Karolinska Institutet
Department of Medicine Huddinge
Division of Cardiology
Stockholm, Sweden

CARDIAC ELECTROPHYSIOLOGIC EFFECTS OF MENTAL STRESS

Per Insulander

Karolinska Institutet

Stockholm 2009

Cardiac electrophysiologic effects of mental stress
ISBN 978-91-7409-343-8
Previously published papers reproduced with permission from the publishers.
Published by Karolinska Institutet.
Printed by larseric Digital Print AB, Landsvägen 65, 172 65 Sundbyberg

To Mona, Therese and Jessica

CONTENTS

ABSTRACT

Background: Mental stress may trigger serious arrhythmias. Mental stress tests cause significant increases in heart rate, blood pressure and cardiac output. When β-adrenergic inhibition is considered for secondary anti-arrhythmic prophylaxis β1 selective agents are often preferred because of less adverse reactions. The importance of the different β-adrenoceptors on the electrophysiologic function is incompletely known.

Methods: In all studies, healthy volunteers were investigated by means of standard electrophysiologic catheterization procedures. Electrophysiologic and hemodynamic variables were measured under different settings of autonomic modulation with the primary goal to characterize the effects of mental stress on cardiac conductive tissues and the importance of β1 and β2 adrenergic receptors in cardiac tissues. To this mean infusion of adrenergic agonists (using epinephrine, dobutamine and salbutamol), a mental stress test (Stroop's Colour Word Conflict Test, CWT) and selective and unselective autonomic tone inhibition, ATI, (using atenolol, propranolol and atropine) were used.

Study I investigated the electrophysiologic effects on the cardiac conduction system elicited by CWT and compared them with the effects of epinephrine. Mental stress produced significant electrophysiologic effects with shortening of all measured electrophysiologic variables except atrial, most markedly those of the sinus and the atrioventricular nodes. During infusion of epinephrine, corresponding effects could only be reproduced at a much higher plasma level than during CWT.

Study II investigated gender differences in the cardiac electrophysiologic effects elicited by CWT and by ATI. During CWT men had shorter QT and JT durations. Women had shorter refractoriness in the atrial tissue and in the AV node. After ATI no gender differences in sinus nodal properties were noted, whereas AV nodal refractoriness and conduction time were shorter in women. QT and JT duration, and the refractory period of the right ventricle, became shorter in men.

Study III assessed the overall electrophysiologic effects of infusion with the β2-agonist salbutamol. To distinguish β2-agonist effects a comparison was made with the β1 selective agonist dobutamine. Salbutamol produced significant changes in electrophysiologic properties both in myocardial and nodal tissues, the effects being greater on nodal properties. The proportional decreases in the AV node parameters were more pronounced than in the sinus node. There was an *increase* in the duration of the QS interval, indicating slower depolarization of the ventricle. QT dispersion increased

Study IV evaluated if the type of β-blocker, β1-selective or unselective, is of importance in inhibition of the electrophysiologic effects of CWT, which was also assessed with each β-blocker combined with atropine. With propranolol as the β-blocker in ATI the electrophysiologic effects of CWT were completely eliminated. ATI with atenolol, though, gives an incomplete inhibition of the effects of mental stress on sinus cycle length, sinus node recovery time, AV nodal and the ventricular refractoriness, and on QT-duration.

Conclusions: Mental stress produces pronounced electrophysiologic effects, most markedly in the sinus and AV nodes and less in the ventricle. Circulating epinephrine plays only a minor direct role as a mediator of mental stress effects on the heart.

During mental stress, women exhibit a more pronounced effect on the AV node and on the sinus node, and men react with a more pronounced effect on ventricular EP properties. After ATI, women have higher heart rate, longer QT and JT intervals, faster AH conduction, shorter AV nodal effective refractory period, and longer ventricular effective refractory period.

β2-stimulation with salbutamol results in significant effects on cardiac electrophysiology, most pronounced in sinus and AV nodes and less on atrial and ventricular parameters. A discordant effect on ventricular conduction, which slowed, and refractoriness of the ventricular myocardium, which shortened, was seen. QT dispersion increased.

Inhibition with atenolol does not eliminate the effects of mental stress on the ventricular myocardium, effects that at least partly appears mediated through stimulation of β2 adrenoceptors. Vagal withdrawal is part of the reaction to mental stress also in the ventricular myocardium.

LIST OF ORIGINAL PAPERS

I. Insulander P, Juhlin-Dannfelt A, Freyschuss U, Vallin H. Electrophysiologic effects of mental stress in healthy subjects: a comparison with epinephrine infusion. *J Electrocardiol.* 2003;36:301-9.

II. Insulander P, Vallin H. Gender differences in electrophysiologic effects of mental stress and autonomic tone inhibition – a study in healthy individuals. *J Cardiovasc Electrophysiol.* 2005;16:59-63.

III. Insulander P, Juhlin-Dannfelt A, Freyschuss U, Vallin H. Electrophysiologic effects of salbutamol, a β2-selective agonist. *J Cardiovasc Electrophysiol.* 2004;15:316-22.

IV. Insulander P, Vallin H. Electrophysiologic effects of mental stress after autonomic tone inhibition – different effects of selective and unselective β-receptor inhibition. *In manuscript.*

.

