From the Department of Internal Medicine in Huddinge
Division of Cardiology
Karolinska Institutet, Stockholm, Sweden

ANALGETIC AND ALGETIC EFFECTS OF ADENOSINE IN HEALTHY VOLUNTEERS AND PATIENTS WITH CORONARY ARTERY DISEASE

Bita Sadigh

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To my family

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There was the Door to which I found no Key;
There was the Veil through which I might not see:
Some little talk awhile of ME and THEE
There was – and then no more of THEE and ME.

Omar Khayyam

ABSTRACT

Background
Adenosine is a bimodal neuromodulator with algesic and analgesic effects. The different effects of adenosine are partly due to the route of administration. Low-dose adenosine infusion induces analgesia at the same magnitude as morphine, while adenosine as bolus injection induces chest pain, similar in character as angina pectoris.
AIMS
To evaluate algesic / analgesic and preconditioning effects of adenosine in patients with silent myocardial ischemia or angina pectoris and healthy volunteers and the possible gender differences and opioid effect on the adenosine-provoked pain.
Methods and results
In paper I, the tolerance to adenosine-provoked pain was tested in patients with silent myocardial ischemia, angina pectoris and healthy volunteers. Patients with silent myocardial ischemia had higher pain threshold compared to the other two groups. This difference was not modified by opioid antagonist, naloxone.
In paper II, the hemodynamic and pain response to high-dose adenosine infusion and the effects of an exogenous opioid agonist, β-endorphin and antagonist, naloxone were studied. High-dose adenosine infusion induced pain of oscillatory character which was not modulated by β-endorphin or naloxone.
In paper III, the influence of gender on adenosine-induced pain and the analgesic effect of β-endorphin were investigated. B-endorphin induced analgesia in men but not in women. Naloxone counteracted the analgesic effect in men.
In paper IV, the preconditioning effect of low-dose adenosine infusion as pretreatment to physical exercise was studied. Adenosine improved the regional ventricular function in the ischemic walls during maximal work loud compared to placebo. No changes in ventricular function were noted in the non-ischemic walls.
In paper V, the preconditioning effect of low-dose adenosine infusion as pretreatment to ischemic pharmacological provocation and its effect on coronary flow reserve were studied. Ventricular function was improved in the ischemic wall segments during peak stress following adenosine pretreatment but not placebo, without affecting the coronary flow reserve.
Conclusions
There are some differences in tolerance to adenosine in patient with asymptomatic and symptomatic ischemic heart disease. Patients with silent myocardial ischemia have decreased sensitivity to adenosine-provoked pain, which is not modulated by naloxone. In contrast no differences are demonstrated in adenosine-provoked pain between the genders. However in males β-endorphin induces analgesia, which is counteracted by naloxone, while in females β-endorphin does not modulate the adenosine-induced pain. High-dose adenosine infusion induces chest pain, which is not continuous and has oscillatory character. The fluctuation of pain is independent of β-endorphin and naloxone.
Low-dose adenosine infusion, in the dose range that induces analgesia, improves the ischemic ventricular function without any effect on coronary flow reserve, ruling out vasodilatation and unloading as the mechanisms for improved ventricular function.

Keywords
Angina pectoris, silent myocardial ischemia, opioid, gender, preconditioning

LIST OF PUBLICATIONS

I. **Sadigh-Lindell B**, Sylvén C, Berglund M, Eriksson BE. Role of adenosine and opioid-receptor mechanisms for pain in patients with silent myocardial ischemia or angina pectoris: A double-blind, placebo-controlled study. *J Cardiovasc Pharmacol 2003;42(6):757-763*

II. **Sadigh-Lindell B**, Sylvén C, Berglund M, Eriksson BE. High-dose adenosine infusion provokes oscillations of chest pain without correlation to opioid modulation: A double-blind controlled study. *J Pain 2004;5(9):459-475*

III. **Sadigh B**, Berglund M, Fillingim RB, Sheps D, Sylvén C. β-endorphin modulates adenosine provoked chest pain in men, but not in women – A comparison between patients with ischemic heart disease and healthy volunteers. *Clin J Pain 2007 in press*

IV. **Sadigh B**, Sylvén C, Berglund M, Brodin LÅ, Quintana M. The preconditioning effect of low dose adenosine infusion during exercise test in patients with ischemic heart disease – A double-blind, cross over, placebo controlled study. *Submitted 2007*

V. **Sadigh B**, Shahgaldi K, Sylvén C, Quintana M, Winter R. Pretreatment with low-dose adenosine infusion improves ischemic wall motion at pharmacological stress without affecting the myocardial perfusion in patients with coronary artery disease – a double-blind, placebo controlled, cross over study. *Submitted 2007*

CONTENTS

LIST OF ABBREVIATIONS

ATP	Adenosine 5´-triphosphate
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CCS	Canadian Cardiovascular Society
CFR	Coronary flow reserve
IVCT	Isovolumic contraction time
IVCV	Isovolumic contraction velocity
IVRT	Isovolumic relaxation time
LAD	Left anterior descending coronary artery
LV	Left ventricular
MI	Myocardial infarction
PSV	Peak systolic velocity
S	Strain
SMI	Silent myocardial ischemia
SR	Strain rate
TDE	Tissue Doppler echocardiography

A great truth is a truth whose opposite is also a great truth.

Nils Bohr

1 INTRODUCTION

The term angina pectoris was first introduced by Herberden in 1772. Patients with this symptom are still the main category of cardiac patients admitted to hospital and targeted for therapy [1]. Ischemic cardiac pain - angina pectoris – is complex in character and usually located central in the chest with a radiating referred pain. Chest pain occurs relatively late in the ischemic cascade and is preceded by metabolic disturbances, wall abnormality movements and ECG-changes. A puzzling fact is the poor correlation between the underlying pathophysiology and the symptoms. In patients with diagnosed coronary artery disease (CAD), a majority of the ischemic episodes are without symptoms [2], in contrast to syndrome X patients with normal coronary angiography but disabling angina pectoris-like chest pain.

Cardiac pain was first proposed to be caused by a distension of the ventricular wall – “mechanical hypothesis”. However, later studies showed that ventricular dilatation during spontaneous transient ischemia was similar during painful and pain-free ischemic episodes [3]. Mechanical factors may however play a role in the activation of sensory receptors at the peripheral levels [4].

In the 1930s, the “chemical hypothesis” was introduced, which proposed that ischemic pain may be provoked by the intramyocardial release of pain-producing substances induced by ischemia. Lewis formulated three conditions for a pain producing substance: i. Release in substantial quantities during ischemia. ii. Neuronal activation and iii. Provocation of pain [5]. Although a number of substances have been suggested to play a role as pain messengers during myocardial ischemia, so far only adenosine has been demonstrated to fulfill the criteria postulated by Lewis [6].

Later, the intensity theory gained popularity by suggesting that pain is the result of excessive stimulation of nonspecific sensory receptors, which function as

chemoreceptors, mechanoreceptors or polymodal receptors. In contrast, the “specificity hypothesis”, proposed that, pain is a result of the excitation of a specific type of receptors, so-called nociceptors, that are only stimulated by noxious stimuli [7]. Further research led to a merger of the two theories into a modified intensity hypothesis, which suggested that cardiac pain results from extreme excitation of a spatially restricted population of afferent fibers, a stimulation that in turn can be reduced by central inhibitory mechanisms [8]. This theory was not supported when adenosine injected in different arterial beds provoked pain in different parts of the body [9].

1.1 ADENOSINE

Purines are essential components of all living cells. Adenosine 5´-triphosphate (ATP) is an energy source for nearly all cellular activity. Due to their ubiquitous nature purines have evolved and become important molecules for both intracellular and extracelullar signaling, roles that are strictly distinct apart from their activity related to energy metabolism and genetic transmission of information. Many of the actions of adenosine either reduce the activity of the excitable tissues (e.g. by slowing down the heart rate) or increase the delivery of metabolic substrates (e.g. by inducing vasodilatation) and thus, help to couple the rate of energy expenditure to energy supply. Adenosine plays a variety of different roles as an intracellular messenger [10]. In 1963, Berne presented experimental evidence for the “adenosine hypothesis”, which suggested that adenosine, an endogenous dilator of coronary vessels, is released during reduced myocardial O_2 supply or increased myocardial workload [11]. Early studies showed that arterial inflow to the heart is precisely regulated by the coronary vasculature over a wide range of cardiac activities, maintaining a nearly constant and high level of O_2 extraction by the myocardium, resulting in low coronary venous O_2 saturation levels [12]. Since the high level of O_2 extraction is maintained even during basal condition, little reserve capacity remains for increasing O_2 extraction during stress. Furthermore, the myocardium has a

very limited capacity for anaerobic metabolism, and is largely dependent on oxidative metabolism. The blood flow in the myocardium seems to be under the control of three different regulating mechanisms. These are (i) the nervous system (neurogenic control), (ii) the arterial myogenic mechanism and (iii) chemical substances originating from the myocytes (metabolic autoregulation). The latter is an indirect regulation, in which hypoxia leads to metabolic changes that modify the flux of vasoactive substances, which in turn act on the coronary smooth muscles to cause vasodilatation [12]. Hence, vasoactive substances therefore act as error signals that increase in concentration when myocardial perfusion is inadequate and decrease as the balance between myocardial energy demand and the supply by the coronary artery flow is restored [12, 13]. This theory favors adenosine as a primary mediator (Figure 1), although there is evidence that in addition to adensosine, ATP and peptides such as calcitonin gene-related peptide, substance P and neurokinin A, may also be involved [14].

