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Cardiovascular diseases

Models of myocardial ischemia

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The heart is highly dependent on aerobic metabolism. Given the widespread occurrence of coronary atherosclerosis in the human, myocardial ischemia is a huge clinical problem. Myocardial ischemic is a molecular disease of cardiomyocytes, but also, as a consequence of reduced heart function, becomes a disease of the whole organism. Experimental models range from large animal models designed to mimic the human clinical situation, to gene modified mice, isolated hearts and cell cultures.

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Introduction

Heart disease includes a large number of different diseases such as genetically derived defects in ion channels, congenital disease, heart valve disorders, cardiomyopathies, myocardial infarction and heart failure. In human medicine, it is assumed that the combat of ischemic heart disease and its consequences will be a worldwide challenge for years to come. Ischemic heart disease is listed among the degenerative diseases and diseases of an aging population. Some of the available Internet resources describing the impact of ischemic heart disease are listed in Links.

To establish useful models for ischemic heart disease, we not only need models of disease mechanisms to understand how and why injury and malfunction occur (Box 1), but also need models for further development of interventions like surgery, revascularization and tissue engineering as well as good models for large scale drug testing. When investigating molecular mechanisms, mice models have been increasingly popular in the latest years owing to the possibility of studying gene-modified animals and this area is rapidly expanding. Genetically engineered mice strains with single gene knockout and the development of heart-specific conditional knockout techniques are continually expanding our understanding of the gene products regulating susceptibility towards ischemia.

Furthermore, the use of cell imaging and cell culture technique has given a new momentum to ischemia research. Techniques previously only used within cell proliferation and cancer research have proven useful. The ischemic heart and ischemic heart disease involves an organ with severe dysfunction and injury at the molecular level, but with great consequences for the integrated organism. This is the background for the diversity of experimental methods needed in this research field.

In vivo models

Experimental models aimed at studying myocardial ischemia in the integrated organism are important in human medicine given the limited possibilities for well-controlled human studies. Whether the investigator wants to be close to the situation in human medicine or studies the influence of extra-cardiac factors in ischemic heart disease, the model of choice will be an *in vivo* model. This also includes development of new clinical methods, instruments and pharmacological agents. Most *in vivo* experimental models of myocardial ischemia have been established in the dog, pig, rabbit, rat or mouse. Although other species have also been used, the accumulated knowledge of the most often used laboratory animals in models of myocardial ischemia cannot be underscored.

In vivo models are usually divided into *chronic* or *acute* models. In addition, myocardial ischemia can be studied in

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Glossary

Hibernation: is defined as reversible reduction in contractile function due to reduced coronary perfusion. The original definition relating to chronic ischemia is extended to also include acute hibernation. Hibernation may represent an endogenous regulation resulting in reduction in function to reduce oxygen demand and preserve cellular integrity. With chronic hibernation normalization of ultrastructure and recovery of function may be delayed after normalization of blood flow. Because collateral flow development is sparse in the pig heart, this species is often used for hibernation studies.

Ischemic postconditioning: is protection against ischemia-reperfusion injury caused by one or multiple very brief, transient ischemic episode (30–60 s) followed by reperfusion (30–60 s) at early reperfusion after a prolonged ischemic insult. The protective mechanism that limits injury is rapidly activated and maintained for several hours, but it can only be activated close in time to the start of reperfusion. Postconditioning can be instituted *in vivo* as well as *ex vivo* in isolated perfused hearts.

Ischemic preconditioning: is protection against ischemic injury (mainly infarction) caused by one or multiple, transient ischemic episode before a prolonged ischemic insult. Five to ten minutes transient ischemia is usually needed for preconditioning to be triggered. This results in acute (1–2 h) as well as delayed (24–72 h) protection. Preconditioning as phenomenon has proven to be very robust with respect to experimental models and species. Acute preconditioning can be instituted in the *in vivo* situation as well as in *ex vivo* isolated perfused hearts.