ABBREVIATIONS

AERP	Atrial effective refractory period
AF	Atrial fibrillation
AFL	Atrial flutter
AH	AH conduction time = conduction time through the AV node
ATI	Autonomic tone inhibition
AVNERP	AV nodal effective refractory period
AVNRT	AV nodal reentrant tachycardia
AVRT	Atrioventricular reentrant tachycardia
CSNRT	Corrected sinus node recovery time
CWT	Stroop's Colour Word Conflict Test
HV	HV conduction time = infranodal conduction time
LQTS	Long QT syndrome
QTc	Corrected (for rate) QT interval
SCL	Sinus cycle length
SNRT	Sinus node recovery time
VERP	Ventricular effective refractory period
VF	Ventricular fibrillation
VT	Ventricular tachycardia

BACKGROUND

EFFECTS OF MENTAL STRESS

Emotional stress and sudden death

The concept that you can die of fright is well established in our history and culture. Since ancient times, case reports describing sudden death in the context of strong emotions have been reported in the literature [1-5]. The scientific explanation for this lethal experience is less well explored. From modern published reports we know that emotional stress may trigger arrhythmias both in individuals with structural heart disease and in healthy individuals. Serious arrhythmias, including ventricular tachycardia and ventricular fibrillation, have been reported [6-14].

Early case reports of sudden death

In *Acts of the Apostles 5:1-11* we read how Ananias, after having sold a possession but withholding a part of the money for his own disposal instead of offering all to the apostles, is confronted and charged by Peter, who ends his indignant accusation with these words: "*…thou has not lied unto men, but unto God!"* Hearing this, Ananias fell dead to the ground. A similar fate befell his wife Sapphira [1].

The Roman emperor Nerva is described to have died suddenly due to "*a violent excess of anger*" during an animated discussion with a senator who had offended him [2].

Examples of sudden death caused by overwhelming grief, fear, or even joy are abundant in history. Pope Innocent IV died in 1254 shortly after his army was defeated, his death attributed to "*the morbid effects of grief on his system*" [3]. The Spanish King Philip V was described to have succumbed to a classical sudden death when he was informed that the Spanish army had been defeated in 1746 [4].

The 18th century Scottish surgeon and anatomist John Hunter, known for his temper, was well aware of the relation between strong emotions and sudden death and correctly predicted the manner of his own death: "*My life is at the mercy of any scoundrel who chooses to put me in a passion"* [5].

Modern case reports

The American physiologist Walter Cannon performed important work forming the basis of our understanding of the response system linking strong emotions such as fear or acute threat with illness. He coined the expression "fight or flight" to describe the neurophysiologic-behavioural response pattern in a situation of acute threat or fear [15].

In a paper 1942 entitled "Voodoo Death" [16] he cited numerous examples from the anthropology literature of death from fright, although few of these examples were what we would today define as sudden death. He argued that

> *"fear is one of the most deeply rooted and dominant of the emotions… Associated with*

it are profound psychological disturbances, widespread throughout the organism… When [fear or rage is] roused they bring into action an elemental division of the nervous system, the so-called sympathetic or sympathico-adrenal division, which exercises a control over internal organs and also over the blood vessels. If these powerful emotions prevail, and the bodily forces are fully mobilized for action, and if this state of extreme perturbation continues in uncontrolled possession of the organism for a considerable period, without the occurrence of action, dire results may ensue…"

Missing from Cannon's explanation is the hormonal stress response, the cascade of hormones released from the brain, pituitary gland, and adrenal gland within minutes of exposure to any sort of stressor. In 1942, when the article was written, many of these hormones were yet to be discovered [17].

In 1971, George L. Engel [4] collected 170 accounts of sudden death caused by strong emotional stress such as the impact of the death of a spouse, acute grief, personal danger or threat of injury, or on loss of status or self-esteem. Several of these anecdotal deaths are described in quite detail, as the following example:

"A 71-year old woman arrived by ambulance at the emergency room, accompanying her 61-year old sister who was pronounced dead on arrival. The patient collapsed at the instant of receiving the news. An ECG showed AV dissociation or a nodal rhythm with retrograde conduction, left bundle block and myocardial damage. Shortly she developed ventricular fibrillation and died."

In 1976, Gradman et al. [18] described a 53 year old male with known coronary heart disease and three previous myocardial infarctions and frequent PVCs. The latter were evaluated with a treadmill test, which was negative for ischemia although he was sent home with a Holter recorder. On the same day, while viewing an exciting basketball game, he died. The recording showed increased PVC frequency the hour before death; the terminal arrhythmia was a single PVC initiating a rapid ventricular flutter degenerating into ventricular fibrillation. Whether this death was caused solely by an emotionally triggered arrhythmia or if, for instance, a ruptured plaque contributed to his death is of course not known.

Natural disasters and war

Natural disasters such as earthquakes have been reported to increase the incidence of sudden cardiac death. Trichopoulos et al. [19] found an excess of deaths also from cardiac causes on the days after a major earthquake in 1981, when a comparison was made with the same periods the previous and the following year. Leor et al. [20] found an increase in sudden deaths from cardiac causes that were related to atherosclerotic cardiovascular disease. The increases went from a daily average of five cases the preceding week to 24 cases on the day of an earthquake 1994.

The incidence of acute myocardial infarction and sudden death during the Iraqi missile attack on Israel during the Gulf war 1991 was studied by Meisel et al. [11]. They found a significant increase in the incidence of myocardial infarction and an increase of more than 80% in sudden out-of-hospital deaths.

The concept of stress

The stress syndrome was described by Hans Selye in a paper published in Nature 1936 [21]. He focused on steroid hormones as mediators of the stress effect. He published a theoretical outline for of the stress concept in 1946, where he termed the response to stress as the general adaptation syndrome [22]. Selye later defined biologic stress as the non-specific response of the body to any demand made upon it.