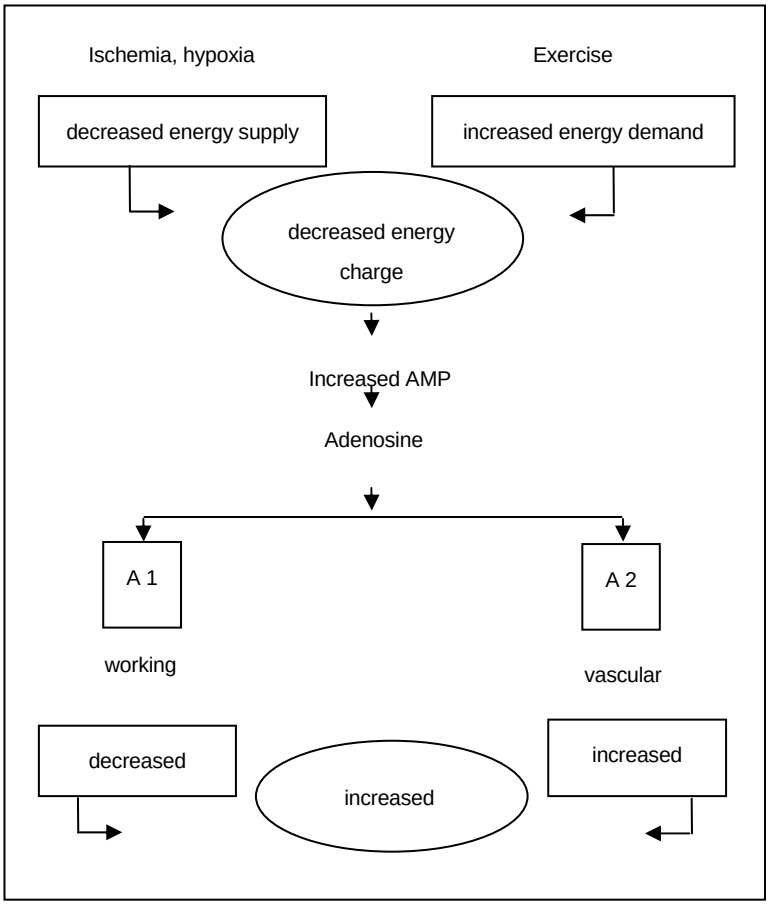


Figure 1. The role of adenosine in the energy supply-demand balance [13].

1.1.1 Adenosine and algesia

Adenosine has been shown to have neuromodulatory effects and depending on activation of the receptors and their differing effects on different targets, its net effects can be either to activate or inhibit various functions, such as heart rate, blood pressure, vascular tone and neural activity [15]. The algesic properties of adenosine in man following intravenous bolus injection was first reported by Sylvén et al in 1986 [16]. In healthy volunteers angina pectoris-like symptoms were induced without signs of myocardial ischemia. The intensity of pain was dose-dependent and could be reproduced in patients with known ischemic heart disease [17]. The algetic effect of adenosine was concluded to be exerted at extracellular membrane-bound receptors, because i.v. administration of theophylline counteracted and dipyridamole enhanced the adenosine-provoked anginal pain. Furthermore, substance P [18] and nicotine [6] potentiated the adenosine-provoked pain. The first metabolite of adenosine, inosine did not provoke any pain [19] and autonomic blockade (β-blockade and atropine), opioid antagonist (naloxone), nitroglycerine, clonidine and cyclooxygenase inhibition did not counteract adenosine-provoked pain [20]. Furthermore, the pain-producing mechanism of adenosine in skeletal muscle did not relate to vasodilatation. The adenosine receptor blocker, theophylline counteracted ischemically provoked pain, suggesting that endogenous adenosine acted as a pain-provoking substance [21].

The mechanism behind the adenosine-induced pain is not well-established. Adenosine dose not cause electrocardiographic signs of myocardial ischemia or other physiological changes suggesting an unfavourable myocardial oxygen demand to supply ratio. These observations support the hypothesis that adenosine is an early messenger between myocardial ischemia and pain in angina patients. On the other hand, adenosine has well documented hemodynamic effects, such as vasodilatation and

a coronary steal phenomenon due to redistribution of myocardial blood flow could thus be involved. Lagerqvist et al demonstrated that steady state infusion of adenosine induced ST-depression which was not the case during intracoronary bolus injection [22]. Also vasodilatation in other ways – i.e. by nitrates or physiological stimuli – dose not per se cause pain. Adenosine as an early pain messenger of ischemia was further studied in skeletal muscle [21]. Since the half life of adenosine in blood is a matter of seconds [23] and appearance of pain after adenosine administration could be influenced by the mode of administration, intravenous bolus injection of adenosine would be expected to result in higher concentrations in the myocardium than in neighbouring organs, while infusion to steady state should result in a more even distribution between organs. This is due to the fact that the coronary arterial tree is the first systemic vascular bed with high relative blood flow, compared to the neighbouring organs.

1.1.2 Adenosine and analgesia

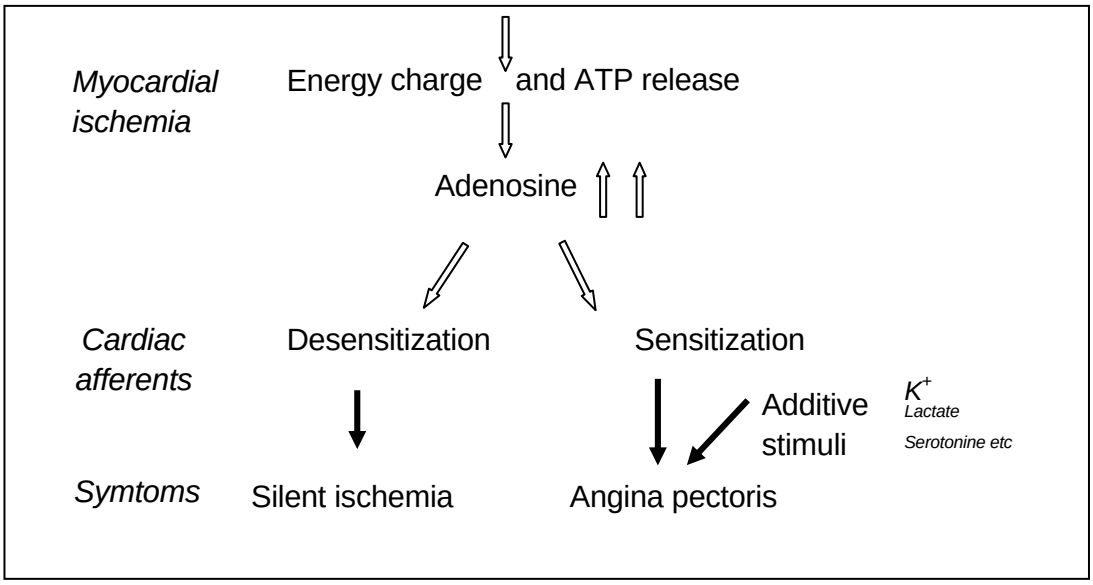
Adenosine acts as a bimodal neruromodulator with algesic and analgesic effects [6]. Analgesic effects of adenosine on ischemic pain, provoked by the forearm tourniquet, on heat pain threshold and on mustard oil-provoked allodynia have been reported [24, 25]. During ischemia such effects are similar in magnitude to those of morphine. The regional analgesic effects of adenosine is counteracted by theophylline, which suggests a peripheral site of action to be excited at membrane bound adenosine receptors [26]. Adenosine is also considered to be involved in opioid-induced antinociception. Systemic administration of the adenosine receptor antagonist aminophylline, inhibits the antinociceptive effects of morphine [27]. In biochemical experiments, morphine produces a dose-dependent opioid receptor-mediated release of adenosine in synaptomoses from the dorsal but not from the ventral half of the spinal cord [28]. The adenosine release occurs via an action of the μ-opioid receptors and requires activation

of voltage-gated Ca^{2+} channels. In the periphery, the adenosine A_1-receptor has been proposed to be associated in a complex fashion with the α_2-adrenergic and the μ-opioid receptors [29].

Sylvén et al reported a decrease in exercise-provoked chest pain during low dose adenosine infusion [30]. Other studies have shown that intrathecal administration of adenosine analogues reduces hypersensitivity in animals after peripheral inflammation and nerve injury [31]. Adenosine infusion may also partially alleviate spontaneous pain, allodynia and hyperalgesia in patients with neuropathic pain [32] and reduce hypersensitivity by activation of local spinal noradrenergic terminals to release nor epinephrine [33].

Several studies have suggested that the antinociceptive effects of adenosine are exercised in the central nervous system, mainly in dorsal horn neurons at the spinal level, where adenosine may inhibit small diameter sensory fibers by both pre- and post-synaptic mechanisms [34]. Other investigations imply that the A_1-mediated analgesia is eliminated by pretreatment with theophylline [35]. These animal studies suggest that the analgetic effect of adenosine is at least in part mediated by peripheral membrane-bound adenosine receptors. The fact that caffeine - an adenosine receptor antagonist - counteracts the analgesic effect of transcutanous electrical nerve stimulation, indicates a neural release of adenosine, causing activation of adenosine receptors [36]. Thus depending on the mode of release, adenosine may play a role in induction of angina pectoris and silent myocardial ischemia (Figure 2).

Figure 2. The peripheral algesic and analgesic effects of adenosine in myocardial ischemia [6].



1.2 OPIOIDS

Opioid receptors belong to a superfamily of G-protein-coupled receptors. Molecular cloning has led to the identification of three different types of opioid receptors, OP_1 (δ), OP_2 (κ) and OP_3 (μ) [37]. The diverse effects of opioid drugs depend on their affinity to the different receptors, and whether they act as agonists (like morphine), antagonists (like naloxone) or mixed agonists / antagonists (like buprenorphine).

Opioids inhibit adenylate cyclase activity, which leads to a decrease in cAMP, a reduction in Ca^{2+} conductance and thus an attenuation of neurotransmitter release. It is hypothesized that endogenous opioids are released via a disinhibitory process and subsequently antinociception is produced [38].

Earlier studies suggested that opioid receptors may be G-protein-coupled to K^+ channels and therefore have a duality in their function [39]. In addition to their effects mediated through G-proteins, they act as openers of the ATP-gated K^+ channels and have the ability to modulate K^+ efflux [40]. Opioids do not bind directly to the K_{ATP}-channels and there is no cross tolerance between opioids and K_{ATP} openers [41].

Minoxidil, an ATP-gated K^+ channel opener, produces a dose-dependent antinociception, which dose not increase the release of endogenous opioid peptides, but is sensitive to opioid antagonists [42].

1.2.1 B-endorphin

Endogenous opioids are intimately involved in the very complex function of the so called “endogenous analgesic system”. Changes in circulating levels of endogenous opioid peptides, in particular β-endorphin, may modulate both somatic and visceral pain perception by actions on the peripheral and central nervous systems [43, 44]. Further, infusion of β-endorphin but not metenkephalin counteracts adenosine provoked chest pain, indicating that peripheral antinociception at opioid receptors primarily occurs at the μ-subtype opioid receptors, although a role of other subtypes cannot be excluded [45]. Increased plasma concentrations of β-endorphin has been reported to alter peripheral pain threshold but not the angina threshold [46]. In patients with varying tolerance to painless ischemia, similar plasma concentration of β-endorphin and metenkephalin were measured in the basal state and during induction of forearm ischemia [44]. On the other hand, plasma β-endorphin concentrations in subjects with silent myocardial ischemia (SMI) were approximately twice as high as in patients with anginal symptoms, which suggests that increased concentrations of plasma β-endorphins may indeed play a role in the decreased sensitivity to pain reported in these patients [47]. This view is also supported by another study which reported higher postexercise plasma β-endorphin concentrations in patients with asymptomatic myocardial ischemia and a significantly positive correlation between postexercise β-endorphin concentration and the time to the onset of angina [43]. The same group reported that in patients with coronary artery disease and angina pectoris, psychological stress e.g. caused by public speaking produced increased cardiovascular

reactivity and an increase in plasma β-endorphin that was significantly correlated to pain threshold [48].

