient to block the complete signaling pathway. This phenomenon could at least in part be responsible for the observed stronger cardioprotection as described in part IV chapter 1 and part V chapter 3 of this thesis.

The three different triggers, adenosine- bradykinine- and opioid receptor activation, converge in a common pathway: activation of protein kinase C (PKC). Blockade of PKC inhibits cardioprotection after stimulation of one of the triggers mentioned above (6).

Besides the receptor mediated activation of PKC, a reactive oxygen species (ROS) mediated PKC activation plays a role in signaling of preconditioning (8). Treatment with a ROS generator induces cardioprotection, which again is blocked by a PKC inhibitor, indicating that ROS mediated cardioprotection is located upstream of PKC (9). In part III chapter 1 of this thesis we demonstrated that ROS generation is not only necessary in ischaemic induced EPC, but also in isoflurane anaesthetic induced preconditioning.

How PKC mediates cardioprotection is until now not resolved. PKC has a lot of potential targets. It is known that PKC interacts with several mitochondrial structures such as mitochondrial permeability transition pore (mPTP), mitochondrial ATP sensitive potassium channels (mK_{ATP}) or the apoptosis pathway.

Opening of the mPTP is involved in cellular necrosis after ischaemia and reperfusion (10), most likely due to uncontrolled influx of Ca^{2+} ions and a burst of ROS. Prevention of mPTP opening could be one of the true end-effectors of preconditioning. Besides the interaction of PKC with mPTP, also the mitochondrial calcium sensitive potassium channels (BKCa) interact with mPTP. Activating of BKCa prevents opening of mPTP at the onset of reperfusion. In part II chapter 2 we investigated whether morphine induced EPC is mediated via inhibition of mPTP opening. However, we were not able to demonstrate in our setting that morphine induces EPC. This was due to the containts of the buffer used in the Langendorff preparation, containing glutamine. This indicates one of the problems in research where results from laboratories all over the world are not always reproducible due to small differences in the experimental conditions. In another set of experiments, on another location and without glutamine, we could demonstrate that morphine induced preconditioning is mediated by activation of BKCa as shown and discussed in part III chapter 3 of this thesis.

In the beginning it was thought that preconditioning protects via an anti-ischaemic effect. In the last years it became more evident that preconditioning induces a phenotype of the myocyte that confers cardioprotection. These mechanisms are mainly the activation of PI3 kinase, Akt and the MEK1/2 and ERK 1/2 at the onset of reperfusion. Hausenloy et al. named theses kinases “reperfusion injury survival kinases (RISK)”, as pharmacological blockade of one of these kinases abolishes the preconditioning induced cardioprotection (11). The closed link between the RISK and mPTP opening was demonstrated by Juhaszova et al. in cardiomyocytes *in vitro* (12).

In young and healthy animals, EPC can easily be induced. However, in experimental models of disease (i.e. diabetes) or in aged myocardium, preconditioning is abolished. In part V chapter 2 of this thesis we addressed whether mitogen activated protein (MAP) kinases or heat schock protein (HSP) 27 are blocked through diabetes in rat hearts i*n vivo*. MAP kinases and HSP 27 are

downstream targets of PI3 kinase. We demonstrated that in our model, neither MAP kinases nor HSP 27 are affected by diabetes in rat hearts *in vivo*, suggesting that the blockade of EPC by diabetes occurs downstream of these targets.

With ageing, the potency of cardioprotection by preconditioning decreases (13). In part V chapter 1 of this thesis we demonstrated that in aged rats mitochondrial respiration is depressed and that the regulation of mitochondrial respiration through BKCa is age dependent. Opening of mPTP is regulated by phosphorylation of glycogen synthase kinase (GSK)-3β (12) . In aged rats inhibition of GSK-3β does not prevent the opening of mPTP in vitro (14). However these results were all gained in in vitro studies and therefore it is difficult to conclude if these effects are responsible for the decreased effectiveness of EPC in aged myocardium in vivo

Endothelial dysfunction is common in the ischaemia reperfusion situation, possibly aggravating the injury through inflammation processes and or occlusion of blood vessels (15). De Fily et al. demonstrated that preconditioning reduces endothelial dysfunction after ischaemia and reperfusion (16). Several interactions between endothelial cells and constituents of the blood occur and might recruit circulating leukocytes to the site of damage. Mainly cell adhesion molecules (CAM) are activated. Release of pro-inflammatory cytokines as tumor necrosis factor (TNF) α proceeds the expression of CAM. In part III chapter 2 of this thesis we investigated if anaesthetics (isoflurane, xenon and nitrous oxide) or the opioid morphine prevent the expression of CAMs in human umbilical vein endothelial cells after stimulation with TNF-α. All four agents prevented the expression of intracellular CAM. Intravascular CAM 1 expression was only blocked by the volatile anaesthetics isoflurane, xenon and nitrous oxide. None of the given pretreatments affected the expression of E-selectin. However, the increased transcriptional activity of the nuclear transcription factor κB was abolished by all four agents. These results indicate that anaesthetics and morphine interact with CAM. Interestingly, nitrous oxide was not able to induce preconditioning in rat hearts *in vivo* as shown in part III chapter 4 of this thesis.