Before Seley's work, the neuroendocrine response to non-specific injury was thought to be restricted to the release of catecholamines as described by Walter Cannon. Selye's hypothesis addressed the non-specificity of the neuroendocine response. He called the stress-causing agents stressors [23].

In a 1955 article ("Stress and disease") published in *Science* [24], he made a number of accurate predictions confirmed by later studies:

> "*Among the derailments of the general adaptation syndrome that may cause disease, the following are particularly important: (i) an absolute excess or deficiency in the amount of adaptive hormones (for example, corticosteroids, ACTH, and STH) produced during stress; (ii) an absolute excess or deficiency in the amount of adaptive hormones retained by their peripheral organs during stress; (iii) a disproportion in the relative secretion during stress of various antagonistic adaptive hormones; (iv) the production by stress of metabolic derangements, which abnormally alter the target organ's response to adaptive hormones ("conditioning"); and (v) although the hypophysis-adrenal mechanism plays a prominent role in the general-adaptation syndrome other organs that participate in the latter (for example…the nervous system…) may also respond abnormally and become the cause of disease during adaptation to stress…"*

Selye's hypothesised that stressors very different in nature (e.g. excessive heat or cold, exercise, chemical, biologic and psychological agents) always elicit the same neuroendocrine (non-specific) response. Among his last contributions to the stress concept was the recognition that despite our different psychological and cerebral reactions, both negative and positive stressors elicit virtually identical corticoid/catecholamine response [25]. This laid the broad base for stress research for years to come.

However, later Mason [26], among others, presented evidence that distinctive cardiovascular and endocrine responses are specific to certain types of stressors. Furthermore, there is evidence that the stress response is not caused by the type of stressor per se, but by the ability of the individual to cope with the stressor [26]. Lundberg et al. [27] have shown that the increases in epinephrine, norepinephrine and cortisol levels differ significantly between different kinds of mental stress tests.

Emotional stress and cardiac arrhythmias

Already moderate emotional stress may cause arrhythmias. In one early study, ECG was recorded in experienced car drivers while driving their own cars along well-known routes. In subjects with stable coronary heart disease development of multiple ventricular ectopic beats while driving was a common finding and even short runs of ventricular tachycardia. No palpitation was experienced by the participants during these arrhythmias [8].

Using a questionnaire, Hansson et al. [28] analyzed triggering factors in one hundred randomly selected patients with idiopathic paroxysmal atrial fibrillation leading to hospitalization. They found that mental stress was the most common factor triggering arrhythmia (54%), followed by physical exertion (42%), tiredness (41%), and infections (22%).

The psychological distress caused by the attack on the World Trade Center September 2001 increased occurrences of adequate ICD therapies for ventricular arrhythmias among patients in New York. Shedd et al. [29] reported that in the 30 days following the attack, a total of 14 patients (11%) had ventricular tachyarrhythmias, compared with five (3.8%) in the preceding 30 days. A similar result was found by another group who reported a 2.3-fold increase in risk of ventricular arrhythmias during this period [30]. They also noted that the first arrhythmic event did not occur until three days after the attack.

Strong emotions associated with sports events have also been reported to cause an increase in the risk of acute cardiovascular events including sudden death. Wilbert-Lampen et al. [31] examined the relation between emotional stress and the incidence of cardiovascular events during the FIFA World Cup in football 2006 in Germany. They found an increase in the incidence of myocardial infarction by a factor of 2.5 and in cardiac arrhythmias causing major

symptoms by a factor of 3.1 on days the German team competed.

Lampert et al. [32] performed an experiment where patients with ICD were given diaries to record levels of defined mood states. The reported anger level during the period preceding a shock of a confirmed ventricular arrhythmia was significantly higher compared with a control period. The same group compared the morphologic and initiation pattern between ventricular arrhythmias triggered by anger and those that were not. They found that polymorphic ventricular tachycardia was more common in anger-triggered events than in non-angered triggered events [33]. In a another study from this group on patients with ICD and inducible ventricular tachycardia, they found that mental stress induced by a stress test altered the tachycardia cycle lengths and made termination more difficult [34].

Not only acute stress triggers ventricular arrhythmias. Lane et al. [35] interviewed 25 survivors with idiopathic ventricular fibrillation and matched controls. The patients surviving ventricular fibrillation more often reported severe or moderate stress during the six months before the cardiac event as well as during the last 24 hours before the event compared with control patients.

Effects of mental stress on heart rate and ECG

Already moderate stress results in profound cardiac electrophysiologic changes. Stroop's Colour Word Conflict test, an often-used mental stress test, causes a 31% to 47% increase in heart rate in healthy subjects [36-38].

In one study, 30 healthy physicians were studied with ambulatory ECG while on duty during night. When the subjects were aroused from rest or sleep by alarm calls heart rate increased from 55 to 112 beats per minute. The T wave showed inversion in 38% with moderate ST segment depression. The QT interval shortened only slightly and was significantly longer than for similar heart rates during stable conditions [39].

In another study, T-wave alternans was evaluated during a mental stress test and exercise. The authors found that T-wave alternans increases during mental stress and occurs at lower heart rates compared with exercise in patients with ICD and coronary artery disease [40].

Using spectral analysis of RR variability, Pagani et al. [41] studied the changes in the sympathetic and vagal activities regulating heart rate caused by mental stress in healthy subjects and in patients with a recent myocardial infarction. Mental stress induced marked changes in the sympathovagal balance, which moved toward sympathetic dominance, in healthy subjects but not in post-myocardial patients (in whom medication was discontinued at least 24 h before the study).