1.2.2 Naloxone

Studies, designed to investigate the effect of the opioid receptor antagonist naloxone, have not shown significant changes in anginal or peripheral pain threshold. Low doses of naloxone (2 mg and 1.2 mg) did not induce angina pectoris in patients with SMI [49, 50]. However, patients with symptomatic ischemia undergoing exercise treadmill test using a higher range dose of naloxone (6 mg followed by 0.1 mg/min during exercise test) exhibit a significant change on their electrical pain threshold while the angina threshold seems unaltered [44].

1.3 SILENT MYOCARDIAL ISCHEMIA

Twenty-five percent of the persons who suffers a sudden cardiac death and has severe coronary atherosclerosis on post mortem examination had never showed clinical symptoms of CAD [51]. The prevalence rate of SMI ranges according to different studies from 9 to 57% [52, 53]. This broad range is probably due to differences in the populations studied, such as age of the patients, duration of the underlying disease, inclusion or exclusion of patients with high-risk factors or symptoms of CAD and the definition of SMI. Thus there are a considerable number of persons with SMI in the population at large.

SMI is defined as a transient alteration in myocardial perfusion, function or electrical activity in the absence of chest pain or the usual anginal equivalents [54]. Cohn has classified this group into three subgroups. Type I patients are those who are asymptomatic and have no history of myocardial infarction or angina pectoris. Type II patients had previous myocardial infarction and have evidence of inducible, but asymptomatic, ischemia, usually on exercise testing. Type III patients are those with

angina but also episodes with silent ischemia [55]. The detection, prevalence and prognosis of SMI vary according to patient type. The prevalence of conventional risk factors added to SMI increases the risk of cardiac death significantly [56]. Thus in a population with high likelihood of coronary artery disease, chest pain tends to lose its apparent value as the only clinical parameter predicting cardiac morbidity and mortality. There are small differences in the magnitude of perfusion abnormalities between those with SMI and symptomatic ischemia. Previous studies in victims of sudden cardiac death and survivors of cardiac arrest show no differences in the extent of CAD in those with a prior history of angina versus those with SMI [57]. Furthermore, the Asymptomatic Cardiac Ischemia Pilot (ACIP) study suggests that in a well-described group of patients with SMI, revascularization offers significant prognostic advantages [58]. Medical therapy aimed to reduce ischemia appeared to be prognostically better than symptomatic therapy but not as effective as revascularization [59]. Special reference should be made to the role of gender in CAD. Men appear to have a higher incidence of SMI compared to pre-menopausal women, but this difference changes quickly in post-menopausal women, mainly due to the risk factors typically associated with CAD [60]. There are also some psychological correlations with SMI. Patients with SMI report less depression, anxiety, somatic awareness, sensitivity to pain and discomfort [61, 62]. They have also decreased health care use compared to patients with symptomatic myocardial ischemia [63].

1.4 GENDER DIFFERENCES AND PAIN

Gender differences are reported from animal pain studies. Gonadal steroids have been suggested to partly account for gender differences in the antinociceptive responses to morphine [64, 65]. Morphine, a common μ-receptor agonist has greater antinociception in male rats, due to greater activation of periaqueductal gray in male rats compared to

female rats [66]. Several epidemiological studies suggest that women are overrepresented among those who report pain and they also more often report multiple pain sites, more frequent pain and pain with longer duration [67, 68]. Further, it has been reported that women exhibit lower pain threshold and tolerance than men [67]. The response to somatic pain such as musculoskeletal pain was different between men and women in a population-based Dutch study [69]. Women with musculoskeletal pain sought more health care, while men required more time off work due to low back pain only [69]. Moreover, women experienced better pain relief with butorphanol, a κ-agonist than morphine (μ-receptor agonist), while men reported more pronounced effects on their pain score with morphine [70]. The effect and side effect of morphine during epidural anesthesia was tested in male and female patients. Although there were no differences in the dosage of epidural morphine, women experienced significantly more nausea and vomiting as side effects [71]. Differences between men and women have also been reported throughout the entire spectrum of ischemic heart disease. Atypical symptoms such as multiple non-chest pain, older age at the onset of symptoms and higher prevalence of risk factors may partly explain the higher risk of complications and the adverse prognosis in women [72-74].

1.5 PRECONDITIONING

Ischemic preconditioning protects the heart against long periods of ischemia by initially exposing it to brief episodes of ischemia. The cardioprotective effect of ischemic preconditioning seems to be biphasic, with an early opioid mediated phase occurring within minutes from the initial ischemic insult, lasting for 2 to 3 hours, and a late phase becoming apparent 12 to 24 hours later, lasting for 3 to 4 days and requiring de novo protein synthesis [75, 76]. The possible beneficial effects of ischemic preconditioning include the preservation of the left ventricular (LV) systolic and diastolic functions

[77], preservation of adenosine triphophate levels in myocardial biopsy specimens [77] and antiarrhythmic effects through minimizing QT and JT dispersion [78]. Some studies indicate that pathologies such as diabetes mellitus [79], hypercholesterolemia [80] and remodeling of the LV may interfere with ischemic preconditioning, although certain therapies such as AT_1-receptor blockade could preserve preconditioning [81]. The molecular basis of ischemic preconditioning is the release of adenosine, bradykinin and opioids, which eventually leads to the opening of
ATP-sensitive potassium channels either in the cardiac sacrolemmal membrane or mitochondrial membrane [82-84]. K_{ATP} openers such as nicorandil and bemakalin [82] have shown preconditioning effects, while K_{ATP} blockers such as glibenclamide can actually block the ischemic preconditioning [83, 84]. Furthermore receptor or signaling "cross talk" seems to be important in adenosine-mediated cardioprotection. Such interaction between adenosine and opioid receptors has been demonstrated in the in situ rat myocardium during acute protection [85]. Opioid receptor antagonism inhibited adenosine receptor-mediated protection, and vice versa. Adenosine mediates its cardiac protection via several receptor subtypes [86, 87]. The A_1 receptor is the most extensively studied adenosine receptor subtype within the context of cardiac protection. Preconditioning mimetic agents that stimulate the biochemical pathways of ischemic preconditioning without inducing ischemia seem to be at least theoretically beneficial as adjunct during ischemia. Several clinical studies have therefore investigated the preconditioning effect of adenosine during acute myocardial infarction (MI) [88, 89]. The AMISTAD trials indicated reduced infarct size mainly in patients with anterior MI [88, 90]. However morbidity and mortality were not affected. In the ATTACC study, the LV systolic function was not affected by adenosine but trends towards improved survival were observed in patients with anterior MI [89].

2 AIMS OF THE THESIS

To test the hypotheses that:

Patients with symptomatic and asymptomatic myocardial ischemia respond differently to adenosine provoked pain due to different opioid sensitivity.

Opioids modulate the pain response induced by high dose infusion of adenosine.

Gender influences the adenosine induced chest pain and modulates the pain response by opioid agonism and antagonism.

Low dose adenosine infusion, in doses that induce analgesia has a preconditioning effect on the heart.

3 MATERIAL AND METHODS

3.1 PATIENTS AND HEALTHY VOLUNTEERS

Paper I

Thirteen patients with SMI (Cohn I-II) [54], mean age 58 ± 10 years, ten patients with angina pectoris, mean age 59 ± 9 years and ten healthy volunteers, mean age 52 ± 7 years, all male were included in the study. Myocardial ischemia was defined as either ST-depression of $\geq$ 1 mm 60 milliseconds after the J point or a reversible perfusion defect at myocardial scintigraphy during an exercise or pharmacological stress test.

Paper II

Ten male healthy volunteers, mean age 26 ± 3 years were studied.

Paper III

Twenty patients, 10 male (mean age 59 ± 7) and 10 female (mean age 61 ± 9) patients with significant CAD, according to coronary angiography and 20 healthy volunteers, 10 male (mean age 56 ± 7) and 10 female (mean age 57 ± 5) were included. In the male patient group, 4 had prior (MI). Six male patients had three vessel disease and 4 had two vessel disease. Nine male patients were scheduled for coronary artery by pass grafting (CABG). Among female patients, 3 had experienced previous MI. Two had three vessel disease and 2 had two vessel disease and the rest had one vessel disease. Seven female patients were planned to undergo CABG.

Paper IV

Nine male patients, (mean age 74 ± 7) years, (Canadian Cardiovascular Society (CCS) class II – III) participated in the study. Eight patients had three vessel disease and one, two vessel disease. Except for one patient with mildly depressed global left ventricular (LV) function, the rest had normal LV function. Three had earlier MI. All patients were scheduled for CABG. One patient had earlier undergone CABG.

Paper V

Thirteen patients (one female and 12 male), mean age 63 ± 6 years, CCS class II) were recruited for the study. All patients were planned for CABG. Two patients dropped out and one patient underwent only the two dobutamine stress tests due to an earlier set operation date.

3.2 METHODS

Paper I-III

Blood pressure was measured by a finger cuff on the right hand using the Finapres™ (Ohmeda, USA) device. Skin electrodes were placed on the right hand to record sympathetic-induced skin resistance (sudomotor). The subjective intensity of chest pain was determined using an inter-modal matching technique called hand algometer (proprioceptive analogue scale, PAS) on the left hand [91, 92]. The participant adjusted the distance between the thumb and the index finger attached to two metal arms (Figure 3) that were connected to a linear potentiometer. The output voltage was recorded on a polygraph continuously during the adenosine infusion. A maximal and comfortable spread of the finger was assigned the value of 100. The patient was instructed to produce continuous finger spans related to the corresponding subjective intensity of

chest pain caused by the infusion. Pain onset was recorded as the start of deflection of the potentiometer output. Pain duration was recorded as the time during which the potentiometer was deflected from zero. Pain mass was calculated as the integral of pain over time (area under the curve). Pain peak was defined as the maximum amplitude during a pain episode (Figure 4). After each pain episode, the intensity of the pain was also rated verbally according to the Borg category ratio scale [93] with verbally anchored intensities ranging from 0-10 (CR-10 scale). This scale has been evaluated concerning reproducibility and validity [94]. One lead ECG was continuously monitored during the experiment. A PowerLab ADInstrument (UK) was used to record and analyse the measurements.