As demonstrated in part III chapter 2, morphine interacts with NFκB in HUVECs in vitro. NFκB has divergent functions within the myocardium. Depending on the physiological context and the cellular type it can protect cardiovascular tissues from injury or contribute to pathogenesis (17). This transcription factor

plays a prominent role in signaling of late preconditioning (LPC). Next to different time courses (EPC occur immediately after the preconditioning stimulus and LPC with a delay of 24 hours), the main difference between EPC and LPC regarding the signalling is that EPC relies on phosphorylation of kinases and LPC on *de-novo* synthesis of proteins.

In part II chapter 1 of this thesis we demonstrated that morphine induced late preconditioning in rat hearts *in vivo*. This cardioprotection was triggered and mediated through opioid receptors. Morphine increased NFκB activation, and this effect was abolished by blockade of opioid receptors. These results indicate that morphine and the opioid receptors interact with NFκB.

Cyclooxygenase (COX)-2 is regulated by NFκB and involved in LPC (18). In part III chapter 3 of this thesis we tested the hypothesis that the anaesthetic noble gas xenon induces LPC and that this cardioprotection is mediated by COX-2. The data show that xenon induced LPC and pharmacological blockade of COX-2 by the selective COX-2 inhibitor NS-398 abolished this cardioprotection. However, on a molecular level we were not able to show that xenon induces COX-2 translation and expression. This is in contrast to our findings in the same study that ischaemic induced LPC does increase levels of COX-2 messenger RNA and protein expression. These results indicate that ischaemic and xenon induced LPC have in part different signal transduction pathways.

In the beginning of preconditioning research, it was thought that preconditioning is an “all-or- nothing” phenomenon. That means, once induced, additional stimuli do not increase the extent of cardioprotection. Nowadays we know that increasing the number of stimuli makes the signal transduction more robust against counteracting mechanisms. For example, blockade of the β-adrenorecpetors increases the threshold to induce preconditioning in rabbits *in vivo* (19). We tested the hypothesis that a multiple cycle protocol induces preconditioning compared with a single cycle protocol in humans undergoing coronary artery bypass grafting procedures. Here, we could clearly demonstrate that a multiple cycle protocol induces preconditioning, whereas as single cycle protocol does not (part IV chapter 1 of this thesis). These results were confirmed by Bein et al. (20)

When we performed these experiments there was weak evidence that multiple cycle anesthetic induced preconditioning confers stronger protection compared with a single cycle protocol (21). We therefore decided to provide more experimental evidence that multiple cycle anaesthetic induced preconditioning leads to stronger cardioprotection. Additionally, we tested the hypothesis that aprotinin blocks anaesthetic induced preconditioning. As shown in part V chapter 3 of this thesis multiple cycle preconditioning induces stronger cardioprotection with a maximum after three cycles. Aprotinin, used in open heart surgery to prevent blood loss, completely abolished anaesthetic induced preconditioning via blockade of endothelial NOS.

The fact that volatile anaesthetics induce cardioprotection led to a change in clinical practice. Before, most centers dealing with open heart procedures used an high dose opioid based anesthesia. This was mainly caused by the idea that volatile anesthetics are negative inotrope and potent vasodilators and therefore counteracts the aim of achieving stable hemodynamics. However, nowadays, volatile anesthetics are widespread used during open heart procedures or in patients who are at risk for perioperative myocardial ischemia, due to its cardioprotective properties. In the current guidelines on the perioperative cardiovascular evaluation and care for non-cardiac surgery from the American College of Cardiology and the American Heart Association it is recommend to use volatile anesthetics in hemodynamic stable patients who are at risk for myocardial ischemia (22).

In conclusion, this thesis provides evidence that the opioid morphine and almost all inhalational anaesthetics, except nitrous oxide, induce preconditioning and the thesis gives an insight in the signaling transduction cascades. Preconditioning can be influenced by age, diabetes or the chosen experimental conditions. In humans, anaesthetic induced preconditioning strongly depends on the chosen protocol and co-administered drugs.

References

1. *Halothane-induced decrease in experimental myocardial ischemia in the non-failing canine heart.* **Bland JH, Lowenstein E.** 1976, Anesthesiology, Vol. 45, pp. 287-93.

2. *Isoflurane improves the tolerance to pacing-induced myocardial ischemia.* **Tarnow J, Markschies-Hornung A, Schulte-Sasse U.** 1986, Anesthesiology, Vol. 64, pp. 147-56.

3. *Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium.* **Murry CE, Jennings RB, Reimer KA.** 1986, Circulation, Vol. 74, pp. 1124-36.

4. *Preconditioning: state of the art myocardial protection.* **Lawson CS, Downey JM.** 1993, Cardiovasc.Res., Vol. 27, pp. 542-50.