Folino et al. [42] evaluated the influence of sympathetic stimulation, induced by a verbal mental arithmetic stress test, on signal-averaged ECG in healthy subjects and in patients with pervious myocardial infarction but not currently on betablocker therapy. They found that mental stress could influence the results of signal-averaged ECG in healthy subjects, probably by influencing intraventricular conduction, but not so in patients with ischemic heart disease.

Other cardiac effects of mental stress

There are several epidemiologic studies exploring the relationship between high-stress jobs and increased cardiovascular risk. Theorell et al. [43] studied full-time working men who suffered a first myocardial infarction and found that a combination of high work demands and low control was an independent predictor of risk for myocardial infarction. In another study a more than 2-fold increased risk for new coronary heart disease was found in men who experienced a marked discrepancy between effort and reward at work [44].

Data from the Stockholm Female Coronary Angiography Study showed that stress from family or work life may accelerate coronary disease processes in women [45]. Also marital stress has been linked to an increased incidence of myocardial infarction in women [46].

Supporting these findings are studies showing that social networking has a protective effect against harmful stress. In a study of 783 participants (both men and women), social network indices such as being single or widowed were associated with increased risk for coronary artery calcification, independent of age and coronary risk factors [47].

A number of studies using different types of mental stress tests have demonstrated induced myocardial ischemia in substantial subsets (40% to 70%) of patients with coronary artery disease during mental stress [26]. Some studies have also shown constriction of epicardial coronary arteries due to endothelial dysfunction during stress tests in some patients [48]. Several studies have also demonstrated that mental stress can induce wall motion abnormalities as well as reduce ejection fraction in patients with coronary artery disease [49,50].

Transient left ventricular apical ballooning syndrome, *Tako-Tsubo cardiomyopathy*, is a cardiac condition that mimics the clinical presentation of acute coronary syndrome but without any evidence of obstructive atherosclerotic coronary artery disease. Intense emotional or physiologic stress, with profoundly increased catecholamine levels, is presumed to be the triggering factor and to play a pathogenic role. Over 90% of reported patients are women [51].

Acute versus chronic emotional stress

Most definitions of stress agree on the following descriptions:

1. stress is a process that occurs when environmental demands exceed the adaptive capacity of the organism
2. this process results in psychological and/or biologic changes that may have consequences for health
3. individual interpretations are important in determining responses to stress [26].

The stress reaction is mediated through several neurocardiac stress effector systems, among which the autonomic nervous system, the adrenomedullar hormonal system, and the hypothalamic-pituitary adrenocortical axis system are the most important.

Major neurocardiac stress effector systems

Sympathetic nervous system:
Transmitter: *norepinephrine*.
Increases heart rate, contractility, conduction, vasoconstriction, automaticity, peripheral vascular resistance

Adrenomedullar hormonal system:
Transmitter: *epinephrine*.
Increases heart rate, contractility, conduction, automaticity, platelet aggregation

Parasympathetic nervous system:
Transmittter: *Acetylcholine*
Decreases heart rate, contractility, conduction

Hypothalamic-pituitary adrenocortical axis system:
Transmitter: *Cortisol*
Decreases cardiovascular response to catecholamines,
Influences sodium retention and circulatory integrity

Modified from Soufer et al., ref. 231

A distinction must be made between acute and chronic stress. Acute stress often has an abrupt onset, short duration, and is both unpredictable and uncontrollable (such as trauma, disasters or serious threat). Chronic stress is long standing and chronic stressors include persistent conditions that often are associated with an individual's occupation, social relationships, or socio-economic environment [52].

The acute stress reaction is primarily mediated through the autonomic nervous system and is characterized by cardiac and visceral responses to increased sympathetic stimulation and vagal withdrawal. This is the classic "fight-or-flight" response.

Chronic stress, on the other hand, results from chronic elevation of catecholamines, cortisol, and other mediators and produces a chronic "wear and tear" on the cardio-

vascular system that in the long run can increase the risks for cardiovascular events such as stroke and myocardial infarction.

The term "allostasis", introduced by Sterling and Eyer [52], refers to how the body responds to daily events and maintains homeostasis. McEwen et al. [52-54] introduced the term "allostatic load or over-load" to describe to the negative effects that result from either too much stress or from inefficient management of allostasis, e.g., not turning off the response when it is no longer needed or not turning on an adequate response in the first place.

The parasympathetic nervous system plays an important regulatory role in allostasis, since it generally opposes the sympathetic nervous system. Parasympathetic activity also seems to have anti-inflammatory effects [55].

In this context it is also important to distinguish between mental stress as induced by mental stress tests compared to emotion and overall panic. Emotion and panic are characterized by producing a sympathetic stimulation that is both more sudden and strong. In regard to arrhythmia it is not clear whether the most important factor for a particular substrate is the intensity of the stimulation, its suddenness or the type of stimulation [56].

Several studies have provided evidence of a catecholamine hyperreactivity in personality type A compared with type B individuals, although any difference regarding adrenocortical responsiveness is less clear [57,58].

Coping strategies seem to influence the hormonal response both to mental stress tests and real-life stressors. The use of an active, direct problem-focused coping strategy is related to less psychoendocrine reactivity, whereas a coping strategy based on denial and avoidance has been associated with an increased neuroendocrine responsiveness [59,60].