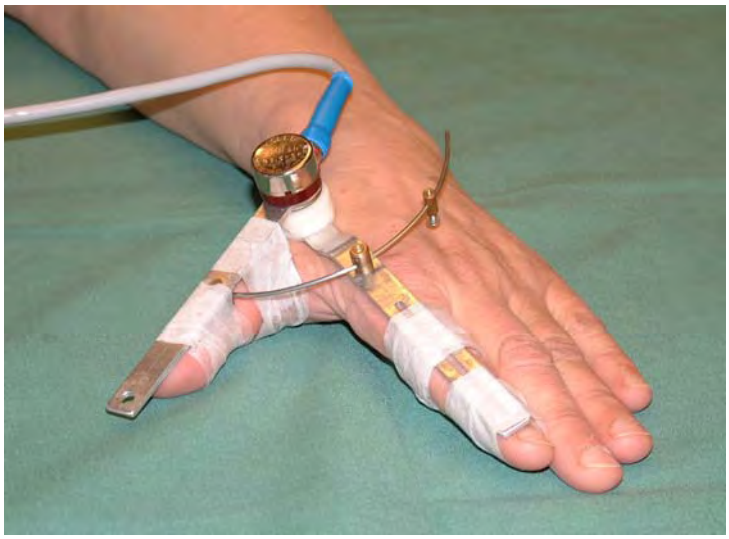


Figure 3. Hand algometer

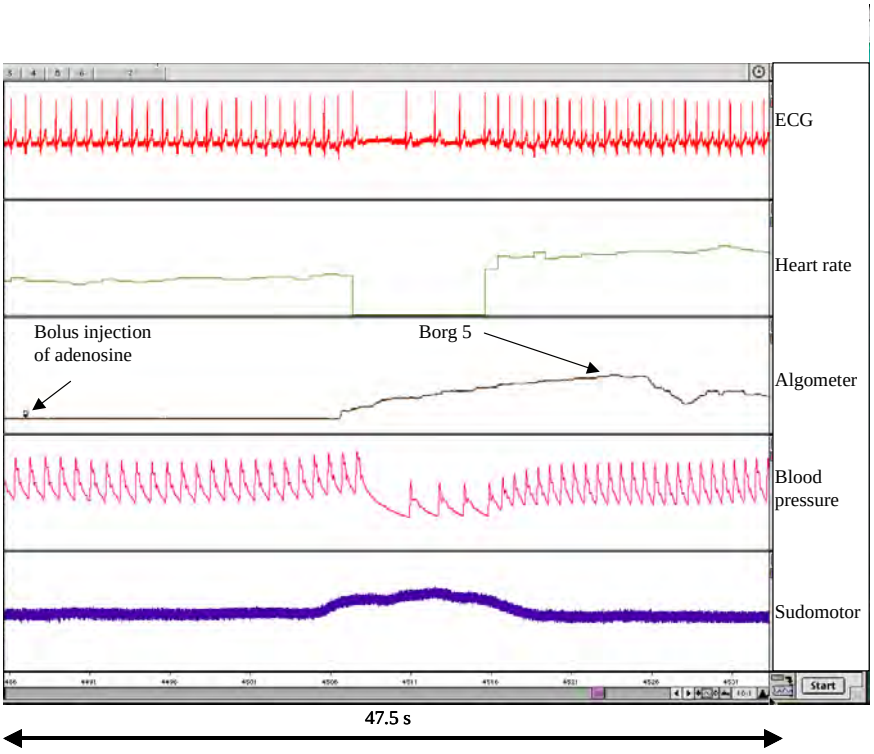


Figure 4. Online recording during one bolus dose of adenosine. ECG, heart rate, hand algometer, blood pressure and sudomotor activity. Pain intensity was estimated online by hand algometer and verbally by Borg CR-10.

Adenosine bolus injection and infusion

In paper I and III, the effect of adenosine bolus injection was studied. At first the maximum tolerable dose of adenosine was determined individually by increasing doses of adenosine injected rapidly into an antebrachial vein followed by a flush of 5 ml of saline with an initial dose of adenosine of 2.5 mg and increment of 2.5 mg. The hemodynamic and perceptual effects of adenosine disappeared within one minute. After one minute of rest and before the next dose, the subject was asked whether any symptoms remained and if not, permission was obtained to continue with the next test dose. If the subject did not wish to proceed, the last dose given was taken as the maximum tolerable. The dose was not increased, if 2nd and 3rd degree of atrioventricular block with duration of more than 2 seconds appeared on ECG. After establishing the maximum tolerable dose of adenosine the subjects were given 4 doses (placebo, 1/3, 2/3, 3/3 of maximum tolerable dose of adenosine) in a double-blind,

randomized order. The procedure was repeated after an injection of a non-selective opioid antagonist (naloxone 0,4 mg) in paper I. In paper III, the protocol was divided into three parts. First, the subjects were given placebo, 1/3, 2/3 and 3/3 of maximum tolerable dose twice in a double blind randomized order. Second after a rest of ten minutes, the subjects were given a bolus injection of 13.5 nmol of β-endorphin, followed by β-endorphin infusion (9.1 nmol/min) [45, 95]. After 5 minutes of ß-endorphin infusion, the same 8 injections of adenosine were given in a double-blind randomized order. Third, after a second rest of ten minutes, patients received a bolus injection of naloxone (0.8 mg) and after 5 minutes, the same 8 double-blind randomized injections of adenosine were given.

In paper II, the subjects received high-dose adenosine infusion (140µg/kg/min) for 22 minutes at 3 different days. After 5 minutes of infusion, the subjects were randomized to either placebo (NaCl bolus for 2 minutes, followed by infusion for 15 minutes, or β-endorphin bolus (13.5 nmol) followed by β-endorphin infusion (9.1 nmol/min) [45, 95] or naloxone bolus (0.8 mg) followed by NaCl infusion.

Paper IV

Echocardiography with superimposed tissue Doppler echocardiography (TDE) images using 2.5 MHz transducer with commercially available equipment (Vivid 7, GE Vingmed, Horten, Norway) was performed at rest, with standard parasternal short and long axis views and apical two, three and four chamber view during five consecutive cardiac cycles. Thereafter the patients underwent stress test at two separate occasions. In paper IV, the stress provocation was induced by an exercise stress echocardiography test in semi-supine position on a specially designed, table-mounted bicycle ergometer. The initial workload was 50 Watts with increments of 10 Watts per minute. At each stage, heart rate, systolic blood pressure and a 12-lead ECG were recorded. Standard

echocardiographic images of five consecutive cardiac cycles containing TDE information at baseline, low dose infusion, sub-maximal workload (heart rate = 70% of the expected maximal heart rate), maximum workload, and recovery phases (one to two minutes after the exercise) were digitally recorded and stored.

All echocardiographic images were analysed off-line using specially designed software (EchoPac PC, SW BT04 Vivid 7, GE Vingmed Ultrasound, Horten, Norway). A sample volume was positioned on the region of interest at the basal segment of the four LV walls (inferoseptal, anterolateral, inferior and anterior) to obtain a myocardial velocity profile (Figure 5). Both the systolic and diastolic phases of the velocity profile were analyzed. Peak systolic velocity (PSV) was defined as the peak velocity during ejection. Further we measured peak velocity at early diastole (E´-wave) and peak velocity at the late diastole (A´-wave). We also calculated the longitudinal A-V plane displacement by time integration of the PSV, and calculated the degree of myocardial compression/deformation as strain (S) and strain rate (SR).

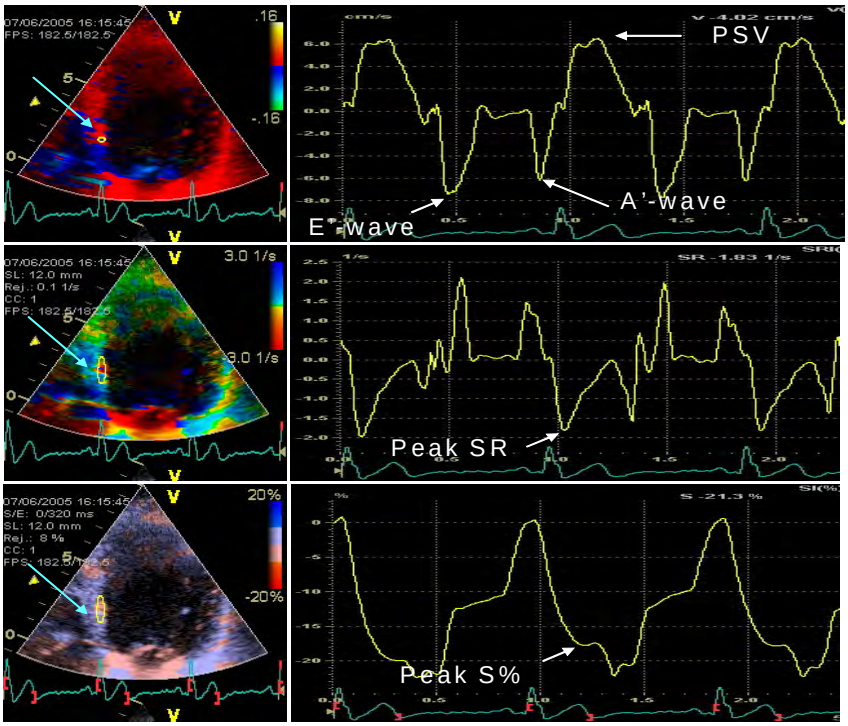


Figure 5. Online recordings of tissue Doppler echocardiography variables. PSV= peak systolic velocity, SR= strain, S%= strain rate.

In order to assess the LV regional systolic function and to detect the presence of regional myocardial ischemia, the LV walls (Figure 6) were categorized as ischemic or non-ischemic: an LV wall was defined as ischemic if a reduction, no increment, or an increment of <15% in PSV, was observed during maximal work load as compared to baseline preceding placebo-infusion; otherwise, the LV walls were defined as non-ischemic.

