Porcine myocardial ischemia-reperfusion studies on cardioprotection, ventricular arrhythmia and electrophysiology

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Gothenburg 2009

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ISBN 978-91-628-7921-1
http://hdl.handle.net/2077/21176

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Cover drawing by Joel Grip.
Printed by Geson Hylte Tryck, Göteborg, Sweden 2009

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ABSTRACT

Background: Coronary artery disease is the primary cause of death in adults in the industrialised world and ventricular fibrillation associated with myocardial ischemia is the main cause of sudden cardiac death. Restoration of blood flow and preservation of myocardial integrity throughout ischemia and reperfusion is essential to improve clinical outcome. Alteration in calcium handling and its consequences are central features of these events. Sympathico-vagal imbalance and electrophysiological alterations are important predisposing factors for malign ventricular arrhythmia and sudden cardiac death.

Aims: To investigate whether ultra-short acting calcium antagonism or spinal cord stimulation (SCS) could reduce myocardial ischemia and infarct size in a porcine closed-chest model. Furthermore, the feasibility of endocardial electromechanical mapping for defining myocardial viability during acute infarction was evaluated. Finally, non-invasive electrophysiological characteristics of ischemia-reperfusion and the occurrence of ventricular arrhythmias were investigated as well as the effects of SCS on these measures and events.

Methods: Myocardial infarction was induced by 45 minute coronary occlusion in closed-chest landrace pigs. An ultra-short acting calcium antagonist, clevidipine, was administered into the myocardium at risk. Myocardial viability was assessed by Evans Blue, tetrazolium and endocardial electromechanical mapping and the correlation between these methods was investigated. Three-dimensional vectorcardiography was continuously recorded, analysed offline with regard to depolarisation and repolarisation parameters, and later correlated to myocardial ischemia and ventricular arrhythmia. In a second series of experiments, the effects of SCS were investigated with regards to haemodynamics, infarct size, ventricular arrhythmia and electrophysiology.

Results: Clevidipine did not reduce infarct size. Electrical and mechanical activities were both impaired within the infarct zone, but the precision of electromechanical mapping to identify an infarct was poor, and due to intersegmental variability and arrhythmia. All T vector loop parameters changed in response to ischemia. Ventricular arrhythmia was more prevalent during proximal left anterior descending coronary artery occlusion, which was associated with more pronounced electrophysiological alterations. In the SCS group, ventricular arrhythmia occurred less frequently in association with signs of less ischemia and electrical alterations. SCS did not reduce infarct size.

Conclusions: Infarct size was neither reduced by ultra-short acting calcium antagonism nor by SCS, but the latter seemed to have cardioprotective properties as it reduced the occurrence of ventricular arrhythmia. Endocardial electromechanical mapping was not feasible for acute myocardial viability assessment.

Keywords: porcine; myocardial ischemia; ventricular arrhythmia; sudden cardiac death; electrophysiology; vectorcardiography; endocardial mapping; spinal cord stimulation

ISBN 978-91-628-7921-1; http://hdl.handle.net/2077/21176 Gothenburg 2009

LIST OF ORIGINAL PAPERS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals:

I Odenstedt J, Månsson C, Grip L.
Failure to demonstrate myocardial protective effects of the ultra short-acting calcium antagonist clevidipine in a closed-chest reperfusion porcine model.
Journal of Cardiovascular Pharmacology 2004;44(4):407-415.

II Odenstedt J, Månsson C, Jansson SO, Grip L.
Endocardial electromechanical mapping in a porcine acute infarct and reperfusion model evaluating the extent of myocardial ischemia.
Journal of Invasive Cardiology 2003;15(9):497-501.

III Odenstedt J, Rubulis A, Grip L, Bergfeldt L.
Distorted T-vector loop and increased heart rate are associated with ventricular fibrillation in a porcine ischemia-reperfusion model.
Journal of Electrocardiology 2009;42(3):267-273.

IV Odenstedt J, Linderoth B, Bergfeldt L, Ekre O, Grip L, Mannheimer C, Andréll P
Effects of spinal cord stimulation on myocardial ischemia, infarct size, ventricular arrhythmia and non-invasive electrophysiology in a porcine ischemia-reperfusion model.
In manuscript.

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ABBREVIATIONS

3-D	three-dimensional
AAR	area at risk
AP	action potential
APD	action potential duration
ATP	adenosine triphosphate
CABG	coronary artery bypass grafting
CAD	coronary artery disease
ECG	electrocardiography
IPC	ischemic preconditioning
IPost	ischemic postconditioning
IQR	interquartile range
IS	infarct size
LAD	left anterior descending artery
NSVT	non-sustained ventricular tachycardia
PCI	percutaneous coronary intervention
ROS	reactive oxygen species
SCD	sudden cardiac death
SCS	spinal cord stimulation
SD	standard deviation
SEM	standard error of mean
STVM	ST vector magnitude
SVT	sustained ventricular tachycardia
TENS	transcutaneous electrical nerve stimulation
UPV	unipolar voltage
VCG	vectorcardiography
VF	ventricular fibrillation
VR	ventricular repolarisation
VT	ventricular tachycardia

INTRODUCTION

Coronary artery disease (CAD) is the primary cause of death in adults in the industrial world. Sudden cardiac death (SCD), ascribed mainly to ventricular fibrillation, is the single most important contributor accounting for 13% of all natural deaths and for ~50% of all cardiovascular mortality.[1-4] The underlying cause of SCD is CAD in 80%, cardio-myopathy in 15% and primary electrical disorders in 5%.[5] Besides immediate mortality, ischemic heart disease causes considerable morbidity such as post-infarction left ventricular failure and angina pectoris. Coronary occlusion induces metabolic, ionic and neurohumoral imbalances that might conclude with lethal myocardial injury and arrhythmia. To limit these harmful effects and to improve clinical outcome preservation of myocardial integrity throughout ischemia and reperfusion is essential.

ACUTE MYOCARDIAL ISCHEMIA AND REPERFUSION

Reversible and irreversible acute ischemic injury

Ischemic myocardial cell injury can be either reversible or irreversible.[6, 7] Acute ischemia, postischemic dysfunction (stunning) and myocardial hibernation are all forms of reversible myocardial dysfunction. In myocardial stunning, the recovery of myocardial function is delayed despite the restoration of coronary artery flow. In hibernation, there is a persistent dysfunction of viable myocardium at rest due to regional hypoperfusion. For this condition function normalises only after successful revascularisation.[8] Abrupt coronary artery occlusion, causing cessation of myocardial perfusion, eventually results in irreversible myocardial cell injury if collateral flow is absent and reperfusion does not occur.[7, 9] After 20-30 minutes of experimental ischemia in canine and porcine models cell death begins to appear in the endocardium, the region with the most pronounced metabolic demand and the least developed collateral flow reserve, and propagates gradually towards the epicardium in a wavefront manner.[7, 10-13] Diminished myocardial oxygen supply inhibits mitochondrial adenosine triphosphate (ATP) synthesis, metabolism becomes anaerobic and generates an accumulation of metabolic products.[7] Dysfunction of membrane ion pumps causes intracellular accumulation of H^+, Na^+ and Ca^{2+} and eventually permanent contractions, membrane fragmentation and cell swelling.[14] The amount of irreversibly damaged myocardium proceeds over time, and restoration of coronary blood flow, i.e. reperfusion by fibrinolysis, percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG), is pivotal in order to salvage reversibly endangered myocardium.[10, 11, 15, 16]

Identification of reversible and irreversible myocardial injury

In both the acute and chronic stages of CAD it is essential to separate viable and reversibly injured myocardium from irreversible injury, as this has both diagnostic and therapeutic implications. Modalities such as positron emission tomography, myocardial perfusion imaging and stress echocardiography have been developed to identify and quantify myocardial viability as revascularisation of hibernating myocardium may improve clinical outcome in CAD patients.[17]

Newer techniques, such as magnetic resonance imaging, contrast echocardiography and electromechanical mapping, have emerged.[17] The latter imaging technology is a computerised nonfluoroscopic, catheter-based endocardial mapping system that enables the simultaneous assessment of electrical and

mechanical myocardial activity as well as anatomic characterisation, displayed as 3-dimensional (3-D) electromechanical maps.[18-21] Preserved electrical myocardial activity indicates viability and reduced mechanical activity can either represent infarcted or ischemic myocardium.[22] Mapping has, in chronic CAD, shown good agreement with many other techniques and can, in addition, be employed in conjunction with coronary angiography for diagnostic as well as for therapeutic (gene or cellular transfer) purposes.[17, 23-25]

Reperfusion injury

Irreversible reperfusion-induced injury to viable myocytes has been shown in both animal models and clinical studies.[26-30] Reperfusion *per se* can induce cascades of harmful events in the ischemic myocardium, manifested as stunning, no-reflow phenomenon, arrhythmia and lethal reperfusion injury.[26, 31, 32] The latter causes irreversible reperfusion injury to ischemic, but still viable, cardiomyocytes and contributes to the final infarct size. In preclinical studies, various interventions targeting lethal reperfusion injury have reduced the final infarct size by up to 50%, although, in general, these achievements have not been translated into clinical benefit.[3, 33]

The pathophysiological mechanisms underlying lethal reperfusion injury have been subjected to extensive research and, although not yet fully understood, ascribed to mitochondrial reenergisation, reactive oxygen species (ROS), rapid normalisation of pH and an exacerbated overload of intracellular Ca^{2+}. Myocardial reoxygenation and restarting of the mitochondrial ATP synthesis after a prolonged period of severe ischemia initiate a cascade of harmful events leading to cellular damage.[3, 14, 31, 34] Ionic pump activity is resumed in order to correct for the intracellular excess of Na^+, Ca^{2+} and H^+, and acidosis is promptly corrected by H^+ efflux. Subsequently, intracellular Na^+ is exchanged for Ca^{2+}, adding to the intracellular Ca^{2+} burden. Calcium will be sequestered via an ATP-dependent pump into the sarcoplasmic reticulum, but if its capacity is exceeded, Ca^{2+} oscillations will commence. If Ca^{2+} is not extruded from the cytosol, myofibrillar permanent hypercontractions are initiated and might, together with hydrolytic proteins in the sarcolemma and cytoskeleton, lead to membrane damage. Hypercontraction, cyto-skeletal damage and contraction band necrosis[13, 35] propagate from cell to cell via Na^+ through interconnecting gap junctions and a subsequent rise in calcium. Inflammation will attract neutrophils and add to the damage by microvascular plugging and release of degradative enzymes and reactive oxygen species. Last but not least, apoptosis contributes to the final infarct size.[3, 14, 31]

Although not within the scope of my experimental studies, ROS, inflammation and the mitochondrial permeability transition pore (mPTP) in relation to pre- (IPC) and post- (IPost) conditioning will be briefly described as these pathways enlighten important cardioprotective mechanisms relevant to the present thesis.