There also seems to be a gender difference in the response pattern to psychological stress. Frankenhauser [61,62] found a stronger epinephrine response to mental stress in men and Davidson [63] observed higher norepinephrine levels in men during mental stress tests. Also other hormones show a gender difference. Luteinizing hormone (LH) plasma levels were lower in male subjects before and after an academic examination while TSH levels were higher during examination [64]. In a similar study women showed higher levels of cortisol and T3 than men did [64].

Mental stress tests

A number of mental stress tests are well validated. Mental arithmetics is one of the most commonly used in stress tests in a laboratory setting. The psychoendocrine response to mental arhithmetics is mainly characterized by catecholamine release from sympathetic nerves and the adrenal medulla [64].

Public speaking is another frequently used test. Public speaking is a more complex source of stress, including both the anticipation of possible failure and a mental task. This test induces an endocrine response characterized by both elevated catecholamine levels as well as an increased level of cortisol [64].

Other studies have used presentation of films and videotapes having strong emotional implications. Also provocative interviews have been applied, although the endocrine response to the latter shows no consistent pattern [64].

Stroop's colour word conflict test (CWT)

There is vast experience with Stroop's Colour Word Conflict Test (CWT) as a stressor in the laboratory setting, especially in our institution. It is a test that with good reproducibility evokes a cardiovascular response resembling the classical defence reaction, with significant increases in the heart rate, blood pressure, and cardiac output, and vasodilatation in peripheral muscle tissue [36-38].

The hemodynamic effects of CWT are well documented. In a group of healthy subjects, this test raised heart rate from 60 to 88 beats per minute and cardiac output from 6.3 to 10.9 L per minute [36]. In patients with

coronary heart disease, the heart rate and the systolic and diastolic blood pressure increased significantly during mental stress tasks. In this category of patients, mental stress also produced a significant decrease in the left ventricular ejection fraction [65].

Elevated catecholamine levels are a constant finding in response to CWT, while the hypothalamic-pituitary adrenocortical axis system axis does not appear particularly sensitive to this test [64].

THE BRAIN-HEART CONNECTION

Introduction

The autonomic nervous system regulates cardiac functions - heart rate, contractility and conduction velocity - through the sympathetic and the parasympathetic nervous systems. While vagal effects are solely neurogenically mediated, sympathetic influences may be mediated via neurogenic and humeral pathways. The effects differ depending on whether they are neurogenically or humorally mediated. The neurogenic sympathetic and the vagal effects are sudden and the duration is shorter compared with the humorally mediated sympathetic effect [56].

The sympathetic system has a stimulatory effect, increasing heart rate, contractility and conduction velocity, and shortening refractoriness. The parasympathetic nervous system generally inhibits and opposes the effects of the sympathetic system. The autonomic nervous system acts via adrenergic receptors and muscarinic acetylcholine receptors.

Anatomy

Kawashima [66] recently published results from submacroscopic anatomic investigations of the entire human autonomic cardiac innervation, modifying earlier results. In this series of 18 cadavers, he found that the cardiac sympathetic ganglia include a superior cervical ganglion (communicates with C1–3) and a cervicothoracic (stellate) ganglion (communicates with C7–8 –T1–2). The superior, middle, and inferior cardiac nerves from these ganglia innervate the heart by simply following the great arteries. The thoracic cardiac nerves in the posterior mediastinum follow a more complex course to the heart because of the long distance to the middle mediastinum. Parasympathetic innervation comes from the vagus and is divided into superior, thoracic, and inferior branches.

The sympathetic and parasympathetic nervous systems innervating the heart could not be distinguished at the macroscopic level because of the cardiac plexa at the inlet/outlet level of the great vessels of the heart. Areas particularly rich in autonomic innervations are the pulmonary vein-left atrium junctions (so called pulmonary vein autonomic plexa).

The functional pathways of the autonomic innervation to the canine ventricles have been worked out by Zipes et al. [67]. In both ventricles sympathetic axons are superficially located in the epicardium with branches penetrating through the myocardium. Vagal axons penetrate the myocardium just beneath the AV groove and transverses close

to the endocardium with branches penetrating the mycocardium in the opposite direction in regard to the sympathetic fibres.

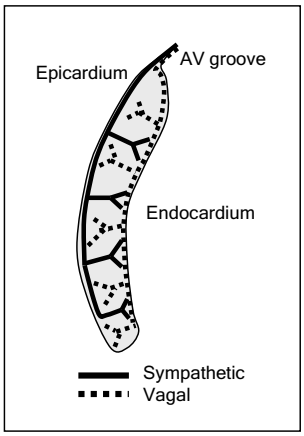


Figure 1. Pathways of vagal and sympathetic innervation to the left ventricle. Modified from ref. 89.

Receptors

In the early 1900s Langley, Dale, Erlich, and others showed that specific receptors mediate the physiologic effects of adrenal extracts and sympathetic nerve endings [68]. In 1948, Ahlquist found that two different types of adrenoceptors existed, which he named α and β [69]. Twenty years later Lands et al. [70] recognized that each of these adrenoceptors could be categorized into two distinct subtypes: α1 and α2, and β1 and β2. Later studies have demonstrated further subtypes. Today three classified subtypes each of α1, α2 and β adrenoceptors have been identified [71].

β1- and β2- adrenoceptors

The sympathetic innervation shows a high density in the left ventricle, with a small gradient from the base to the apex, with the highest density at the base. This density also seems to be greater in the endocardial wall than in the epicardial wall. β-adrenergic receptors have a distribution pattern that is inversely related to the innervation density; they have a density gradient extending from the apex to the base and from the subepicardial layer to the endocardial layer [67].