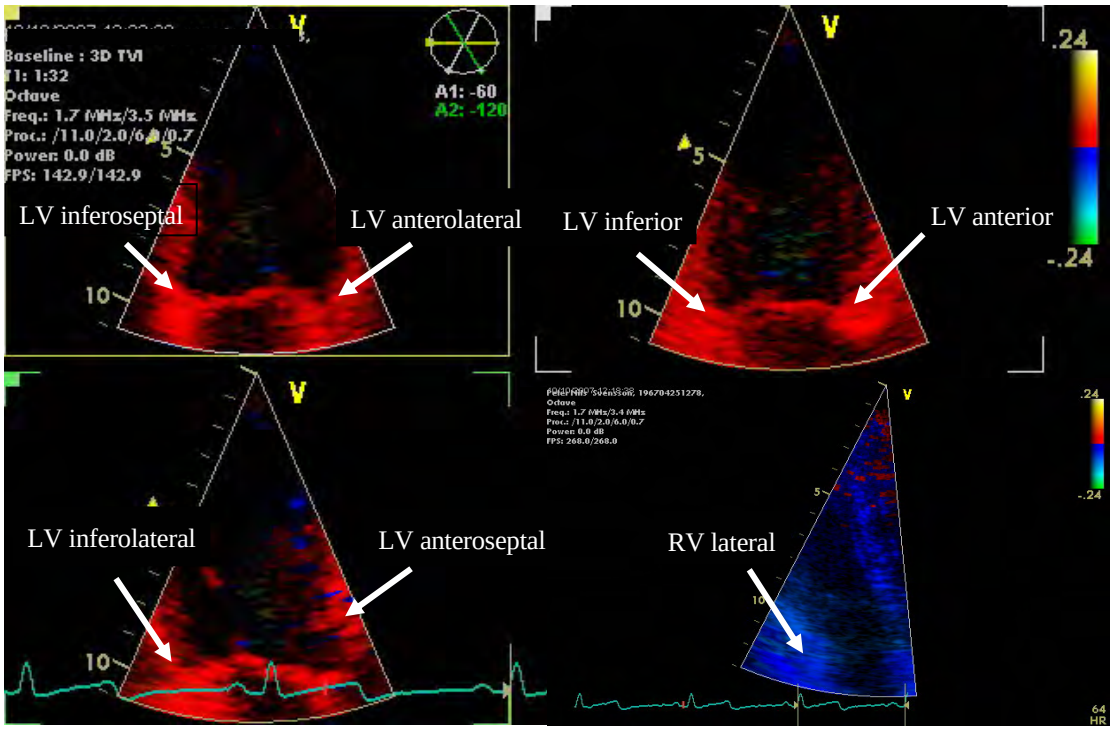


Figure 6. Left and right ventricular walls

Paper V

After performing the echocardiography at rest, the stress test was pursued pharmacologically with dobutamine stress echocardiography. Dobutamine was infused intravenously for 3 minutes at 10, 20, 30 and 40 µg/kg/min. At the final stage, intravenous atropine was added if necessary in 0.25 mg increments every minute up to a maximum dose of 1 mg to reach a target heart rate of 0.85 x (220 – age). At rest, maximal workload and recovery phase (five minutes after maximal dobutamine stress),

standard echocardiographic images of three consecutive cardiac cycles containing TDE information were obtained and digitally stored.

Myocardial perfusion was indirectly measured using contrast echocardiography for coronary flow reserve (CFR) measurement in the distal left anterior descending coronary artery (LAD). The perfusion measurement was performed at two separate occasions using Sonovue® infusion at 0.7 ml/min at rest and during high dose adenosine infusion at 140 µg/kg/min for maximal vasodilatation. After visualizing the middistal LAD, using colour Doppler with a high frequency pediatric transducer, 7S 3.5-8 MHz, the diastolic flow velocity profile was recorded using pulse wave spectral Doppler in the distal LAD at rest and during high dose adenosine infusion for at least three minutes. CFR was quantified using the peak/baseline ratio of maximal diastolic velocity in distal LAD. We predefined a normal flow reserve as a ratio > 2 [96].

All echocardiographic images were analysed off-line as previously described. A circular sample volume with a diameter of 6 mm was positioned at the basal segment of the right ventricle´s lateral wall and the six left ventricular walls (inferoseptal, anterolateral, inferior and anterior, inferolateral, anteroseptal) to obtain a myocardial velocity profile. Both systolic and diastolic phases of the velocity profile were considered for analysis. PSV and E´-wave were measured. Isovolumic relaxation time (IVRT) was defined as the time measured from the end of the ejection phase, crossing zero line to the beginning of the E´-wave. Isovolumic contraction time (IVCT) was measured from the end of the A´-wave (peak velocity at the late diastole), crossing zero line to the beginning of the ejection phase and finally isovolumic contraction velocity (IVCV) was defined as the maximal velocity during the positive wave on the tissue velocity curve during myocardial isovolumic contraction period (Figure 7). Using a regional velocity increment of 25% or less during peak dobutamine infusion as the cut-off for significant ischemia, the left ventricular (LV) walls were categorized as

ischemic or non-ischemic; thus a LV wall was defined as ischemic if a reduction, no increment or an increment of < 25% in peak systolic velocity (PSV) was measured during maximal stress compared to baseline preceding placebo-infusion; otherwise, the LV walls were defined as non-ischemic.

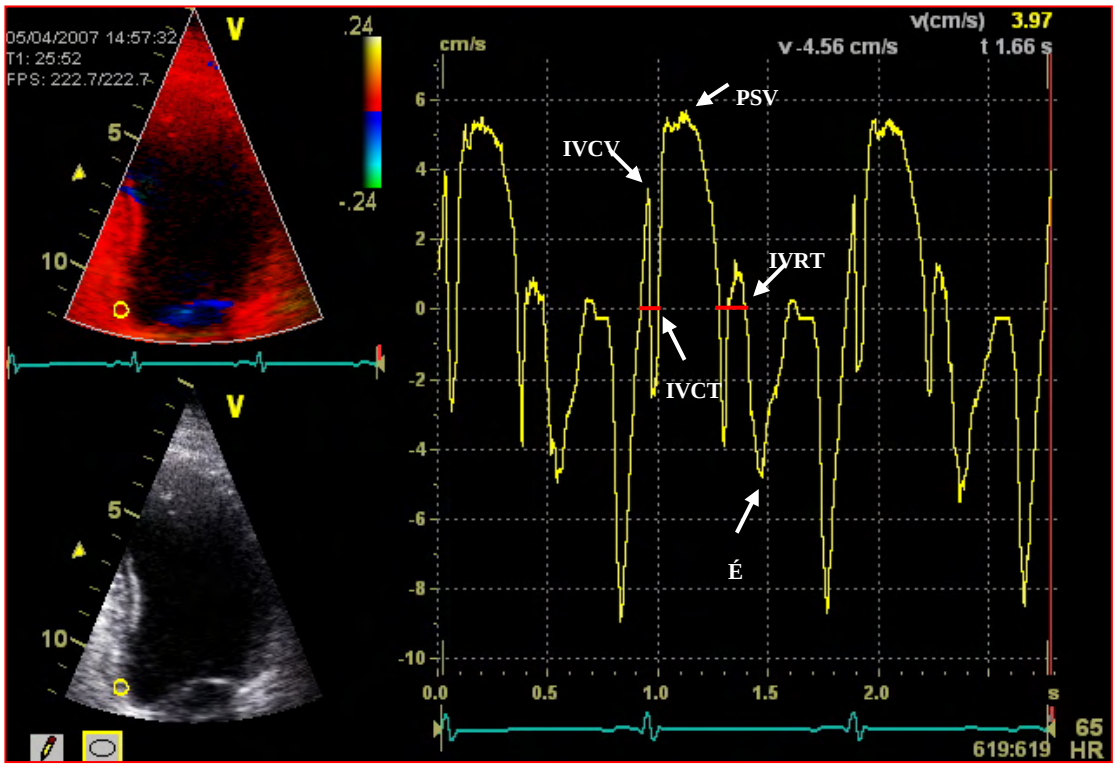


Fig 7. Online recording of tissue Doppler echocardiography. PSV= peak systolic velocity, IVRT= isovolumic relaxation time, IVCT= isovolumic contraction time, IVCV= isovolumic contraction velocity.

Coefficient of variation (CV) for measurement of PSV and E´-wave at TDE were (11-18%) and (11-40%) [97] respectively. CV for measurement of CFR was < 7% [98].

Adenosine infusion

Preceding the stress and perfusion tests an intravenous infusion of either low dose adenosine (35 µg / kg / min) or placebo (saline infusion) was given in a double-blind manner during 10 minutes in paper IV and 15 minutes in paper V. A cross-over protocol was applied where the sequence of infusion was inverted at the second test, at least one day after the first occasion.

4 STATISTICS

Paper I

Statistical comparisons between the three groups (patients with SMI, patients with angina pectoris and healthy volunteers) were performed using the Mann-Whitney U test and comparisons within the groups (only adenosine, naloxone and adenosine) were performed using the Wilcoxon signed ranked test. A p value < 0.05 was considered significant. The Bonferroni correction was used for multiple comparisons between the three measurements (1/3, 2/3, 3/3 of maximum tolerable dose of adenosine). Thus a p value < 0.0167 was considered as significant. If at least two of three possible measurements in each comparison were significant, the outcome was defined as significant. If only one measurement was significant, the outcome was defined as having a tendency for significant difference.

Paper II

Statistical comparisons between the interventions (only adenosine, β-endorphin and adenosine, naloxone and adenosine) were performed by two-tailed Student t test for paired samples. Statistical non-homogeneity for repeated measures was tested with analysis of variance (ANOVA). A p value < 0.05 was considered significant.

Paper III

Statistical comparisons between the groups (male and female patients, male and female healthy volunteers, males and females) and interventions (only adenosine, β-endorphin and adenosine, naloxone and adenosine) were calculated with 2-way analysis of variance (ANOVA). Statistical comparisons within groups were performed by 2-tailed Student t test for paired and unpaired samples.

Paper IV

All echocardiographic measurements were analyzed blinded. Statistical comparisons were performed by analysis of variance (ANOVA) with repeated measures. Post hoc comparisons between the groups (placebo, adenosine) were performed with the Tukey test.

Paper V

All echocardiographic measurements were analyzed blinded. Statistical comparisons were performed by 2-way analysis of variance (ANOVA) with repeated measures. Post hoc comparisons between groups were performed with the Fisher Protected Least Significance test.

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5 ETHICS

All the studies were approved by the local Ethics´ committee. All experiments were performed in a fully equipped laboratory with intensive cardiac care equipments available. All the participants gave their informed consent.

6 RESULTS

6.1 PAPER I

Role of Adenosine and Opioid-Receptor Mechanisms for Pain in Patients with Silent Myocardial Ischemia or Angina Pectoris: A Double-Blind, Placebo-Controlled Study

Aims

To investigate whether patients with SMI has decreased sensitivity to adenosine provoked pain and if so, whether the induced pain response is counteracted by non-selective opioid antagonist.

Methods

Thirteen patients with SMI, 10 patients with angina pectoris and 10 healthy volunteers, all male, were included in the study. After establishing the maximal tolerable dose of adenosine, the participants were given 4 doses (placebo, 1/3, 2/3, 3/3 of maximum tolerable dose) in a double-blind, randomised order and the procedure was repeated after an injection of an non-selective opioid antagonist (naloxone 0.4 mg). Central chest pain was quantified by psychophysical methods.