Reactive oxygen species (ROS): Despite extensive experimental efforts, the role of oxidative stress in humans is still debated, as clinical studies have been inconclusive. ROS are highly unstable compounds that are generated during normal cellular metabolism via incomplete oxygen reduction and are involved in the regulation of fundamental cellular activities. However, ROS are released during ischemia and increase rapidly upon reperfusion, serving as an important mediator of reperfusion injury.[14, 36, 37] Potential injury mechanisms involved are ion pump inhibition (Ca^{2+} overload), membrane lipid peroxidation (cell swelling), chemotaxis and activation of neutrophils (plugging of capillaries and a potent source of ROS). ROS are generated from endothelium,

cardiomyocytes (mitochondria and cytoplasma) and by activated neutrophils.

Inflammation: The role of inflammation is complex and remains controversial despite experimental evidence of harmful effects.[14, 38, 39] Myocardial ischemia gradually initiates neutrophil infiltration, which peaks after 2-4 days. Reperfusion accelerates and increases the inflammation in endothelial cells and cardiomyocytes and this inflammation is thought to play an important role in lethal reperfusion injury.

Neutrophils are activated by and attracted/adhere to factors derived from the myocardium (endothelium, myocytes and mast cells), such as complement fragments and cytokines, ROS and lipid mediators. The activated neutrophils mediate their harmful effect via proteolytic enzymes and ROS. The interaction with endothelial cells is a central feature of the inflammatory response and neutrophils will eventually migrate into the interstitial space and adhere to myocytes. As a consequence of the inflammatory response, the endothelial vasodilatory capability is reduced, vasoconstrictive molecules released and platelets activated leading to distal plugging and diminished coronary flow (no-reflow). Furthermore, many of the mediators released by the neutrophils are directly cytotoxic and contribute to myocardial necrosis.

Mitochondrial permeability transition pore (mPTP): Recent studies have highlighted the possible importance of the state of the mPTP for cell survival. The mPTP is normally closed and remains closed during ischemia due to the acidic milieu. At the time of reperfusion, rapid normalisation of pH together with phosphate overload, ATP depletion, excess of ROS and calcium triggers the mPTP to open and enable influx of Ca^{2+} and H^{+}. This results in loss of mitochondrial integrity, swelling and rupture. The proposed mechanisms by which IPC and IPost limit infarct size are either via an indirect (intracellular calcium handling, ATP preservation, ROS and pH correction) or direct (signal transduction pathways) inhibition of the mPTP. Both IPC and IPost activate adenosine, bradykinin and opioid G protein-coupled receptors on the sarcolemma, which trigger signal transduction pathways and, finally, inhibit mPTP opening. In addition to reduced ROS at the onset of reperfusion, both IPC and IPost induce ROS signalling that trigger protective transduction pathways.[40]

Calcium antagonism

Numerous experimental myocardial ischemia-reperfusion studies have investigated the effect of various calcium antagonists and shown a significant reduction in infarct size.[41] Clinical evidence, however, remains inconclusive.[33] The exact cardioprotective mechanisms are unclear, but have been suggested to be related to bradykinin and nitric oxide[42, 43], activation of K^{+} ATP channels[44], a protective effect against oxygen free radicals[45], attenuated neutrophil accumulation[46] and amelioration of ischemia-induced endothelial cell permeability.[47] The long-lasting effect of the calcium antagonists has made it difficult to discern local myocardial from systemic actions. Furthermore, the timing of drug administration in relation to ischemia and reperfusion is critical in order to achieve protective effects.

Clevidipine, a third generation dihydropyridine L-type Ca^{2+} channel blocker developed from felodipine, is characterised by ultra-short acting arterial selective properties. The initial half-life in man is less than three minutes and arterial steady-state is reached within two minutes.[48] Clevidipine was investigated in a series of open-chest porcine ischemia and reperfusion studies.[49] Segawa et al. demonstrated a reduced infarct size by local delivery of clevidipine during the early phase of ischemia or at the time of reperfusion, phases when intracellular Ca^{2+}

overload is known to occur.[50-52] Gourine et al. suggested, from experiments using the same porcine model, that the cardioprotective effect was mediated by mechanisms related to bradykinin and nitric oxide.[53, 54]

SUDDEN CARDIAC DEATH

Sudden cardiac death is defined as death from an unexpected circulatory arrest, usually due to cardiac arrhythmia occurring within one hour from the onset of symptoms.[4]

Mechanisms

Ventricular tachycardia (VT) degenerating to ventricular fibrillation (VF) and eventually asystole seems to be the dominating sequence in SCD associated with CAD according to ambulatory electrocardiogram (ECG) recordings.[5, 55] Lethal arrhythmia usually requires an underlying substrate, its modulation and a triggering factor.[56] Trigger factors include stress, drugs, ischemia-reperfusion, acidosis, electrolyte imbalance, inflammation, hypoxia, hemodynamic changes and stretch and imbalance in the autonomic nervous system.[56] Absence of interaction between substrate and modifying and triggering factors can explain why not more CAD patients suffer from lethal arrhythmias.

The risk of malignant ventricular arrhythmia is highest within the first 30 minutes of experimental acute myocardial ischemia and a majority of triggers for VF occur in the infarct boarder zone.[57, 58] Reduced excitability, slowed conduction, altered refractoriness and increased wall stress promote malignant arrhythmia, together with other factors.[2, 57, 59] A combination of impaired conduction and altered (heterogeneous) repolarisation might set the stage for re-entrant ventricular arrhythmias and is considered to be the dominant mechanism of VT/VF.[60] Triggered activity due to delayed afterdepolarisations caused by calcium overload may also be a contributing factor.[2, 56, 61]

Cardiac cellular electrophysiology

Cardiac action potential

Electrical signalling of the heart involves passage of Na^+, K^+, Ca^{2+} and Cl^- through transmembranic ion channels. The concentration of K^+ ions is relatively high inside the cell, whereas Na^+ ions have a higher concentration outside. The normal transmembrane resting potential of cardiac myocytes is approximately the equilibrium potential for K^+ (-90mV, negative inside).[62]

The cardiac action potential (AP) is the result of a complex interaction between depolarising inward and repolarising outward currents. The depolarising currents originate mainly from Na^+ and Ca^{2+}, whereas repolarisation results from K^+. The five phases (0-4) of the AP are briefly described below and illustrated in Figure 1.[62]

Phase 0: Initial Na^+ influx causes rapid reversal of the membrane potential and membrane depolarisation. Phase 1: A transient outward K^+ current rapidly repolarises the membrane to nearly 0mV. Phase 2: Subsequently, an extended plateau phase is maintained by inward Ca^{2+} currents and counterbalanced by outward K^+ currents. The inflow of Ca^{2+} triggers massive Ca^{2+} release from the sarcoplasmic reticulum, increasing the cytosolic calcium level and causing contraction. Phase 3: Finally, the resting membrane potential is restored by K^+ efflux and removal of Ca^{2+} from the cytosol, deactivating contractile proteins, thereby relaxing the cardiac muscle. Phase 4: During the diastolic phase the membrane potential remains relatively constant and near the K^+ equilibrium because of an inward K^+ current in the working myocardium. In contrast, there is spontaneous depolarisation during phase 4 in pacemaker cells (primarily in the sinus node).

Ventricular activation and recovery

Ventricular depolarisation

The QRS complex reflects the sum of the spatio-temporal vectors of ventricular activation. In humans, ventricular excitation rapidly spreads along the specialised intracardiac conduction system (His-Purkinje system) in the endocardium, starting on the septal surface, down and around the anterior free walls to the posterior and basal regions in an apico-basal direction. Consequently, most of the endocardial surfaces of both ventricles depolarise within several milliseconds. The activation front then propagates from cell to cell towards the epicardium.[63] Slow intraventricular conduction is a key component of arrhythmic substrate, and QRS prolongation might therefore be expected to predict the risk of SCD.[64]

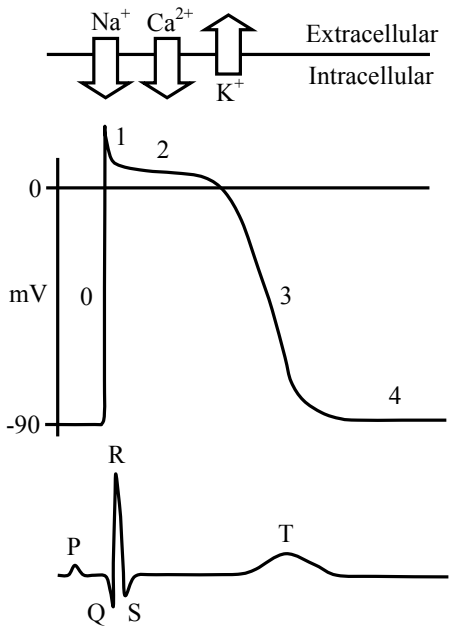


Figure 1. The myocardial action potential with corresponding electrocardiogram and main ion currents. See text for details.