5. *Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart.* **Liu GS, Thornton J, Van Winkle DM, Stanley AW, Olsson RA, Downey JM.** 1991, Circulation, Vol. 84, pp. 350-6.

6. *Ischemic preconditioning: from adenosine receptor of KATP channel.* **Cohen MV, Baines CP, Downey JM.** 2000, Annu.Rev.Physiol, Vol. 62, pp. 79-106.

7. *Ischemic Preconditioning : Differences in Protection and Susceptibility to Blockade With Single-Cycle Versus Multicycle Transient Ischemia.* **Sandhu R, Diaz RJ, Mao GD, Wilson GJ.** 1997, Circulation, Vol. 96, pp. 984-95.

8. *Ca2+- and phospholipid-independent activation of protein kinase C by selective oxidative modification of the regulatory domain.* **Gopalakrishna R, Anderson WB.** 1989, Proc.Natl.Acad.Sci.U.S.A, Vol. 86, pp. 6758-62.

9. *Reactive Oxygen Species Precede the epsilon Isoform of Protein Kinase C in the Anesthetic Preconditioning Signaling Cascade.* **Novalija E, Kevin LG, Camara AK, Bosnjak ZJ, Kampine JP, Stowe DF.** 2003, Anesthesiology, Vol. 99, pp. 421-8.

10. *Regulation and pharmacology of the mitochondrial permeability transition pore.* **Zorov DB, Juhaszova M, Yaniv Y, Nuss HB, Wang S, Sollott SJ.** 2009, Cardiovasc.Res., Vol. 83, pp. 213-25.

11. *New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway.* **Hausenloy DJ, Yellon DM.** 3, 2004, Cardiovasc Res, Vol. 61, pp. 448-60.

12. *Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore.* **Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, Fishbein KW, Ziman BD, Wang S, Ytrehus K, Antos CL, Olson EN, Sollott SJ.** 2004, J.Clin.Invest, Vol. 113, pp. 1535-49.

13. *Protection in the aged heart: preventing the heart-break of old age?* **Juhaszova M, Rabuel C, Zorov DB, Lakatta EG, Sollott SJ.** 2005, Cardiovasc.Res., Vol. 66, pp. 233-44.

14. *Cardioprotection of the aged rat heart by GSK-3beta inhibitor is attenuated: age-related changes in mitochondrial permeability transition pore modulation.* **Zhu J, Rebecchi MJ, Glass PS, Brink PR, Liu L.** 2011, Am.J.Physiol Heart Circ.Physiol, Vol. 300, pp. H922-H930.

15. *Ischemic and non-ischemic preconditioning: Endothelium-focused translation into clinical practice.* **Jabs A, Fasola F, Muxel S, Munzel T, Gori T.** 2010, Clin.Hemorheol.Microcirc., Vol. 45, pp. 185-91.

16. *Preconditioning protects coronary arteriolar endothelium from ischemia-reperfusion injury.* **DeFily DV, Chilian WM.** 1993, Am.J.Physiol, Vol. 265, pp. H700-H706.

17. *Role of nuclear factor kappaB in cardiovascular health and disease.* **Van der Heiden K, Cuhlmann S, Luong lA, Zakkar M, Evans PC.** 2010, Clin.Sci.(Lond), Vol. 118, pp. 593-605.

18. *Inducible Nitric Oxide Synthase Modulates Cyclooxygenase-2 Activity in the Heart of Conscious Rabbits During the Late Phase of Ischemic Preconditioning.* **Shinmura K, Xuan YT, Tang XL, Kodani E, Han H, Zhu Y, Bolli R.** 2002, Circulation Research, Vol. 90, pp. 602-8.

19. *Desflurane-Induced Preconditioning Has a Threshold That Is Lowered by Repetitive Application and Is Mediated by beta(2)-Adrenergic Receptors.* **Lange M, Redel A, Smul TM, Lotz C, Nefzger T, Stumpner J, Blomeyer C, Gao F, Roewer N, Kehl F.** 5, 2009, J.Cardiothorac.Vasc.Anesth., Vol. 23, pp. 607-13.

20. *The effects of interrupted or continuous administration of sevoflurane on preconditioning before cardio-pulmonary bypass in coronary artery surgery: comparison with continuous propofol.* **Bein B, Renner J, Caliebe D, Hanss R, Bauer M, Fraund S, Scholz J.** 2008, Anaesthesia, Vol. 63, pp. 1046-55.

21. *Dual exposure to sevoflurane improves anesthetic preconditioning in intact hearts.* **Riess ML, Kevin LG, Camara AK, Heisner JS, Stowe DF.** 2004, Anesthesiology, Vol. 100, pp. 569-74.

22. *ACC/AHA 2007 guidelines on perioperative cardiovascular evaluation and care for noncardiac surgery: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines.* **Fleisher LA, Beckman JA, Brown KA, Calkins H, Chaikof EL, Fleischmann KE, Freeman WK, FroehlichJB, Kasper EK, Kersten JRRiegel B and Robb JF.** 17, 2007, Circulation, Vol. 116, pp. 418-499.