Results

Pain responses measured as pain duration, intensity and pain mass were shorter in patients with SMI compared to patients with angina pectoris and healthy volunteers. The differences in pain response persisted also after injection of naloxone. Naloxone tended to increase the pain response in healthy volunteers.

Conclusions

Patients with SMI has a decreased sensitivity to adenosine-provoked chest pain compared to patients with angina pectoris and healthy volunteers. The decreased pain response is not related to opioid receptor activity.

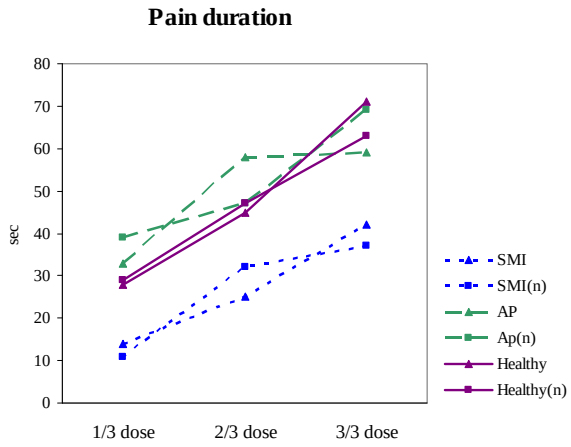

Figure 8. Duration of pain after injection of adenosine in patients with silent myocardial ischemia (SMI), patients with angina pectoris (AP) and healthy controls before and after administration of naloxone (n).

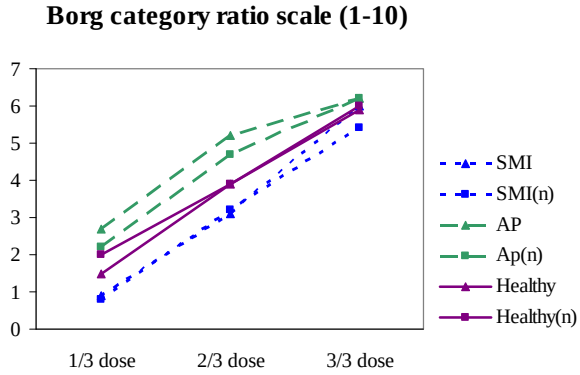

Figure 9. Intensity of pain after injection of adenosine in patients with silent myocardial ischemia (SMI), patients with angina pectoris (AP) and healthy controls before and after administration of naloxone (n).

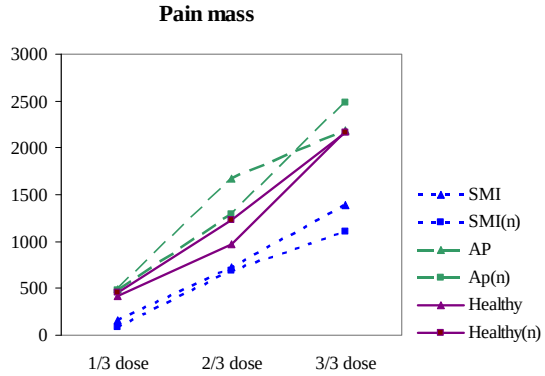

Figure 10. Pain mass (integral of pain over time) after injection of adenosine in patients with silent myocardial ischemia (SMI), patients with angina pectoris (AP) and healthy controls before and after administration of naloxone (n).

6.2 PAPER II

High-Dose Adenosine Infusion Provokes Oscillations of Chest Pain Without Correlation to Opioid Modulation: A Double-Blind Controlled Study

Aims

To characterize the pain response during high-dose adenosine infusion and the effect of μ-receptor agonist, β-endorphin and non-selective opioid antagonist, naloxone on adenosine provoked chest pain.

Methods

Ten healthy volunteers with mean age 26 ± 3 years were included. The study was performed at 3 separate sessions. High-dose adenosine (140 μg/kg/min) was infused for 22 minutes at each session. After 5 minutes of high-dose adenosine infusion, the subjects were randomized to either NaCl bolus for 2 minutes, followed by NaCl infusion for 15 minutes (placebo), or β-endorphin bolus (13.5 nmol) followed by β-endorphin infusion (9.1 nmol/min), or naloxone bolus (0.8 mg) followed by NaCl infusion. Hemodynamic and pain parameters were monitored.

Results

All participants experienced chest pain with oscillation of painful and pain-free episods. There were no significant differences in hemodynamic or pain response between only high-dose adenosine infusion compared to high-dose adenosine in combination with β-endorphin or naloxone. Painful episodes were preceded by an increase in sudomotor activity and were accompanied by as increase in sympathetic activity, expressed as increased heart rate and blood pressure.

Conclusions

High-dose adenosine infusion induces chest pain which is not continuous, but shows oscillation between painful and pain-free intervals of about 35s. The oscillation is not modulated by opioids.

	BORG mean	Number of oscillations	Pain Duration (s)	Pain free Duration (s)	Integral Pain over time	GSR onset Pain onset (s)
NaCl	3 ± 1.3	16 ± 3.6	35 ± 11.4	34 ± 7.9	599 ± 422	8 ± 4.3
ß-endorphin	2 ± 0.9	17 ± 5.2	37 ± 18.1	39 ± 25.5	627 ± 575	7 ± 4.7
Naloxone	3 ± 1.1	15 ± 2.5	34 ± 5.9	37 ± 10.7	550 ± 281	7 ± 4.1
ANOVA (p-value)	0.754	0.634	0.865	0.847	0.926	0.883

	Heart Rate At max Pain (bpm)	Blood Pressure at max pain (mmHg)	Double Producte at max Pain (bpm * mmHg)
NaCl	89 ± 16.9	130 ± 14.1	11 522 ± 2 599
ß-endorphin	89 ± 14.3	130 ± 16.2	11 525 ± 2 223
Naloxone	92 ± 10.3	131 ± 16.8	12 099 ± 2 313
ANOVA (p-value)	0.825	0.988	0.833

Table 1. Data are given as mean ± SD.

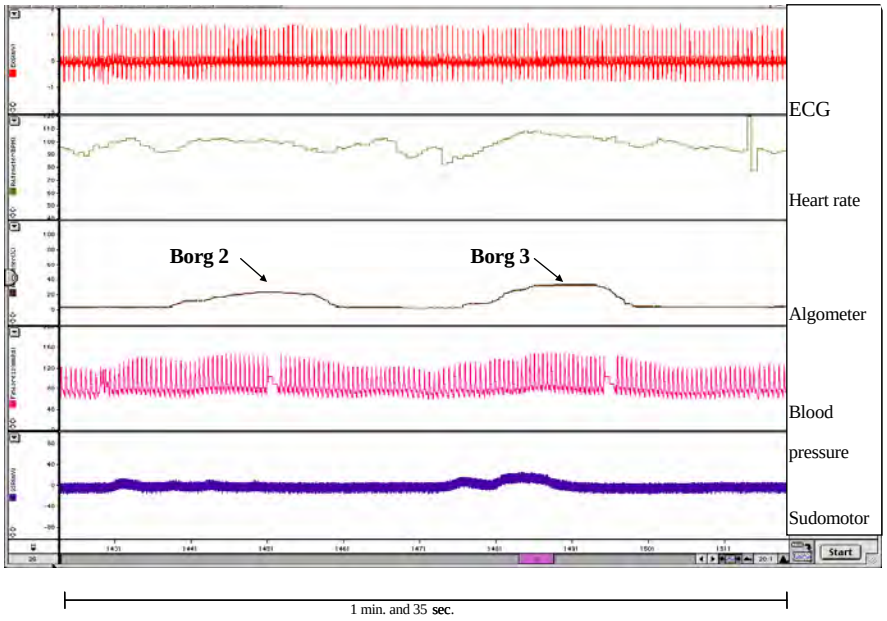

Figure 11. Online recording during adenosine infusion. Sudomotor (sympathetic induced skin resistance). Pain was estimated both online by hand algometer and verbally by Borg CR-10.

6.3 PAPER III

B-endorphin Modulates Adenosine Provoked Chest Pain in Men, but not in Women – A Comparison between Patients with Ischemic Heart Disease and Healthy Volunteers

Aims

To investigate the influence of gender on the adenosine-provoked chest pain and the effect of the opioid receptor agonist and antagonist on adenosine-provoked chest pain.

Methods

Twenty patients (10 male and 10 female) with significant CAD and 20 healthy volunteers (10 male and 10 female) were included. Chest pain was measured as pain intensity, using Borg CR-10 and pain peak using hand algometer. After establishing the maximal tolerable dose of adenosine, the subjects received (placebo, 1/3, 2/3, 3/3 of maximum tolerable dose) in a double-blind manner. This procedure was repeated after bolus injection followed by infusion of β-endorphin and finally after bolus injection of naloxone.

Results

Maximal tolerable dose of adenosine did not differ between the genders. Pain, estimated by two different modalities (hand algometer and Borg CR-10) correlated ($r=0.77$).

In males, β-endorphin counteracted the adenosine induced pain, expressed as pain intensity ($p < 0.02$) and pain peak ($p < 0.04$). The analgesic effect of β-endorphin was even more evident in the patient group ($p < 0.15$). In males, naloxone tended to increase the pain response compared to the pain response during β-endorphin infusion. β-endorphin and naloxone did not affect the pain response in the female group.

Conclusions

β-endorphin did not modulate the adenosine-provoked chest pain in women, but in men β-endorphin induced analgesia.

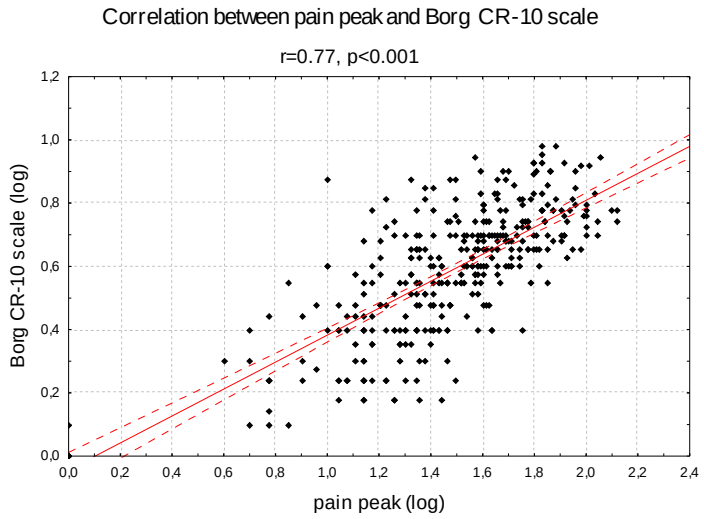

Figure 12. Correlation between pain estimates by hand algometer (pain peak) and the Borg category-ratio (CR-10) scale.