Ventricular repolarisation

Repolarisation occurs roughly in the reverse order compared with depolarisation. Action potential duration (APD) is more extended in endocardial layers (activated first) and thus minimises the transmural dispersion arising from the delayed activation of epicardial layers (activated later). Consequently, repolarisation tends to be homogeneous although some degree of heterogeneity exists on the regional (base vs. apex, anterior vs. posterior, right vs. left ventricle), transmural (epi- vs. mid- and endocardium) and temporal (beat-to-beat) level.[63] The AP dispersion is highly relevant, but large dispersion between distant regions might exist without pro-arrhythmic consequences, whereas heterogeneity between adjacent sites can trigger arrhythmia.[65]

Effects of myocardial ischemia

Compared with the contracting myocytes, the specialised conduction system (Purkinje network) is more resistant to acute ischemia and thus related electrophysiological consequences are less intense and more delayed.[1] Furthermore, the amplitude of QRS is more affected than its duration during acute myocardial ischemia. Depolarisation is less susceptible to ischemia than repolarisation (ST-T).[66]

As a consequence of severe, acute myocardial ischemia, intracellular Na^+ increases and contributes to loss of intracellular K^+, extracellular K^+ accumulation, and increase in intracellular Ca^{2+}. The latter might cause triggered arrhythmias via delayed afterdepolarisations. Opening of K^+ channels also causes accumulation of extracellular K^+, which will partially depolarise and thereby decrease the transmembrane potential (less negative) within the ischemic myocardium. The rate of Na^+ influx decreases, and consequently, conduction velocity declines due to a reduced rate of rise and amplitude of the initial rapid

depolarisation (phase 0).[2, 62] In addition, excitability and conduction velocity are more rapidly depressed epicardially than in the endocardium, leading to transmural heterogeneity.[1]

Voltage gradients (local injury currents) both between normal and ischemic regions and transmurally are represented as ST deviations on the ECG.[63] Potassium concentration modulates the cardiac automaticity, excitability and refractoriness. The increased extracellular K^+ (peaked T waves) causes a large dispersion of the repolarisation across the ischemic border zone.[1] The duration of the AP is shortened by acute ischemia[63], but refractoriness extends beyond the AP during ischemia.

Risk markers for sudden cardiac death

Several non-invasive and invasive risk markers have been studied. They are based on clinical data, electrophysiological principles (e.g. conduction, intervals, late potentials, T wave alternans, programmed stimulation and many more), sympathetic-parasympathetic balance (e.g. heart rate variability and baroreceptor sensibility) and ventricular function (e.g. left ventricular ejection fraction). Although informative on group level, the positive predictive value of single or combined risk factors hardly exceeds 20%, i.e. at least five patients need to be treated with implantable cardiac defibrillator to save one from SCD. In contrast, several of these risk factors have a very high negative predictive value, i.e. severe cardiovascular events are rare in their absence (Figure 2).[4, 68-70]

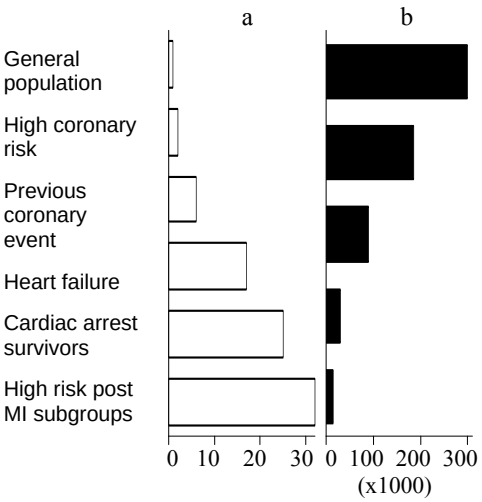


Figure 2. Epidemiology of sudden cardiac death: percentage per group (a) and number per year (b). Adapted from Myerburg RJ et al.[67]

VECTORCARDIOGRAPHY

A 12-lead ECG can be synthesised with good approximation from vectorcardiography (VCG) according to the Dower transformation.[71] An inverse Dower transformation is also feasible in order to synthesise a VCG from a 12-lead ECG.[72] In the present thesis, continuous digital 3-D VCG was performed (MIDA 1000 and CoroNet; Ortivus, Sweden) using eight electrodes positioned according to the Frank orthogonal lead system, modified for the porcine model (Figure 3).

The electrical field of the heart can be represented by a single vector. The length of the vector represents the magnitude and the direction depicts the spatial orientation of the electrical forces. When all the instantaneous single vectors are plotted consecutively, a continuous vector loop is formed in 3-D space.[73] The spatial VCG consists of three consecutive vector loops (afterwards: loop), the P loop (atrial depolarisation), the QRS loop (ventricular depolarisation) and the T loop (ventricular repolarisation), and can be analysed according to several parameters: the magnitude and direction of the maximum vector in space, the loop morphology and the angle between the maximum QRS and T vectors. In humans, the maximum QRS and T vector are usually oriented in a left, inferior and anterior direction. The T vector increases and point towards the ischemic area in response to left anterior descending coronary

artery (LAD) occlusion and, as a consequence, the QRS-T angle widens.[74] Theoretically, the QRS-T angle represents the spatial deviation between de- and repolarisation and reflects the heterogeneity of APD relevant to arrhythmogenesis.[75] The shape of the T loop has been related to increased ventricular repolarisation (VR) heterogeneity, i.e. a more distorted and circular T loop.[74, 76-82] Tarea changes in parallel with Tamplitude and Tpeak-end and seem to reflect important aspects of VR, such as repolarisation gradients and heterogeneities.[82]

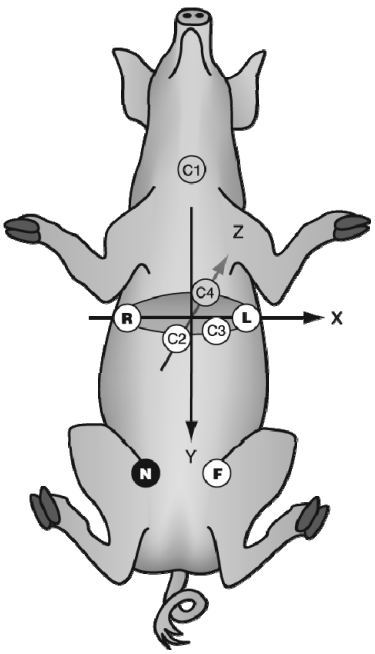


Figure 3. The eight vectorcardigraphic electrodes were positioned according to the Frank orthogonal lead system (X, Y, and Z): C1, in the center of the dorsal side of the neck; C2, over the heart, in the center of the ventral part of the thorax; C3, in between C2 and L; C4, in the center of the dorsal part of the thorax (corresponding to C2); R, to the right of C2 and on the "mid-lateral" line; L, to the left of C2 and on the "mid-lateral" line; F, ventral and medial of the left hind leg; N (ground), ventral and medial of the right hind leg. Modified after Näslund et al.[83]

The VCG has several advantages over the conventional 12-lead ECG in describing repolarisation: 1) the ability to provide information about the spatial orientation of repolarisation[73]; 2) the opportunity to construct an anatomically representative T vector loop; 3) T loop analysis is less dependent on determination of the T wave end than scalar ECG analysis of the QT interval and QT dispersion.[84, 85] The association between the increased heterogeneity of repolarisation and changes in T loop morphology has been demonstrated[76-78], but not been correlated to the occurrence of ventricular arrhythmia. The mechanistic aspects and clinical relevance of alterations in VCG parameters remain to be elucidated.

SPINAL CORD STIMULATION

Refractory angina pectoris, defined as a chronic condition with reversible myocardial ischemia not controlled by medical therapy, PCI or CABG, can be relieved by non-pharmacological interventions and the susceptibility to SCD can be reduced by both pharmacological and non-pharmacological means.[86-88] Some neuromodulation techniques, such as transcutaneous electrical nerve stimulation (TENS) and spinal cord stimulation (SCS), have been applied in refractory angina pectoris since the mid-1980s.[89-93] There is scientific evidence that SCS offers symptomatic benefits, improves functional status and reduces stress-induced ST depression in CAD patients.[88, 94] The anti-ischemic effect of SCS seems to be related to a decrease in myocardial oxygen consumption rather than to an increase in coronary blood flow[94], but the underlying mechanism is incompletely understood. Stimulation-induced effects on sympathetic activity, myocardial metabolism and redistribution of coronary blood flow have been suggested.[95-99] SCS has in experimental studies been demonstrated to reduce both ischemia-induced ST elevation in canines[100]

and infarct size in an ischemia-reperfusion rabbit model.[101]

Heterogeneous increase in sympathetic activity and loss of parasympathetic activity are important predisposing factors for life-threatening arrhythmia and SCD in heart failure and myocardial infarction, whereas sympathetic blockade and parasympathetic stimulation can be protective.[2, 86, 87] SCS has, based on animal experiments, shown antiarrhythmic effect, which has been ascribed to sympatholytic and vagomimetic actions, and to the reduced infarct size.[86, 87, 101]