Stunning: refers to reversible post-ischemic dysfunction in the setting of normalised coronary perfusion. The term came into use at the beginning of the 1980s and gained increasing interest after reperfusion became clinically possible. This also led to the understanding that stunning was not only a laboratory phenomenon but occurred also in humans subjected to either therapeutic or spontaneous reperfusion. The stunning phenomenon was extensively reviewed by Mareban and Bolli in 1999 [38] who also stressed the importance of the following statement: stunning is sometimes inappropriately applied to situations in which the persistence of contractile abnormalities in postischemic tissue is due to other causes (such as myocellular death, persistent ischemia or nonischemic injury). It is important to note that the stunning phenomenon is observed and can be investigated both *in vivo* and *in vitro* but that the extent and duration of the stunning are highly model-dependent. It is assumed that dysfunction is due to ROS production and decreased myofilamental calcium responsiveness. The ROS-calcium relationship at ischemia-reperfusion seems model dependent and it follows that it will be difficult to design universal treatment regimes.

conscious or *anaesthetized state*. The majority of studies are acute ischemia in the anaesthetized animal. This requires understanding of the impact of the anaesthetic agent in use and inclusion of sham operated animals and timed controls. In the clinical situation spontaneous or intentional ischemia is regional (coronary occlusion, angioplasty) or global (lethal arrhythmias or coronary bypass surgery). Correspondingly, experimental models are aimed at simulating either global ischemia or regional ischemia.

Dog models

Historically, experiments with myocardial ischemia in the anaesthetized dog have led to basic understanding of heart function and overall hemodynamic changes during regional ischemia. Techniques for measurements of oxygen consumption and substrate metabolism under ischemia combined with estimates of heart work were originally established in the *in situ* canine heart. Regional ischemia and infarct models were developed for testing potential cardioprotective compounds. An example demonstrating the use of established knowledge about infarction and tissue lipid metabolism in the dog heart combined with newly raised questions is the use of specific cytochrome P-450 (CYP) antagonists for cardioprotection [1]. The role of arachidonic acid metabolites in myocardial ischemia has been questioned throughout the years, more recently in conjunction with the clinical use of cyclooxygenase COX II blockers and the increasing understanding of the diversity of the various CYP products. Nithipatikom *et al.* 2006 [1] used regional ischemia in anaesthetized dogs to investigate the role of cytochrome *P*-450-hydroxylases and 20-HETE in an infarct model. A reduced level of 20-HETE was associated with reduction in infarct size. COX and lipoxygenase enzymes metabolize arachidonic acid (AA) to 5-, 12- and 15-hydroxyeicosatetraenoic acid (HETE), prostaglandins, prostacyclin, thromboxane and leukotrienes. The third enzymatic pathway for the metabolism of AA is by cytochrome *P*-450 (CYP) to epoxyeicosatrienoic acids (EETs), dihydroxyeicosatetraenoic acids (DiHETEs) and 19- and 20-HETE, and also other HETEs and reactive oxygen species (ROS). Gottlieb and co-workers indicated in their studies that EETs are beneficial and 20-HETEs detrimental in ischemia. It is known that HETEs induce vasoconstriction [2]. Endothelium produced EET are also described as endothelial derived hyperpolarization factors (EDHF).

A second model is a chronic model with a surgically implanted ameroid constrictor placed around the left coronary artery. This results in gradual narrowing of the artery lumen and corresponding gradual reduction in ejection fraction and other contractile parameters of the affected myocardium over a few weeks. The dog heart has a great potential for coronary collateral vessel growth and therefore this model is convenient for studying the regulation of clinically

Box 1. Ischemic injury – a cell perspective

- Contractile failure
  - ATP and CP loss
  - Diastolic calcium overload
  - Cytosolic proton accumulation
  - Increase in cellular free phosphate
- Arrhythmias
  - Partial depolarization of membrane potential
  - Conduction delay
  - Shortening of the action potential
- Cardiomyocyte cell death (necrosis, apoptosis, oncosis)
  - Mitochondrial permeability transition
  - Cytosolic calcium overload
  - Caspases
  - Hypercontraction, sarcolemma rupture