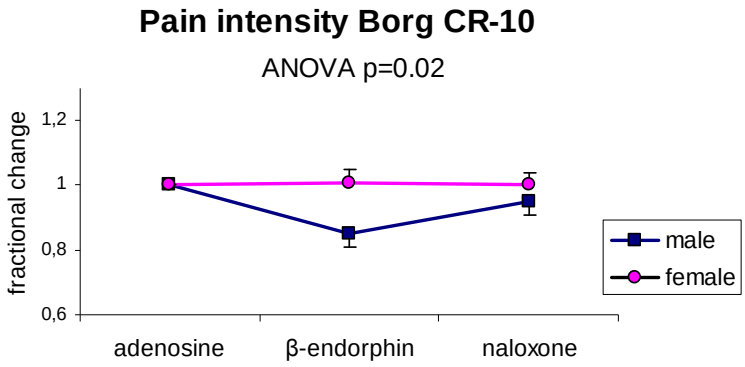

Figure 13. Gender differences in fractional change (intervention / baseline) of pain intensity (Borg CR-10) during in the interventions. Two-way ANOVA, $p < 0.02$. Values are given as mean ± SEM.

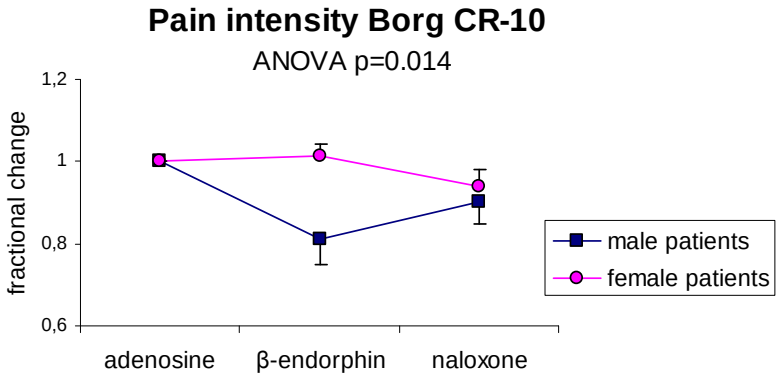

Figure 14. Differences between male and female patients in fractional change of pain intensity (Borg CR-10) during the intervention. Two-way ANOVA, $p < 0.02$. Values are given as mean ± SEM.

6.4 PAPER IV

The Preconditioning Effect of Low-dose Adenosine Infusion during Exercise Test in Patients with Ischemic Heart Disease – A Double-Blind, Cross Over, Placebo Controlled Study

Aims

To study whether low-dose adenosine infusion reduces ischemic burden, induced by physical exercise, expressed as improved regional LV systolic function.

Methods

Nine male patients with CAD were included. Myocardial ischemia was induced by exercise and quantified by tissue Doppler Echocardiography technique (TDE) at two separate occasions with a randomized, double-blind and cross-over protocol. Prior the exercise test, an intravenous infusion of either low-dose adenosine (35 µg/kg/min) or placebo (NaCl) was given during ten minutes. Echocardiographic images were obtained during baseline, adenosine/placebo, submaximal, maximal and recovery phases. The LV walls were further categorized as ischemic if an increment < 15% in peak systolic velocity was measured during maximal exercise compared to baseline preceding placebo infusion.

Results

There were no significant differences in maximal work capacity, chest pain or hemodynamic parameters between adenosine and placebo pretreatment. According to the predefined ischemia criterion, the ischemic wall segments had higher PSV ($p<0.0001$), E´-wave ($p<0.001$), AV-plane displacement ($p<0.002$) and strain after adenosine infusion compared to placebo. A´-wave and strain rate was not modulated by adenosine. There were no significant differences in TDE variables in the non-ischemic walls after adenosine or placebo infusion.

Conclusions

Low-dose adenosine infusion reduces the ischemic burden measured as improved regional LV systolic function in the ischemic walls.

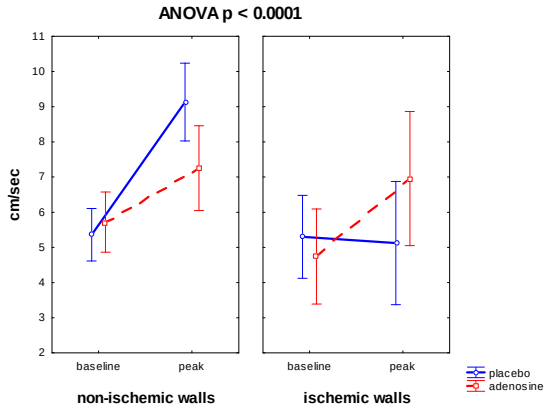

Figure 15. Peak systolic velocity at baseline and peak during adenosine vs placebo in ischemic and nonischemic left ventricular walls. Data are presented as mean ± SD.

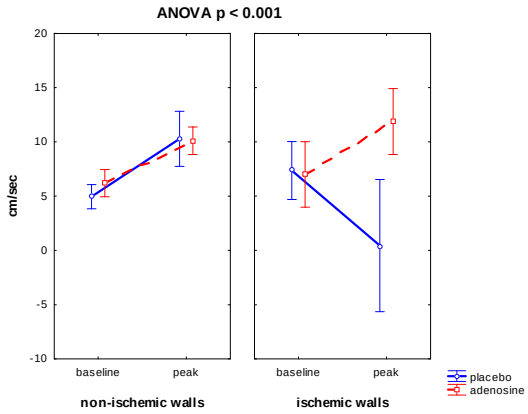

Figure 16. E´-wave at baseline and peak during adenosine vs placebo in ischemic and nonischemic left ventricular walls. Data are presented as mean ± SD.

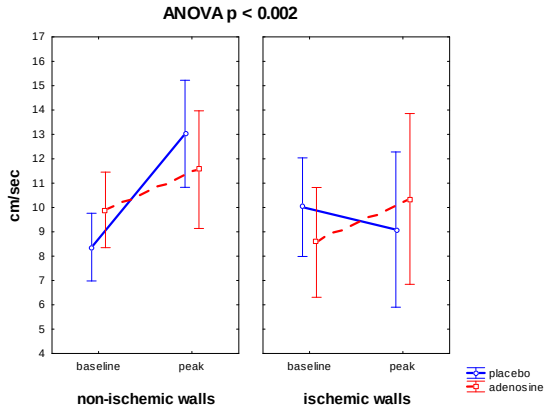

Figure 17. AV-plane displacement at baseline and peak during adenosine vs placebo in ischemic and nonischemic left ventricular walls. Data are presented as mean ± SD.

6.5 PAPER V

Pretreatment with low-dose Adenosine Infusion Improves Ischemic Wall Motion at Pharmacological Stress without Affecting the Myocardial Perfusion in Patients with Coronary Artery disease – A Double-blind placebo controlled, cross over study

Aims

To study whether low-dose adenosine infusion reduces the ischemic burden induced by dobutamine stress test, measured as increased myocardial velocities, without affecting the myocardial perfusion, estimated by coronary flow reserve.

Methods

Eleven patients with CAD were included in the study. Myocardial ischemia was induced by dobutamine stress protocol and quantified by TDE on two separate occasions. CFR was also quantified at two separate occasions at baseline and during hyperaemia induced by high-dose adenosine infusion. Prior to the dobutamine stress and CFR measurement, low-dose adenosine (35 µg/dose/min) or placebo was infused over 15 minutes in a double-blind fashion. During dobutamine stress, echocardiographic images of right and left ventricular walls were recorded during baseline, adenosine /placebo, submaximal, maximal and recovery phases. Further, a wall segment was considered as ischemic, if an increment < 25% was measured during maximum stress in PSV compared to baseline in the placebo group .

Results

PSV increased significantly at maximum stress both after placebo and adenosine pre-treatment in the non-ischemic walls but in the ischemic walls, PSV increased only after adenosine pretreatment ($p < 0.001$). There were no differences in the hemodynamic parameters or coronary flow reserve between the two pretreatments.

Conclusions

Low-dose adenosine infusion decreases the ischemic burden in the ischemic wall segment measured as improved regional systolic LV function, without any effect on

hemodynamic parameters or CFR, suggesting that the observed myocardial improvement in systolic function may be due to preconditioning.

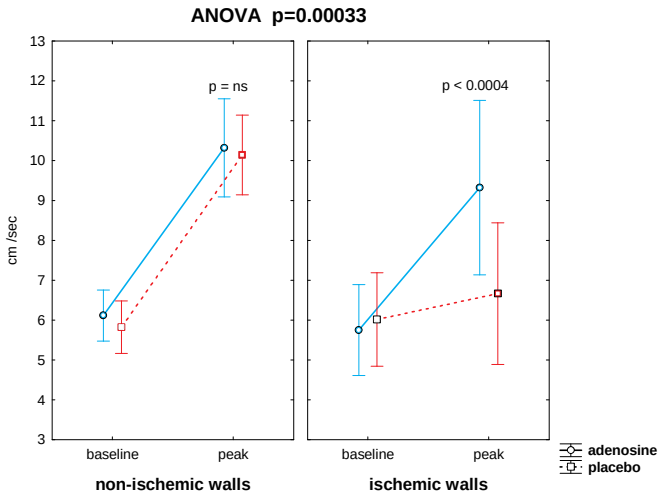

Figure 18. Peak systolic velocity at baseline and maximum stress after low-dose adenosine vs placebo infusion. Values are given as mean ± SD. ns= non significant

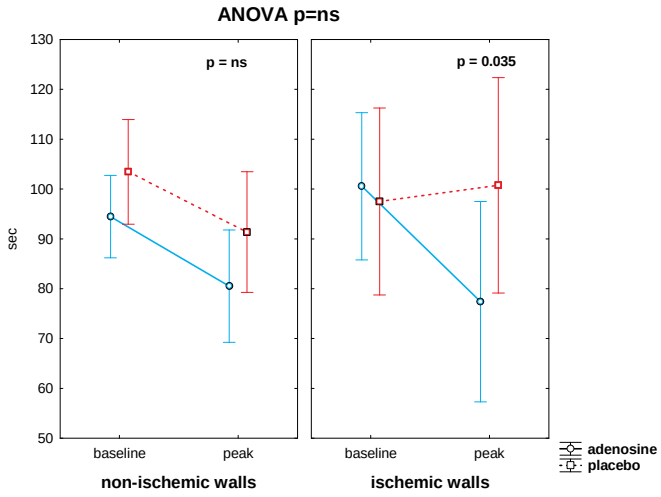

Figure 19. Isovolumic relaxation time at baseline and maximum stress after low-dose adenosine vs placebo infusion. Values are given as mean ± SD. ns=non significant

	CFR baseline	CFR peak	CFR fractional change
Adenosine	0.32 ± 0,18	0.39 ± 0.21	1.37 ± 0.83
Placebo	0.29 ± 0.07	0.43 ± 0.19	1.56 ± 0.87

Table 2. Coronary flow reserve (CFR) during baseline and after three minutes of adenosine infusion (peak) and fractional change (peak / baseline). Values are given as mean ± SD.