β1- and β2-adrenoceptors have been well described in the heart in radio ligand studies and later in functional studies [71]. The number of β-adrenoceptors is evenly distributed in the heart's muscular tissues although the proportions seem to differ. The β1:β2 proportion in atria is 60-70:40-30 and in the ventricles 70-80:30-20 [72-75]. It has also been shown that the β-adrenoceptor density in the nodal tissues is up to 3-fold higher than in the surrounding atrial tissues. The proportion of β2-adrenoceptors also seems do be higher in both the sinus and the AV nodes [71,76,77].

There is a decline in β-adrenoceptor density with age, a decrease that seems to affect β1-adrenoceptors in the ventricles, while β2-adrenoceptor density is unaltered and the densities of both receptor subtypes in the atria are unaltered [71,78]. In the failing heart, there is a selective decrease in β1-adrenoceptors leading to a shift in the β1:β2 proportions. This decrease is less pronounced in atrial tissues compared with ventricular. In patients suffering from pulmonary hypertension with right ventricular failure the down-regulation is only in the right ventricle [71].

Chronic treatment with β1 receptor inhibitting agents results in a selective increase of cardiac β1-adrenoceptors [79]. Furthermore, treatment with selective β1 blockers seems to sensitize cardiac β2-adrenoceptors since the effects of β2 agonists are markedly enhanced [80,81].

β3-adrenoceptors

Ten years ago it was shown that β3-adrenoceptors are present and functional in the human heart and these receptors are responsible for an unexpected negative inotropic effect of catecholamines. This effect may be involved in the patophysiologic mechanism leading to heart failure [82,83].

The effect of β3 stimulation on the cardiac electrophysiology is not yet fully characterrized. Wheeldon et al. [84] showed that after β1 and β2 adrenoceptor inhibition with nadolol, an investigational β3 adrenoceptor agonist produced a small but significant chronotropic effect in healthy volunteers. Furthermore, an experimental β3 agonist infused in dogs significantly reduced the occurrence of ventricular tachycardia [85].

α-adrenoceptors

Three different α1-adrenoceptors have been identified - α1, α2 and α3. α1-adrenoceptors, possibly several subtypes, exist in the heart. Stimulation of α-receptors has important short-term effects on heart rate and contractility [86]. α-adrenergic agonists have been shown to slow sinus rhythm in animal models [87].

Abundant data demonstrate that α1-adrenoceptors mediate a positive inotropic effect in the human heart [79]. Long-term α-receptor stimulation seems to lead to a spectrum of morphologic and molecular changes that are considered of importance for cardiac hypertrophy [86]. Whether the distribution is homogenous or heterogeneous in different cardiac tissues is not clear [79]. Presynaptic α2-receptors may mediate an inhibiting effect on noradrenaline release in the right atrium [79].

Muscarinic acetylcholine receptors

Five muscarinic receptors have been identified, M1-M5. Cardiac muscarinic receptors are predominantly of the M2-subtype. In contrast to β-adrenoceptors regional differences exist for the distribution of M2-receptors in the heart. The density of M2-receptors is up to 2.5-fold higher in the atria compared with the ventricles. The density, distribution and functional responsiveness do not seem to be altered in chronic heart failure [79]. However, M2-muscarinic receptor density declines with age, which may contribute to the decrease in baroreflex activity with age [88].

Sympathetic-parasympathetic interactions

Vagal effects on cardiac tissues

Both the sinus and AV nodes are richly innervated by vagal nerves, and parasympathetic activation decreases conduction velocity and automaticity and increases AV nodal refractoriness. Respiration causes changes in vagal activity with cyclic modulations of the AV nodal refractoriness [89].

In contrast, vagal activation shortens the atrial refractoriness, an effect that is not uniform in the atria. The probable explanation for this dispersion in refractoriness is that atrial tissue adjacent to the vagal nerve endings exhibits more pronounced parasympathetic effect because of a higher exposition to acetylcholine, e.g., atrial tissue in the vicinity of the sinus node [90].

In the ventricles vagal stimulation prolongs refractoriness. To what extent this effect is direct or caused by opposing the sympathetic activity is not fully understood although some data suggest that vagal activity modulates ventricular refractoriness independent of simultaneous sympathetic activity [89, 91]. An increase in ventricular refractoriness in response to vagal activation has been found in human but not in isolated ventricular tissue. This discrepancy may be explained by the difficulty of completely inhibiting β-adrenergic activity in vivo [92].

Atropine shortens the refractoriness of accessory pathways, suggesting that these pathways have properties of ventricular and not atrial muscle, since atropine lengthens atrial refractoriness [93].

The vagal effects on AV nodal and ventricular refractoriness do have clinical implications, since manoeuvres that increase vagal tone often terminate AVNRT, AVRT, and certain types of ventricular tachycardias.

The antiarrhythmic potentials of muscarinic agonist have been tested in dogs. In one study, a preventive effect was found in 63% of the dogs receiving an agonist [94,95]. Transdermal scopolamine like low doses of atropine results in paradoxical vagomimetic effects [96].

Transdermal scopolamine was tested in 20 patients with myocardial infarction.

Scopolamine significantly increased vagal tone and baroreceptor reflex sensitivity in this study [97].

Age

The cardiovascular autonomic regulation changes with age. At rest, elderly individuals show an increased sympathetic activity parallel with a decreased parasympathetic activity. Postural changes in young subjects result in increased heart rate and myocardial contractility to maintain cardiac output and blood pressure. Elderly individuals maintain blood pressure in the postural position mainly through increased peripheral resistance and show a less increase in heart rate. The influence of gender on these age-related changes in autonomic response seems to be minor [98].