THE PORCINE CLOSED-CHEST MODEL

Species and models are important factors in experimental studies on myocardial ischemia and arrhythmia. Species-specific differences in pre-existing coronary collaterals exist and are one of the major determinants for the progression of necrosis in acute coronary occlusion, but are also important with regard to the development of VF.[102, 103] The porcine model shares many anatomical and physiological characteristics with humans, especially with regard to the cardiovascular system, including the distribution and function of coronary arteries.[104, 105] Pigs have a negligible amount of collaterals, whereas dogs are more resistant to ischemia due to well-developed subepicardial collaterals.[102] Studies have demonstrated almost complete transmural infarcts beyond 60-90 minutes of coronary artery occlusion in pigs, whereas ischemic myocardium can still be salvaged after 3-6 hours in the dog.[10, 12, 13, 102, 106]

Dogs, as compared to pigs, are more similar to humans when it comes to Purkinje fibers and ion channel distribution. This explains some of the species-specific differences in the transmural activation sequence during sinus rhythm and VF.[107]

SCD is the first manifestation of CAD in up to 50% of patients[108], and often occurs in individuals with moderate non-collateralised stenosis in a major coronary artery, especially the LAD.[109] Therefore the porcine model seems to be appropriate with regards to a first event of life-threatening ischemia in humans without pre-existing cardiovascular disease. Furthermore, a porcine closed-chest model is more clinically relevant than an open-chest model with respect to reperfusion treatment of acute myocardial infarction. PCI provides the opportunity of both restoring coronary flow and administering agents locally into the endangered myocardium, with the potential of modulating the processes involved in reperfusion injury.[110]

AIMS

To establish a clinically relevant porcine model and in this model evaluate:

- the effects of an ultra-short calcium antagonist on ischemia-reperfusion injury (paper I)
- the feasibility of electromechanical mapping for defining myocardial viability in the acute phase of infarction (paper II)
- non-invasive electrophysiological characteristics of ischemia-reperfusion and the occurrence of ventricular arrhythmias (papers III and IV)
- the effects of spinal cord stimulation on myocardial ischemia, infarct size, ventricular arrhythmias and non-invasive electrophysiology (paper IV).

MATERIAL AND METHODS

Ethics

The investigations conformed to the *Guide for the Care and Use of Laboratory Animals* published by the United States National Institutes of Health.[111] Approval from the local animal ethics committee was obtained.

Study designs and interventions

In total, 71 normally fed female Swedish landrace pigs (50 kg) were included in the ischemia-reperfusion studies presented in four papers. Papers I-III describe sub-cohorts of pigs originating from the same study, whereas paper IV is based on another study. The pigs were randomly assigned to the interventions in the first part of paper I (phase I below) and in paper IV. The investigator was blinded to the randomisation during the entire experimental protocol and during the analysis of infarct size, electromechanical maps, VCG and arrhythmia.

Ultra-short acting calcium antagonist (paper I)

The effect of the ultra-short acting calcium antagonist clevidipine (AstraZeneca R&D, Mölndal, Sweden) on myocardial ischemia-reperfusion injury was studied in 51 pigs divided into three phases. Phases I and II were closed-chest, whereas phase III was open-chest. The interventional drug was administered antegradely, through the occluded part of LAD and into the myocardium at risk via the central lumen of an angioplasty catheter. Drug administration was initiated at the end of ischemia and maintained during the initial part of reperfusion.

Phase I: Twenty-four pigs were randomly assigned to: A) placebo (saline), B) Intralipid (vehicle), C) clevidipine (bolus+infusion) or D) clevidipine bolus + saline infusion. Because of the inconsistent result in phase I compared with previous reports from other researchers, the protocol was modified and the study expanded with two additional explorative phases. *Phase II:* Nine pigs, all receiving clevidipine, were used to explore the impact of three different anaesthetics on ischemia-reperfusion injury: α-chloralose, isoflurane and sodium pentobarbital. *Phase III:* In another 18 pigs (open-chest model), four different LAD occlusion/reperfusion techniques (details below) were evaluated in five groups, in order to explore the mechanisms of any effect by clevidipine on myocardial ischemia-reperfusion injury (one group received vehicle). To reduce the risk of VF, the β-adrenoceptor antagonist metoprolol was given intravenously prior to reperfusion in all but two groups (phase II).

Electromechanical mapping (paper II)

Endocardial electromechanical mapping (see details below) was performed to investigate its capacity to differentiate between myocardium with evolving necrosis and viable myocardium. Mapping was assessed as part of phase I, together with four additional pigs mapped at baseline.

Measures of ventricular repolarisation (paper III)

VCG (see details below) was recorded, as part of the protocol in phase I and II, in order to further explore previously used non-invasive ventricular repolarisation parameters[74, 78, 79, 81, 82] and their relevance in relation to ischemia-reperfusion-induced VF.

Effects of spinal cord stimulation (paper IV)

Effects on hemodynamic measures, electro-physiology, ventricular arrhythmias and

infarct size were tested in 20 pigs randomly assigned to SCS or sham operation (see details below). The hemodynamic measures were analysed at the same time points as the VCG data (see below). The rate-pressure product was calculated as the product of the systolic blood pressure and the heart rate.

Animal preparation and myocardial ischemia-reperfusion

The pigs were pre-medicated with midazolam and ketamin. General anaesthesia was maintained by α-chloralose, isoflurane or sodium pentobarbital in papers I to III and by sodium pentobarbital in paper IV. For analgesia, buprenorphine was administered in papers I to III and fentanyl in paper IV. The pigs were ventilated with 40% oxygen and hydrated by infusion of intravenous fluids. Body temperature was maintained at around 38°C.

Introducers were inserted into the left common carotid artery for coronary artery access and into the right internal jugular vein for continuous monitoring of mean central venous pressure. Pulse oxymetry, heart rhythm and rate, and arterial pressure were continuously monitored, the latter via a cannula inserted into a superficial branch of the femoral artery. Blood gases were obtained at baseline as well as during ischemia and reperfusion. The pigs were allowed to recover and vital signs to stabilise for at least one hour prior to baseline measurements.

Anti-coagulation therapy was initiated prior to ischemia and maintained during the entire ischemia-reperfusion period. Coronary angiography was performed and myocardial infarction induced by 45 minute occlusion of the LAD; reperfusion was subsequently established and verified angiographically. In the closed-chest model (papers I to IV) an intracoronary balloon was inflated to occlude the LAD distal to its 2nd diagonal branch, except for the 2nd half of paper IV, in which the LAD was occluded distal to its 1st diagonal branch. In the open-chest model (paper I, phase III), a median sternotomy was performed and four different LAD occlusion/reperfusion techniques were evaluated: 1) intracoronary balloon inflation as above, 2) ligation over an intracoronary catheter without balloon, 3) intracoronary balloon inflation + ligature and 4) the LAD was dissected free of surrounding tissue followed by ligation over an intracoronary catheter. After four hours of reperfusion in papers I to III and two hours in paper IV, a third angiography was performed and the LAD re-occluded. Thereafter, 40 ml of 2% Evans Blue were infused via the central venous catheter.

Analysis of area at risk, infarct size and viability

Morphology (papers I to IV)

The right ventricle and the atria were removed from the excised hearts. The left ventricle was cut transversely into ~10 mm slices. The area at risk (AAR) was identified as the region not stained by Evans Blue (see above). The infarct size (IS) was indirectly measured by identifying the non-infarcted myocardium after incubation in 37°C for 10 to 20 minutes with 2, 3, 5-triphenyl-tetrazolium chloride. The stained areas were delineated on both aspects of each slice and subsequently, relative masses of the myocardium at risk and the infarcted myocardium were calculated. In order to compare the endocardial electromechanical maps (see below) with the extension of infarcted myocardium, the non-tetrazolium-stained endocardial surface was calculated.

Electromechanical mapping (paper II)

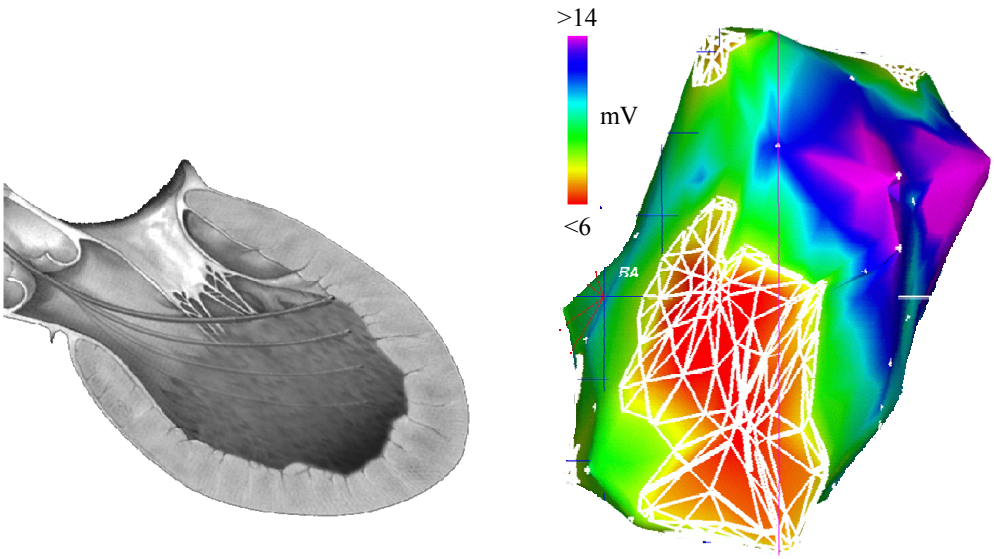
A deflectable-tip mapping catheter was inserted into the left ventricle via the left common carotid and endocardial 3-D electromechanical maps were recorded at baseline and two hours after reperfusion

(Figure 4). The local bipolar electrogram was manually analysed during post-map editing in order to enhance the online criteria used for good catheter-wall contact and stability.