7 GENERAL DISCUSSION

7.1 ADENOSINE AND ALGESIA

Adenosine is a neuromodulatory agent, which depending on activation of the receptor subtypes, can either stimulate or inhibit different functions. Adenosine in bolus and high dose infusion produces dose-dependent chest pain. Patients with SMI comprise a large group in the CAD population. A review of the literature shows prevalence rates of SMI ranging from 9 to 57% due to differences in the population studied [52, 53]. Men seem to have higher incidence of SMI compared to pre-menopausal women, a difference which quickly changes in postmenopausal women [60]. Furthermore, patients with SMI as a group seem to have higher pain threshold to somatic pain. An increased tone in the opioid pain inhibiting system has been proposed by studies in patients with CAD after psychological stress [48, 99] and also during angioplasty [100]. Adenosine bolus injection as a visceral pain model for ischemia was therefore tested in patients with SMI, patients with angina pectoris and healthy volunteers. Patients with SMI had less adenosine induced pain with respect to duration, intensity and burden (defined as the area under the curve produced by hand algometer) compared to patients with angina pectoris and healthy volunteers. These differences in pain response also remained after injection of naloxone. In the healthy participants naloxone had a tendency to increase the pain mass only at one dose. Naloxone did not modulate the pain response in the other two groups with CAD, (SMI and angina pectoris). Therefore the decreased sensitivity to adenosine is not due to the increased opioid activity.

High dose adenosine infusion is a pharmacological option for provocation of ischemia due to steal phenomenon. We have earlier reported that high dose adenosine infusion induces algesia but of oscillatory character with increased plasma endogenous β-endorphin during infusion. [101]. B-endorphin as a predominantly μ-opioid receptor

agonist counteracts adenosine induced pain [45]. In our second paper we investigated the effect of exogenous β-endorphin and opioid antagonist, naloxone on high dose adenosine infusion induced algesia and analgesia. We could demonstrate that high dose adenosine infusion induces episodes of pain which are not continuous and vary in intensity. This phenomenon was not modulated by opioid agonist or antagonist. Oscillations of pain episodes with a cycle time of about 35 s are not common in biological systems. If the oscillations are not opioid dependent peripherally, there may be due to stimulation and inhibitions more central in the central nervous system. Thalamus serves as a primary relay station in various sensory pathways and Rosen suggested that thalamus could have a gating function in the processing of anginal pain leading to an oscillatory response pattern of pain [102].

Another important aspect of cardiac pain is the different manifestation of the symptoms between the genders. Women seem to report higher pain level and exhibit less pain tolerance as explored earlier [67, 68]. Evidence suggests functional differences in the endogenous opioid system as one of several contributing factors to gender differences in pain sensitivity [70, 103, 104]. Research have shown that opioid medication that acts predominantly on κ receptors produces greater pain reduction in women than in men and therefore may indicate enhanced κ-opioid pain modulation in women. Experimental studies have also demonstrated gender differences in the function of μ-opioid receptors [105], with males exhibiting greater antinociceptive effect of μ-receptor agonists than females [106]. In our study, the gender differences in adenosine provoked pain and the effect of opioids on the induced pain were tested. Surprisingly, there were no differences in maximal tolerable dose of adenosine but β-endorphin had an analgesic effect on adenosine-provoked pain only in males ($p < 0.04$). Further, naloxone had a tendency to antinociception only in the male group ($p=0.054$). The present study is limited by use of a standard dose of β-endorphin and naloxone,

without adjusting for differences in body weight between the genders. It should also be pointed out that the small sample size makes these results preliminary. Another uprising issue would be whether the gender differences to opioid antinociception persist in older individuals and premenopausal women.

In paper I, healthy males showed an increase in pain mass at one dose after naloxone. The same pattern was demonstrated in study III, where males showed almost significant increase in pain response after naloxone. In both studies, the dose of naloxone (0.4 and 0.8 mg) was relatively low and not adjusted to the body weight of the subjects but reached almost a significant effect when it was followed after β-endorphin infusion (study III). Thus the given dose seems not to be enough to decrease the endogenous opioid secretion but naloxone may increase the pain response during exogenous β-endorphin induced analgesia.

In females, β-endorphin and naloxone had no effect in pain response, induced by adenosine, suggesting another opioid receptor subtype than μ- receptor, such as κ-receptor, responsible for analgesia, which is in accordance to other reports [70, 103, 104]. Therefore, also among women, naloxone which is a non-selective opioid antagonist, at the given dose is not able to inhibit the endogenous opioid system.

7.2 ADENOSINE AND ANALGESIA

The analgesic effect of adenosine has been studied in several animal and clinical studies and is induced by low-dose infusion. Randomized, placebo-controlled studies in humans, show that low-dose adenosine infusion in dose ranges of 40-80 μg/kg/min significantly reduces the requirement of isoflurane and postoperative opioid usage [107-109]. Other studies compared the analgesic effect of adenosine to remifentanil with better pain relief and less opioid usage in the adenosine group [110, 111]. Interestingly, the effect of adenosine on postoperative pain in these studies outlasted the

duration of the infusion. The same phenomenon was seen in patients with chronic neuropathic pain [32].

Adenosine in analgesic dosage has also been studied in trials of acute MI and reperfusion. Preconditioning is a well-established phenomenon and recent clinical trials such as AMISTAD I and II suggest that preconditioning mimetics such as adenosine may reduce myocardial infarcts size, when given during reperfusion. Numerous experimental studies support the crucial role of adenosine as a trigger of classic preconditioning. ATP-sensitive potassium channels (K_{ATP}) play an important role in mediating the classical preconditioning [112, 113]. The adenosine receptor (A_1) [114] opens K_{ATP} channels which hyperpolarize myocardial cells and consequently reduces calcium influx via voltage-regulated calcium channels. Thus the protection from reperfusion injury by ischemic preconditioning is mediated by activation of A_1-receptors by endogenous adenosine, an effect that is abolished by glibenclamide, a K_{ATP} channels antagonist. A recent clinical study demonstrated that administration of the K_{ATP} channel opener nicorandil prior to reperfusion improved the outcome in acute MI patients undergoing reperfusion therapy [115].

The beneficial effects of the clinical adenosine trials such as AMISTAD may not be due to true preconditioning, since adenosine was given during ischemia and not in a pretreatment fashion. The effects observed on the myocardial infarct size may rather be related to postconditioning (graded reperfusion [116]), anti-inflammatory effect, a reduction in apoptosis or an antiplatelet effect whereby adenosine helped to keep the infarct-related artery open.

As analgesia induced by low-dose adenosine infusion might be due to preconditioning, the last two papers were designed to test adenosine as preconditioning mimetic. As it is difficult to predict the occurrence of an ischemic episode, the patients were subjected to physical exercise (paper IV) to mimic an ischemic episode as

physiologically as possible. Prior to the exercise test, the patients received either low-dose adenosine infusion or placebo. TDE variables during baseline and peak exercise were measured and since the number of patients was small, we predefined a cut-off value for ischemia, which was far lower than in clinical practice. This was done in order to have a fair chance to find any statistical difference. The cut-off value in paper IV was chosen to be a 15% increase and in paper V a 25% increase in PSV at maximum stress compared to the baseline after placebo infusion. In paper IV, we could measure improvement in the ischemic wall segments after pretreatment with adenosine infusion compared to placebo (PSV $p<0.0001$). In the last paper, the patients were subjected to pharmacological stress echocardiography and contrast echocardiography. We could reproduce the results of paper IV, which was an increase in myocardial velocity at peak stress after adenosine pretreatment compared to placebo (PSV $p<0.001$). The increase in PSV in the adenosine group during stress reached a level not different from that observed in the non-ischemic walls. Further, the coronary flow reserve did not show any significant difference between adenosine and placebo pretreatment, ruling out vasodilatation or unloading as effects of adenosine for the improved wall motion.

Despite the evidence that preconditioning is protective in both animal models and humans, the concept has not been applied to routine therapy in clinical medicine. It is possible that postconditioning – in which reperfusion is interrupted with brief coronary occlusions and reperfusion sequences – is more likely than preconditioning to be feasible as a clinical application for in e.g. patients undergoing PCI or cardiac surgery.

8 CONCLUSIONS

Patients with SMI show higher pain threshold, measured as adenosine induced pain, compared to patients with angina pectoris and healthy volunteers. The higher pain threshold is not opioid dependent.

High-dose adenosine infusion induces oscillation of pain which is not modulated by opioid agonist or antagonist.

No gender differences have been demonstrated concerning adenosine-provoked pain but β-endorphin induces analgesia in men which is counteracted by naloxone. B-endorphin does not modulate the adenosine-provoked pain in women.

Low-dose adenosine infusion decreases the ischemic burden, measured as increased myocardial velocities both during physical exercise and pharmacological stress provocation, without affecting the coronary flow reserve.

9 FUTURE OUTLOOK

As discussed earlier postconditioning defined as series of brief interruptions of reperfusion applied at the very onset of reperfusion [117] may be clinically more applicable than preconditioning because the therapy would not have to be administered prior to an ischemic episode. Postconditioning could be applied to the interventional technique during reperfusion for acute myocardial infarction but also for interventions in patients with stable angina pectoris. Clinical trials with adenosine during reperfusion may be the first example of clinical use of postconditioining.

Further, since several studies have indicated gender differences in ischemic heart disease, it would certainly be interesting to evaluate whether women have the same benefit of preconditioning as men and also the effect of opioids as preconditioning mimetic.

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11 RELATED PUBLICATIONS CO-AUTHORED BY BITA SADIGH

1. Eriksson BE, **Sadigh B**, Svedenhag J, Sylvén C. Analgetic effects of adenosine in syndrome X is counteracted by theophylline – A double-blind, placebo-controlled study. *Clinical Science* 2000,98:15-20.
   Related to study I-III.

2. **Sadigh-Lindell B**, Sylvén C, Hagerman I, Berglund M, Terenius L, Franzén O, Eriksson BE. Oscillations of pain intensity during adenosine infusion. Relation to beta-endorphin and sympathetic tone. *Neuroreport* 2001,12(8):1571-1575.
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