Marneffe et al. [99] investigated the variations of normal sinus node function in relation to age. They found that the intrinsic sinus node activity - i.e., after autonomic tone inhibition - was correlated with age and showed a progressive lengthening of mean SCL and SNRT with age.

They concluded that parasympathetic activity seems to dominate in young subjects, whereas sympathetic and parasympathetic tones are equilibrated in older subjects.

Chow et al. [100] studied the autonomic innervation in human hearts ranging from newborn to adolescence. They found a sympathetic dominance in infancy and a sympathetic and parasympathetic balance in adulthood. They also found a reduction in density of innervation of the conducting tissues with age.

Time dependency of autonomic activation

The time dependency of the autonomic interactions regulating heart rate has been well summarized by Matthew Levy [101]. When the sympathetic and parasympathetic parts of the autonomic nervous system are not activated synchronously, the steady-state response to a combined stimulation may vary depending on the relapsed time between the start of respectively excitation. In 1934, Rosenblueth and Simeone [102] were first to report the tendency for vagal activity to attenuate the effects of sympathetic activation, a phenomenon called accentuated antagonism.

Sympatheticovagal interactions are particularly pronounced in the neural control of the heart rate. However, in these experiments the stimulation of the sympathetic and vagal nerves was simultaneous [103].

There is a prejunctional inhibition of norepinephrine by vagally released acetylcholine as well as a prejunctional inhibition of acetylcholine by norepinephrine released from the sympathetic nerve endings. The muscarinic receptors located in the heart also inhibit the release of norepinephrine and probably neuropeptide Y (NPY), and norepinephrine and NPY inhibit the release of acetylcholine. The inhibitory effect of norepinephrine on vagal neurotransmission decreases quickly after the sympathetic activity ceases, while the inhibitory effect of the simultaneously released NPY lasts longer [101].

Habermeier-Muth [104] studied the temporal relationship between vagal and sympathetic stimulation in more detail. They found that when sympathetic nerves were stimulated at short or long durations after vagal stimulation had began, no norepinephrine was released. On the contrary, at very short and intermediate durations of sympathetic stimulations in the presence of vagal stimulation, norepinephrine release was not inhibited.

In summary, when parasympathetic and sympathetic activity begin at the same time, the parasympathetic activity dominates; when parasympathetic activity precedes the sympathetic activity, the released acetylcholine effectively inhibits the release of norepinephrine, although this effect is of rather short duration and the inhibition also depends on the duration of sympathetic stimulation; when sympathetic stimulation begins before parasympathetic stimulation, NPY is released in addition to norepinephrine and NPY has a more enduring inhibitory effect on acetylcholine release.

The effects of the muscarinic antagonist atropine are also dose dependant. At a very

low dose (<3μg/kg), atropine causes bradycardia and salivation, while a higher dose (>0.3 μg/kg) results in the well-known tachycardia and dry mouth. Is has been suggested that the paradoxical effect of low doses of atropine is explained by an inhibitory effect on M1 receptors on post-ganglionic vagal nerve terminals in the heart and salivary glands [105].

Effects of exercise training

Evidently regular exercise protects against cardiac events in both healthy individuals and in patients with cardiovascular disease [106]. The mechanism by which physical exercise exerts this effect is unclear but evidence points towards an enhancement in vagal activity.

A meta-analysis including more than 300 subjects showed that exercise training decreases heart rate and increases the high frequency component of the power spectrum of RR interval (hf), suggesting an increased vagal tone [107]. There is no gender difference demonstrated in this effect on heart rate, in contrast to the effect on QT interval, where trained women show a shorter QT interval compared with sedentary women. Such difference is not observed in men [108].

Secondary prevention – selective versus unselective β-blockade

The importance of β2 receptor inhibition in patients with chronic heart failure and coronary heart disease is not clear. In a meta-analysis of secondary and primary prevention trials after myocardial infarction, β1-selectivity showed a greater risk reduction from sudden death than non-selective β-blockers [109], whereas in a meta-analysis of patients with chronic heart failure the conclusion was that selective agents were inferior to non-selective agents in reducing overall mortality [110]. Several of these studies evaluated the effect of carvedilol, which beside β1- and a β2-inhibiting effect also has a α1-inhibiting effect, which may have influenced the outcome. It is not clear to what degree other characteristics of β–blockers - such as lipophilia, intrinsic activity and membrane stabilizing properties - play a role.

Potentially arrhythmic effects of β2 agonists

Early studies on β2 stimulation with oral and intravenous salbutamol in patients with chronic or acute heart failure showed significant hemodynamic changes with an increase in cardiac output, ejection fraction and heart rate [111-113]. These cardiovascular effects were explained as resulting from a reduced afterload caused by vasodilatation.

However, later studies have demonstrated a direct positive chronotropic effect. Infusion of β2 agonists in subjects pre-treated with a β1 antagonist and atropine still causes an increase in heart rate and blood pressure [114,115], which is best explained by a direct cardiac β2 mediated effect.

An increased risk of death from asthma in association with the regular use of inhaled β2-agonists was reported 1992 [116]. From these data, it was not possible to determine to what extent any of these deaths were due to lethal arrhythmias. However, 14% of the reported deaths might have been sudden.