Electrical maps: At each selected endocardial point, local amplitudes (mV) were obtained. *Mechanical maps:* Local linear shortening was obtained and expressed as a percentage by comparing the end-systolic and end-diastolic distances between neighbouring endocardial points. *Regional parameters:* For regional data analysis between different maps, a fixed polar cylindrical coordinate map was defined with the anatomic apex set as a reference. Nine segments were automatically created and the average value of each segment calculated.

Baseline and reperfusion regional maps were compared with regards to electrical activity and mechanical function.

A receiver operating characteristic curve was constructed to define the optimal unipolar voltage (UPV; mV) threshold for infarct detection and localisation as compared to the morphological endocardial extension of the infarct, which was considered as the true extension of the infarct. Each UPV level, starting at 0 mV and adding 1 mV until the maximum UPV level was reached, was used as threshold level. Regions on the electrical map beneath each threshold level were delineated, regardless of localization, and the corresponding area calculated (Figure 4).



A B

Figure 4. A) Endocardial mapping catheter in the left ventricle. B) A right oblique anterior view of an unipolar voltage (UPV; mV) map of a porcine left ventricle after 45 min of ischemia followed by two hours of reperfusion. The red-yellow colour corresponds to low electrical activity in the apico-septal infarct region, whereas the purple-blue represents normal activity. Regions with UPV activity below a certain threshold was delineated and calculated to assess sensitivity and specificity (receiver operating characteristic curve, see text), illustrated by the white lines in figure B where the threshold is set at 8 mV. Note the low activity in the basal regions.

Vectorcardiography (papers III and IV)

The 3-D VCG recording and analysis procedures have been applied in humans.[74, 78, 79, 81, 82] See the introduction for further details.

Parameters

The RR, PQ, QRS, QTend and Tpeak-end intervals (ms) and Tarea (μVs) were calculated from the averaged 3-D QRST complex. Tarea (μVs) was calculated as $(Tx^2+Ty^2+Tz^2)^{1/2}$. The RR interval was expressed as heart rate (60/RR). The QT interval, defined by the tangent method, was corrected for heart rate according to the Van de Waters formula [QTcV=QT-0.087(RR-1000)] because of the relatively high heart rate. T loop morphology and the direction and magnitude of the maximum QRS and T vectors (QRS and Tamplitude; μV) were determined as follows (Figure 5a-d): 1) The ST vector magnitude[112] (STVM; μV), expressing the ST segment deviation (60 ms after the J point) from the isoelectric level, was used as a reference for determining the degree of ischemia. 2) The direction of the maximum T vector in space was expressed by its elevation (Televation; Figure 5a), its azimuth (Tazimuth; Figure 5b) and its relation to the QRS vector (QRS-T angle). 3) The shape of the T loop was expressed by Tavplan and Teigenvalue and is illustrated in Figure 5 c and d.

Recording protocols and analysis

VCG was recorded continuously from baseline to the end of reperfusion. Signal-averaged 3-D QRST complexes as well as QRS and T vector loops were automatically generated and stored as average cardiac cycles during 30 (paper III) and 60 (paper IV) seconds, and analysed off-line using customised software. Abnormal beats were automatically excluded. The automatic analysis and annotations were reviewed manually and revised as needed. Beats after extrasystoles were excluded.

Paper III: The conventional ECG and VCG parameters were analysed at five time points, including baseline, ischemia and reperfusion. At each time point, the mean of each parameter was calculated during a 3-minute recording period. In addition, before the first VF episode in the 16 pigs, we calculated the mean values from the period between six and three minutes before the event. Because arrhythmia occurred at different time points in the VF pigs, the reference/control values were obtained at the 16 corresponding time points and based on the average from all 17 pigs without VF (paired reference values).

Paper IV: Conventional ECG and VCG parameters were analysed at baseline and at four predefined time points during the pre-ischemic and ischemic phases. At these nine time points the mean of each VCG parameter was calculated. The parameters could not be consistently analysed during reperfusion because of the prevalent ventricular rhythm. In addition, all data recorded during the first 25 minutes of ischemia were analysed minute by minute and used for descriptive graphical presentation.

Arrhythmia recording and analysis (paper IV)

A continuous VCG-derived 12-lead ECG was used for arrhythmia classification. This allowed scrutiny of the rate and morphology of all QRS complexes, starting one hour prior to ischemia and lasting for two hours of reperfusion. Ventricular arrhythmia was manually classified as either non-sustained (NSVT; ≥3 consecutive beats and <30 seconds duration) or sustained ventricular tachycardia (SVT; ≥30 seconds duration) at rates ≥120 beats/minute, or as VF.

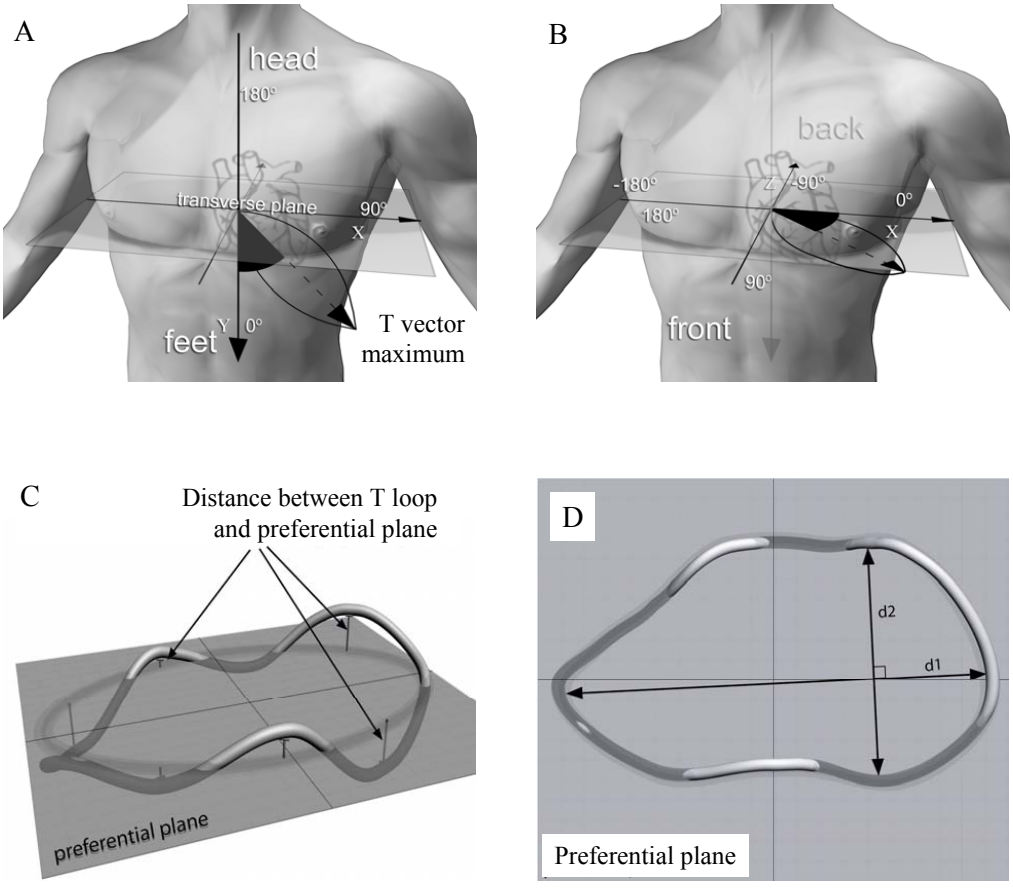


Figure 5. Vectorcardiographic parameters. **A:** Televation describes the angle between the maximum T vector and a craniocaudal axis perpendicular to the transverse (horizontal) plane, which is depicted by the *rectangle* (also in panel B). At 0° the vector points downwards (caudally), and at 180° it points upwards (cranially). **B:** Tazimuth describes the angle between the maximum T vector projected on the transverse plane and the left extremity of the X-axis. At 0° the vector points to the left. Forward motions of the vector (left-front-right) are defined as 0° to 180°, and backward motions of the vector (left-back-right) are defined as 0° to -180°. **C:** Tavplan (in µV) expresses the distortion (bulginess) of the T loop or its deviation from the preferential plane and is measured as the mean distance between the periphery of the loop and this plane. A "healthy" loop deviates little and therefore has a small Tavplan. **D:** Teigenvalue (dimensionless) expresses the shape and geometry of the T loop and is calculated as the ratio between the two highest diameters $(d1/d2)^2$; d1 > d2. A "healthy" loop is elongated and has a high Teigenvalue. The figures are reproduced from Wecke L et al.[82, 113]

Spinal cord stimulation (paper IV)

Implantation technique

A four-pole electrode was introduced through a Touhy needle (loss of resistance technique) into the epidural space of the anaesthetised pigs. The electrode tip was advanced and placed caudally to the C7 vertebra and slightly left of the midline under fluoroscopic guidance, according to clinical routines in humans.[114] The electrode was fixed to the ligaments and the pigs were then randomised to SCS or sham operation (afterwards: non-SCS). Animals were placed in the supine position and the electrode tip location was reconfirmed at the Th1/Th2 (n=18) or C7/Th1 level (n=2); both positions correspond to the clinical target segment T1-T2.

Stimulation protocol

In SCS pigs, test stimulation was initiated at a frequency of 50 Hz and a pulse duration of 0.2 ms, using an external pulse generator. Adequate stimulation was determined by minor muscle contractions induced in the left upper forelimb and left shoulder (motor threshold). The stimulation amplitude was set at 90% of this threshold.[101, 115] The motor threshold was reconfirmed after the pigs were placed in the supine position. The stimulation was started at least 30 minutes before induction of ischemia and maintained during ischemia and the first 30 minutes of reperfusion. The SCS settings chosen were selected to mimic those which are used clinically and practised in multiple animal experiments.[101, 115]

Statistical analysis

Descriptive data are presented as mean (SD or 2SEM) or as median (IQR). All tests were two-sided. Due to multiple testing in paper IV only P-values < 0.01 were considered statistically significant, while those of $0.05 > P > 0.01$ were regarded as trends. In all other tests P-value < 0.05 was considered as statistically significant.

Area at risk and infarct size (papers I to IV)

Non-parametric tests were used in papers I, II and IV, while parametric methods were applied to the larger, pooled, data of paper III.

The Wilcoxon signed-rank test was used for within-group comparisons of IS and AAR in papers I and II and Kruskal-Wallis for between-group comparisons.[116] In paper III, IS and AAR were tested with an unpaired Student's t-test, while in paper IV the non-parametric Mann-Whitney U test was employed for the same parameters. A linear regression model was used for assessing the relation between infarct weights and endocardical infarct areas in paper II.

Electromechanical mapping (paper II): The segmental activities are described by box-plots and statistical testing was performed by non-parametric tests of multiple related data at baseline (Friedman's test followed by two-tailed multiple tests).[116] Serially related reperfusion activity maps were tested with the Wilcoxon signed rank test, and between groups' maps with the Mann-Whitney U test. A receiver operating characteristic curve was used for describing the sensitivity and specificity for detecting an infarct by unipolar voltage maps vs. morphology.

Electrophysiology (papers III and IV) and haemodynamics (paper IV)

The effect of treatment on the overall occurrence of VF was tested with Fisher's exact test. The impact of SCS on the cumulative sums of ventricular arrhythmias was assessed by the Cumulative Wilcoxon rank-sum test stratified for occlusion site (proximal or mid-LAD).

VCG and ECG parameters were, when necessary, $\log_{10}$ transformed to accommodate for skewnesses. Baseline parameters were compared by unpaired Student's t-test in study III and Mann-Whitney U-tests in study IV. Linear mixed models[117, 118] of repeated measurements were fitted – using a 1st order autoregressive correlation structure to accommodate for serial dependence – to estimate the effects of ischemia-reperfusion (papers III and IV), SCS (paper IV) and occlusion site (post-hoc, paper IV) at the defined time points. In paper III, changes in ECG / VCG parameters prior to VF were assessed by pairing the parameter values of the non-VF subjects to the VF-subject's for the corresponding time points; the non-VF data thus become the reference values in paired Student's t-tests.

Statistical analyses were conducted using StatView 5.01 (SAS Institute, NC, USA) for papers I and II, and SPSS 16.1 (SPSS Inc. Chicago Il, USA) for papers III and IV.

RESULTS

Paper I. Effects of ultra-short acting calcium antagonism on myocardial ischemia-reperfusion injury

In the first study, we investigated if the ultra-short acting calcium antagonist, clevidipine, could reduce infarct size in a porcine model, while applying a percutaneous coronary intervention (PCI) technique with closed-chest, assumed to resemble clinical reperfusion therapy in the setting of acute myocardial infarction.

Results: Calcium antagonism did not reduce IS/AAR compared with vehicle or placebo among the closed-chest pigs. However, a cardioprotective effect of calcium antagonism, i.e. reduced IS/AAR, emerged when data from all open-chest pigs were pooled (Figure 6).

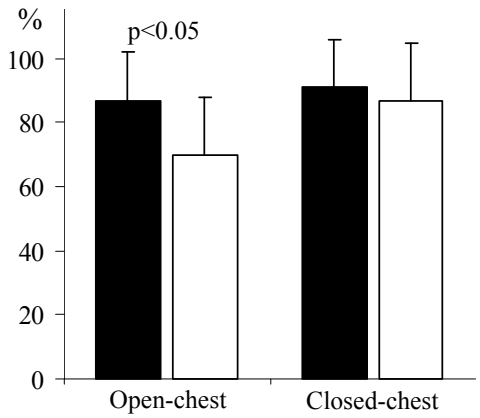


Figure 6. The infarct size / area at risk ratio in pigs treated with placebo/vehicle (filled) or clevidipine (open). Pooled data from open-chest versus closed-chest models. Mean (SD).

Conclusion: No cardioprotective effect was achieved by the ultra-short acting calcium antagonist clevidipine in our closed-chest model. However, the data indicate that model-specific factors may interact with cardioprotective mechanisms. The groups were, however, small and designed merely for explorative purposes, which is why these findings should be interpreted with caution.

Paper II. Assessment of myocardial viability by electromechanical mapping

Endocardial electromechanical mapping was performed in order to investigate its feasibility and capability of differentiating evolving necrosis from viable myocardium shortly after acute myocardial ischemia-reperfusion.

Results: Baseline and reperfusion (paired) maps data of good quality, were obtained in nine pigs. Within each of the nine segments, the mean (SD) number of mapped signals was 6 (4) at baseline and 5 (3) at reperfusion. Twelve hearts were available for both morphologic and map analysis at reperfusion. The mean (SD) IS/AAR was 92 (13) %, AAR 17 (4) % and IS 15 (4) % and the latter correlated well with the calculated endocardial infarct areas (simple linear regression: r^2=0.92). Catheter-induced VF occurred in five pigs during reperfusion. Frequent supraventricular and ventricular arrhythmias during reperfusion rendered fewer points and made the mapping procedure time-consuming, and consequently mapping was not possible in five pigs.

Electrical (unipolar but not bipolar) and mechanical activity were both impaired within the infarct zone. The precision of electromechanical mapping to identify an infarct was, however, poor. Significant intersegmental variability was observed at baseline and the infarct threshold seemed to vary between segments at reperfusion.

Conclusion: Endocardial mapping was time-consuming, had low diagnostic precision and induced ventricular arrhythmia, and was thus not feasible during evolving myocardial infarction.

Paper III. Effects of myocardial ischemia-reperfusion on ventricular repolarisation

Measures of ventricular repolarisation (VR) were investigated in order to further explore previously used non-invasive VR parameters[74, 78, 79, 81, 82] and their relevance in relation to ischemia-reperfusion-induced VF.

Results: Sixteen of 33 pigs developed VF, without relation to calcium antagonism or AAR and IS. VR changes in pigs without VF were characterised and revealed significant changes in all T loop parameters in response to ischemia compared with baseline. The T vector loop diverged towards the ischemic zone and returned to baseline at reperfusion. The QRS-T angle was wide at baseline and decreased significantly during maximum ischemia but then returned towards baseline. Tavplan and Tarea increased, whereas Teigenvalue decreased. The T loop thus became more distorted and more circular (Figure 7). The heart rate contributed significantly to the observed changes in STC-VM (p=0.04), ST-VM (p=0.004) and Tavplan (p=0.048).

When pigs with and without VF were compared, both heart rate (p=0.004) and Tavplan (p=0.028) were higher prior to VF, without any apparent difference in VR at baseline. QTend, QTpeak and QTcV were shorter (p=0.001 for all) before VF, whereas Tpeak-end did not differ.

Conclusion: Aggravated T loop distortion, as well as increased heart rate, preceded VF in this porcine ischemia-reperfusion model and might thus reflect aspects of VR relevant to arrhythmogenesis. The VR response to ischemia was consistent with previous human reports apart from the QRS-T angle, which changed in the opposite direction due to a species-specific difference.

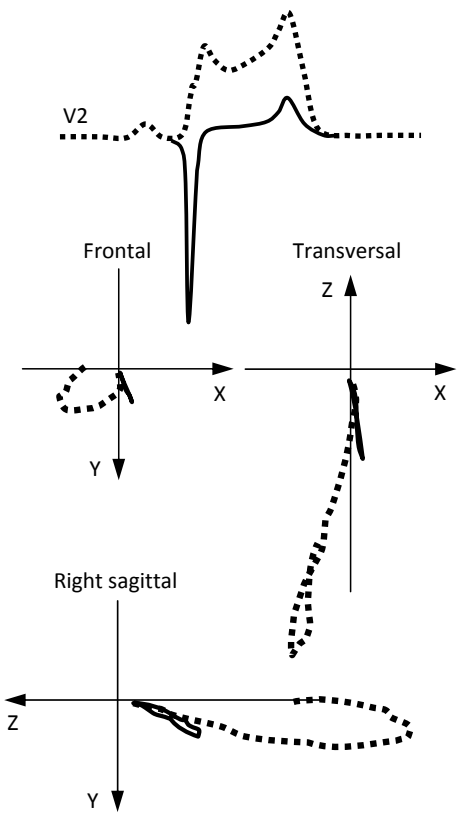


Figure 7. T vector loop and the synthesised precordial lead V2 at baseline (solid line) and after 17 minutes of left anterior descending artery occlusion (dotted line) in a pig. The T vector loops are projected onto three orthogonal planes.

Paper IV. Effects of SCS on myocardial ischemia and infarction, ventricular arrhythmia and electrophysiology

The effect of spinal cord stimulation on myocardial ischemia and infarction, ventricular arrhythmia and non-invasive electrophysiology was the primary objective and was investigated in a porcine ischemia-reperfusion closed-chest model. In a post-hoc analysis the effect of mid versus proximal LAD occlusion was evaluated.

Results: There were no differences between the SCS and the non-SCS group at baseline or during the pre-ischemic phase. During ischemia, heart rate remained unchanged, mean arterial pressure decreased, rate-pressure product tended to be lower and central venous pressure increased. None of these measures was significantly affected by SCS. In response to proximal LAD occlusion, mean arterial pressure was significantly lower and a similar trend was observed for rate-pressure product compared with after mid LAD occlusion.

SCS had no effect on IS, AAR or IS/AAR. Proximal compared with mid LAD occlusion was associated with larger AAR and IS, but the IS/AAR ratio was similar. All VFs, which occurred within 16.5 to 25.5 minutes of ischemia, and a majority of SVT and NSVT occurred in pigs subjected to proximal LAD occlusion (Table 1). The cumulative sum of ventricular arrhythmia episodes (VF, SVT and NSVT) was significantly higher in the non-SCS group than in the SCS group (Table 3).

C*onventional ECG intervals:* Intra-ventricular conduction (QRS interval) was significantly prolonged in a biphasic pattern during ischemia. Despite the prolonged depolarisation, repolarisation, reflected by QT and QTcV intervals, was shortened by ischemia. The Tpeak-end interval, i.e. the dispersion of repolarisation was, however, prolonged by > 50%. SCS had no significant effect on any of these conventional ECG parameters. The impact of proximal compared with mid LAD occlusion showed a significantly longer QRS interval and shortened QTcV, a trend towards shorter QT intervals (p=0.021) and no further prolongation of Tpeak-end. *VCG parameters:* The direction and morphology of the T loop were both consistent with changes observed in paper III (Figure 7; Tavplan, see below). The ischemia-induced increases in Tamplitude and Tarea were significantly reduced by SCS, which furthermore tended to reduce the increase in STVM (p=0.024). The impact of proximal compared with mid LAD occlusion showed increased STVM, Tamplitude, Tarea and Tavplan (bulgier T loop). The latter, however, did not change significantly (p=0.103) during mid LAD occlusion compared with at baseline. Furthermore, Teigenvalue showed no significant difference with regards to occlusion site, i.e. the T loop reached maximal circularity already in response to mid LAD occlusion. QRS-T angle narrowed significantly more in response to proximal as opposed to mid-LAD occlusion.

Conclusion: SCS reduced the accumulated incidence of spontaneous ventricular arrhythmia. Furthermore, this effect was associated with a reduction of repolarisation alterations. SCS reduced signs of myocardial ischemia but not infarct size itself.

Table 1. Number of ventricular arrhythmia events / number of affected pigs in relation to spinal cord stimulation (SCS) and occlusion site (mid or proximal (prox) left anterior descending artery). Numbers within brackets are the total number of pigs within each group.

	Non-SCS		SCS		
	Mid (5)	Prox (5)	Mid (4)	Prox (6)	p
NSVT	22/2	72/5	0/0	45/4	
SVT	15/5	5/5	3/3	5/5	
VF	0/0	3/2	0/0	4/4	
Total	37/5	80/5	3/3	54/6	0.039*

*Cumulative Wilcoxon rank-sum test stratified for occlusion site showed fewer episodes of ventricular arrhythmia in the SCS vs. the non-SCS group. Abbreviations: NSVT=non-sustained ventricular tachycardia, SVT=sustained ventricular tachycardia, VF=ventricular fibrillation.

DISCUSSION

The present thesis was initiated in order to translate potential adjunctive cardioprotective interventions, in the setting of acute myocardial infarction, into clinical practise based on a model resembling treatment with primary PCI in humans. Initially, local delivery of calcium antagonism was investigated but no cardioprotection was established. The ischemia-reperfusion model *per se* triggered ventricular fibrillation in nearly half of the experiments and thus the per protocol recorded VCG offered an opportunity to study non-invasive electrophysiological alterations to elucidate mechanisms and predictors of ventricular arrhythmias. Finally, the effect of neuromodulation by SCS and the impact of the ischemic burden on these alterations and mechanisms were further investigated in the same model.

Protection against ischemia-reperfusion injury

The ultra-short acting calcium antagonist clevidipine has previously been investigated in a series of porcine open-chest ischemia-reperfusion studies and showed promising cardioprotective effects.[50-54] In order to bring this into clinical application, we first intended to investigate the effects of clevidipine in a porcine closed-chest model, with a design more physiologically similar to clinical acute myocardial ischemia and reperfusion therapy than the open-chest model with coronary artery ligation.[119] The overall neutral effect in our study (paper I), however, made the use of clevidipine unlikely to provide benefit for cardioprotection in conjunction with therapies like PCI. On the other hand, the compound offers clinical benefits in perioperative blood pressure control in cardiac surgery[120] and could, at least theoretically, have cardioprotective effects in that context. The inconsistent effect on infarct size, however, indicates that impact of model-specific factors may interact with interventions and thus making results less reliable. The tentative protective effect of mild myocardial hypothermia in open-chest models has been confirmed by others.[121-123] Despite maintained core body temperature, a decrease in myocardial temperature develops[122, 123] and is further reduced in the ischemic region during coronary occlusion.[122] With an ischemia-reperfusion protocol similar to ours, no infarction occurred in an open-chest model at 35°C, but with each 1°C increase in body core temperature, 20% of the AAR became infarcted. Thus, IS/AAR reached 80% at 39°C.[121] Recently, deliberate mild hypothermia was shown to reduce reactive hyperaemia, microvascular obstruction and myocardial infarct size in a porcine model.[124, 125]

The inevitable use of anaesthesia and analgesia in experimental models exert effects that might mimic, interact or counteract with the interventions that are studied. Evidence has accumulated that some anaesthetics and opioids may potentiate or inhibit pre- and postconditioning pathways, although the exact mechanisms remain unclear. Volatile anaesthetics, including isoflurane, have been demonstrated to potentiate such pathways and have been suggested to reduce IS/AAR, compared with pentobarbital, and improve cardiac function after CABG.[126, 127] The anaesthesia and analgesia might have influenced the outcome of our results, although the net effect of all compounds cannot be concluded from our studies.

Evidence of any clinical benefit of direct calcium antagonism remains inconclusive and, besides ROS and inflammation, much research has focused on either alteration in Ca^{2+} handling (ion channels and sarcoplasmic reticulum) or its consequences (hyper-contracture, sarcolemma fragility and mPTP).[33] Recent scientific advancements

have emphasised gradual/staged reperfusion (i.e. postconditioning) for the salvation of threatened myocardium and identified the mPTP as one important target for therapy.[40] Even though the definite pathways remain unclear, mechanical[29, 128] and pharmacological[129] postconditioning has shown promising results that might translate into clinical benefits. Concerning inflammation, an anti-inflammatory acting agent has recently shown promising results in a clinical trial with regard to a reduction of infarct size.[130]

Neuromodulation techniques such as TENS and SCS have been proven to ease pain in refractory angina pectoris and, although the mechanisms have not yet been clarified, reduction in ischemia and infarct size have been proposed (see introduction). Pre-emptive SCS, for example, reduced infarct size in a rabbit model.[101] This effect was suggested to be mediated by preconditioning pathways and by the limitation of reflex activation of intrinsic cardiac sympathetic efferent neurons during ischemia.[101] The beneficial effect on infarct size was eliminated by α-adrenoceptor and blunted by β-adrenoceptor blockade, suggesting the involvement of cardiac adrenergic receptors. The results of this report were corroborated by two canine studies. In the first, angiotensin II induced intrinsic cardiac neuronal activity and ST elevation were both reduced by SCS. ST elevation induced by rapid pacing was, however, unaffected by SCS.[100] In the second study, SCS attenuated sensory inputs induced by transient regional myocardial ischemia, but was unable to overcome excessive sensory inputs arising from global cardiac stress provoked by rapid pacing.[131] Differences in species and design, but not in anaesthesia (pentobarbital in both), between ours and the rabbit model may explain the different effects on infarct size. In the referred open-chest rabbit study, the infarct sizes were considerably smaller. This may indicate less ischemia provocation and the possibility of lowered myocardial temperature, allowing for a window of protection that is lost after more profound ischemia. The lack of infarct reduction in our study (paper IV) could be due to an inability of SCS to suppress enough of the sensory inputs arising from the larger zones of myocardial ischemia; a tentative explanation which is supported by the canine studies[100, 131] and also by the fact that SCS seemed to reduce ischemia as measured by STVM but not infarct size in our study.

Assessment of infarct size and myocardial viability

The use of tetrazolium stain is well established for identifying and quantifying irreversible cellular injury.[132-136] Reperfusion accelerates its ability to delineate the infarct zone even after shorter periods of ischemia.[133, 134] Tetrazolium delineates infarcts in pigs after one hour of ischemia equally well as when followed by one, three or seven hours of reperfusion (IS/AAR 80% at all time points).[134]

Positron emission tomography is the gold standard for the assessment of myocardial function and viability, but due to high costs and logistics issues not widely implemented at PCI centers.[17] Single photon emission computed tomography has been used to define myocardium at risk and infarct size in ST elevation myocardial infarct patients.[137] Cardiac magnetic resonance imaging has in recent years, however, evolved to become a new standard in this population with a better resolution and a potential for significant sample size reduction.[138-141] Magnetic resonance imaging has, furthermore, demonstrated the ability to correlate closely with tetrazolium staining in experimental ischemia-reperfusion studies.[142, 143] We evaluated endocardial electromechanical mapping (paper II), since none of the referred techniques were in general practise in the acute course of myocardial infarction at the

time of our study. In conclusion, invasive mapping was not feasible shortly after reperfusion for arrhythmogenic and reproducibility (inter-segmental variability) reasons. Inter-segmental variability has been confirmed by others.[144, 145] However, the technique has proved its reliability in stable CAD patients with an opportunity for intramyocardial intervenetions.[146] It has been demonstrated that electrical activity decreases gradually after ischemia-reperfusion. Thus, both our relatively short reperfusion period as well as the time delay between mapping (2-3 hours) and tetrazolium staining (4 hours) add to the imprecision of endocardial mapping to delineate an infarct zone shortly after acute myocardial infarction.[147-150]

Electrophysiological alterations in myocardial ischemia and ventricular arrhythmia

Three important factors for the occurrence of ventricular arrhythmia in experimental acute myocardial ischemia are AAR, degree of collateral flow and heart rate, although other factors, such as anaesthetics, stress, mode of coronary artery occlusion, presence of a previous infarction, activity of the autonomous nervous system and hypertrophy in the non-ischemic myocardium, also contribute.[103, 106]

The size of AAR has not only been correlated with the STVM[83, 106], but also with changes in Tavplan and Teigenvalue[151], especially during LAD occlusion[79, 151] and in the presence of left ventricular hypertrophy.[74] In a low-risk cohort of stable CAD patients, left ventricular function and QRS-T angle were independent predictors of cardio-vascular death during an eight year follow-up period.[152] In the same population, distorted repolarisation (i.e. Tavplan and Teigenvalue) was associated with an increased risk of future myocardial infarction. These findings were interpreted as reflecting an increased vulnerability to ischemia in myocardial hypertrophy.[74] The consistency of our data (papers III and IV) with these reports (apart from the QRS-T angle, which is due to species-specific differences) strengthens the relevance of these electrophysiological VR parameters in relation to ischemia, especially with regard to an increased vulnerability to ventricular arrhythmia in larger zones of acute ischemia. The biphasic response pattern of the STVM during 45 minutes of coronary occlusion, with a transient reduction of magnitude between 15 to 25 minutes of ischemia, has been attributed to the onset of cell-to-cell uncoupling and increased ischemic tissue resistivity resulting in a decreased current flowing from the non-ischemic tissue.[59] A brief period, between six to 14 minutes of ischemia, of spontaneous recovery of activation delay explains the biphasic pattern of QRS.[59] The dynamic changes in Tavplan, Tarea and Tamplitude might in part also be attributed to these alterations.

Ventricular arrhythmias, occurring early after induction of acute myocardial ischemia, cluster in two peaks, i.e. Ia (2-10min) and Ib (12-30min), of which the latter is the predominant type in pigs. Depressed excitability, reduced conduction velocity and shortening of repolarisation (APD) during Ia predispose for the occurrence of re-entry, whereas cell-to-cell uncoupling and increased wall stress facilitates re-entry and possibly the occurrence of delayed afterdepolarisation during Ib.[57, 59] Their relative contribution to sudden cardiac death in humans is unknown, but experimental data suggest that the mortality in Ib is higher.[153] The occurrence of most ventricular arrhythmias during Ib in paper IV corresponds well with this, whereas the more scattered development of VF in paper III may also relate to concomitant pharmacological compounds, stress and invasive assessments.

In a recent guinea pig model, repolarisation (measured as APD) shortened in response to regional LAD ischemia and

VR dispersion increased across the boarder zone. These changes were exaggerated in hypertrophied hearts, which also exhibited greater dispersion already at baseline.[60] Together with impaired conduction, decreased excitability and cellular uncoupling, these changes set the stage for ventricular arrhythmia. Our observations are consistent with these mechanisms, as pigs subjected to proximal LAD occlusion and most susceptible to ventricular arrhythmia showed decreased conduction velocity (wider QRS) accompanied by a shortening of VR duration (shorter QTcV) compared with pigs subjected to mid-LAD occlusion. Other measures associated with repolarisation heterogeneity and dispersion, i.e. Tavplan, Tarea and Tamplitude, changed more in the proximal LAD group. These observations fit well with recent studies in humans (cardiac pacing-induced memory and cardiac fatigue), where changes in Tarea occurred in parallel with changes in Tamplitude and Tpeak-end.[82]

In response to acute myocardial infarction, sympathetic activity increases, the haemodynamic state deteriorates and vulnerability to ventricular arrhythmia is augmented.[2, 154] The relative contribution of sympathetic and parasympathetic activity to ventricular arrhythmia is unknown, but it is well appreciated that an elevated adrenergic state increases cardiac vulnerability by impairment of cardiovascular oxygen supply-demand, vasoconstriction, increased workload and wall stress, and altered electrophysiology (impaired conduction and repolarisation heterogeneity).[86, 154] In a canine model, myocardial ischemia heterogeneously diminished efferent sympathetic and parasympathetic innervation in non-infarcted distal segments already 5-20 minutes post coronary occlusion.[155] The denervated, but normally perfused, segments exhibited supersensitivity to β-agonists.[86, 155] Thus, immediate release and diminished re-uptake of norepinephrine in acute myocardial ischemia might cause ventricular arrhythmia.[1] Sympathetic blockade as well as vagal stimulation and improved autonomic balance increase the threshold for ventricular arrhythmia.[86, 87] Surgical transection of sympathetic tracts and thoracic epidural anaesthesia reduce the incidence of VF and improve measures of cardiac performance, including STVM.[156-161] Thoracic epidural anaesthesia also decreased infarct size in a canine model.[162]

Zipes et al. have demonstrated that SCS improves cardiac function and decreases ventricular arrhythmia induced by heart failure alone or in combination with superimposed ischemia in chronic heart failure canine studies.[163, 164] The mechanisms are complex and multifactorial (see introduction), although the authors emphasised that modulation of autonomic tone played an important role in their studies. The intrinsic cardiac nervous system is in constant communication with intrathoracic extracardiac neurons. Regional myocardial ischemia results in the heterogeneous activation of the intrinsic cardiac nervous system and may result in ventricular arrhythmia.[165] Thoracic SCS decreases the basal activity of intrinsic cardiac neurons and, more importantly, suppresses their response to the excitatory effects of local myocardial ischemia.[166, 167] SCS has recently been shown to obtund the capacity of subpopulations of intrathoracic sympathetic extracardiac neurons to transduce signals regarding regional myocardial ventricular ischemia.[131] Furthermore, SCS enhances parasympathetic activity.[168] The finding that SCS significantly reduced the accumulated incidence of spontaneous ventricular arrhythmia in our study (paper IV), in association with a reduction of repolarisation alterations, is consistent with these previous reports. The decreased magnitude of Tarea and Tamplitude, as well as the trend of reduced STVM in the SCS group, might correspond to diminished ventricular gradients and dispersion and to a reduced ischemic burden. The observed relation between Tavplan and heart rate (paper III) possibly reflected an

interplay between sympathetic nervous activity and/or increased myocardial metabolic demand, and the propensity for VF. In paper IV, Tavplan was significantly more distorted during proximal as opposed to mid-LAD occlusion, but remained unaffected by SCS. Pigs subjected to proximal LAD occlusion were more prone to develop ventricular arrhythmia, but the relation between Tloop distortion and arrhythmia mechanisms remains to be elucidated.

CONCLUSIONS

In a closed-chest, porcine coronary occlusion-reperfusion model assumed to be clinically relevant:

- No cardioprotective effect was achieved by the ultra-short acting calcium antagonist clevidipine. However, data indicates that model-specific factors may interact with cardioprotective mechanisms.

- Endocardial electromechanical mapping was not feasible during evolving myocardial infarction for arrhythmogenic and reproducibility (inter-segmental variability) reasons.

- Acute myocardial ischemia induced ventricular repolarisation heterogeneity, including distortion of T loop morphology. Ventricular arrhythmia occurred more frequently in response to proximal, as compared to mid, LAD occlusion and was associated with conduction delay and shorter but heterogeneous repolarisation.

- Spinal cord stimulation reduced the accumulated incidence of spontaneous ventricular arrhythmia in association with a reduction of repolarisation alterations. Furthermore, spinal cord stimulation seemed to reduce signs of myocardial ischemia but not infarct size itself.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to:

Lars Grip, tutor, for your friendship, your patience and faith in me, and for your devotion and never-ending support throughout these days, evenings, nights and years – Thank You.

Lennart Bergfeldt, co-tutor, for your friendship and encouragement, fruitful discussions and support, and for inspiring me on how to communicate academic results.

Paulin Andréll, Clas Mannheimer, Bengt Linderoth and Olof Ekre, co-authors, for your generous academic support and expertise in the field of spinal cord stimulation, for creating an encouraging atmosphere, and for many laughs.

Sahlgrenska Academy, University of Gothenburg and Sahlgrenska University Hospital, for providing me the opportunity to complete this thesis.

Aigars Rubulis, co-author, for your excellent support with the loops.

Chrichan Månsson, for your supreme technical skills and advice during the set up of the porcine model. For your late working hours and friendship, for *the* baseball and volleyball tournaments, and last but not least, for letting me win one set of table tennis.

Cecilia Litborn, for your supreme technical skills, your friendship and encouragement, and for many laughs.

Sven-Olof Jansson, for making a significant difference.

John Gustafsson and Anders Malm, for expert statistical advice and for modelling of cumulative significance.

The Laboratory for Experimental Biomedicine, EBM, for excellent facilities and a friendly atmosphere.

Lennart Gransberg, Gunilla Lundahl and Fredrik Eckardt at Ortivus, for your expertise, timely technical support and software development.

Louise Gracanin, for secretarial assistance.

Colleagues at the PCI clinic, for your supportive attitude and for taking my on-call nights.

Sisters and brothers at the PCI-lab and HIA-ward, for your outstanding commitment in your everyday work, for a friendly atmosphere and many laughs. It is a pleasure to work with you.

Pigs in space.

My parents, for your endless love and support, and for helping me to do it my self.

My wife Annika, our daughter Hilda and her forthcoming sibling, with whom I enjoy life.
Your sacrifices made all of this possible, that is – genuine love. You are the essence of life.

These studies were supported by grants from:
The Swedish Heart-Lung Foundation
The Göteborg Medical Society
AstraZeneca R&D, Mölndal, Sweden

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