Ventricular arrhythmias have been reported to occur during treatment with fenoterol aerosols in patients with chronic airway obstruction [117, 118]. In a recent systematic review of case-control studies and randomized controlled trials, a 2-fold increased risk of adverse cardiovascular events was found in patients treated with β2-agonists. Events included myocardial infarction, congestive heart failure, cardiac arrest, and sudden cardiac death [119].

THE GENDER PERSPECTIVE

Differences in electrocardiographic parameters

Gender differences in electrocardiographic parameters were described early [120]. Women have higher resting heart rate than men, which also persists after autonomic inhibition with propranolol and atropine [121-123].

The reason for this difference has been subject of some debate. Burke et al. [124] studied healthy volunteers during exercise with and without autonomic blockade. They found as previously shown that sinus cycle length is longer in men than women. However, this difference appears to be associated with a gender difference in exercise capacity rather than gender-related properties of the sinus node or differences in autonomic tone.

As early as 1920, Bazett [120] reported that women have longer corrected QT interval (QTc) than men. Rautaharju et al. [125] studied the changes in the QT interval with age and reported that the corrected QT interval in men shortens at puberty and remains so until around age 50. They hypothesized that this effect may be caused by androgen, since this period coincides with the highest androgen levels.

Nakagawa et al. [126] studied the effect of sympathetic stimulation with isoproterenol on the morphology of the T wave and on QTc. The QTc interval was initially prolonged and shortened in both men and women during isoproterenol administration but the QTc prolongation was significantly greater in women. The intrinsic QTc interval after autonomic blockade was also longer in women than in men.

Differences in electrophysiologic parameters

Gender differences in electrophysiologic parameters have also been described, although the results are somewhat conflicting. Taneja et al. [127] studied 396 consecutive patients without structural heart disease who underwent electrophysiologic testing due to supraventricular or ventricular tachycardia, palpitations or syncope/presyncope. They found that men had modest although significant longer sinus cycle length, QRS duration, HV interval and sinus node recovery time.

Liuba et al. [128] studied 203 consecutive patients with typical AV nodal reentrant tachycardia. They found that men had longer sinus cycle length, HV interval, refractoriness in the AV node (the slow pathway), AV block cycle length, and longer tachycardia cycle length; i.e., women had faster tachycardia. Liu et al. [129] studied 895 consecutive patients with AV nodal reentrant tachycardia and atrioventricular reentrant tachycardia. Men had significant longer PR interval when age-matched patients were analyzed. The prevalence of AV nodal dual pathway was not different between men and women.

The differences in dual AV nodal properties between men and women were analyzed in 80 consecutive patients undergoing electrophysiologic study for evaluation of syncope or supraventricular or ventricular tachycardia. Patients with AV nodal reentrant tachycardia were excluded. There was no difference in the presence of dual AV nodal physiology, but women had significantly shorter refractoriness of the slow pathway and in a wider window of

refractoriness, i.e., difference between the effective refractory period of the fast and the slow pathway [130].

Atrial conduction was analyzed with non-contact mapping technique in 16 men and 16 women with atrial arrhythmias. Conduction velocities were analyzed in two different regions in the right atrium. Men had significantly greater conduction dispersion (difference in conduction velocities) compared with women [131].

Hormonal effects

Sinus cycle length is significantly longer in women during the menstrual phase, a finding that seems to be mediated through a change in autonomic tone since autonomic blockade diminished this difference [124]. The conduction properties and refractoriness of cardiac tissues are largely influenced by adrenergic tone [132]. Hypo-oestrogenic states and elevated levels of progesterone increase the levels of catecholamines, which may influence arrhythmias in women [133]. There is also a cyclical variation in the frequency of tachycardia episodes in women with paroxysmal supraventricular tachycardia are more common during the last phase of the menstrual cycle and the frequency and the duration of tachycardia episodes are related to the progesterone level and inversely related to the oestradiol level [134].

Gender differences in the prevalence of arrhythmias

S*inus nodal tachycardia*

Inappropriate sinus tachycardia is a relatively rare arrhythmia characterized by an elevated resting heart rate and an exaggerated response to exercise or mental stress. The proposed mechanism is enhanced automaticity of the sinus node or abnormal autonomic regulation with reduced vagal or increased sympathetic tone. The cause is possibly multifactorial. Patients are usually young and in nine out of ten cases they are women [135,136].

Sinus node reentrant tachycardia is presumed to be caused by microreentry in tissues near the sinus node or the perinodal region along the crista terminalis and should probably be considered a variant of focal atrial tachycardia, see below [137].

Focal atrial tachycardia

Focal atrial tachycardia is either caused by enhanced focal activity or triggered activity. Focal atrial tachycardias are in 75% confined to the right atrium and are in particular found along the crista terminalis, the tricuspid valve or atrial septum. In most reports this arrhythmia is slightly more common among women [138-142].

Cavo-tricuspid isthmus dependent atrial flutter (AFL)

A clear male preponderance has been observed in reports studying the incidence of AFL in the general population [143], where AFL is 2-4 times more common in men, as well as in reports of isthmus ablation procedures, where men constitute up to 80% of the patients [144-146]. The reason for this skewed incidence is unknown. A possibility could be that men have larger hearts, a condition that may facilitate development of large reentry circuits.

Other atrial macro re-entry tachycardia

The majority of these tachycardias are related to scars, usually caused by incisions during surgery [147]. More recently re-entry tachycardias following pulmonary vein ablations have increased in incidence. These tachycardias are more common in men since ablation of atrial fibrillation is more common among men.

Atrial fibrillation (AF)

From the Framingham Heart Study [148], we know that men have 1.5 higher risk of developing atrial fibrillation. The preva-

Gender distribution of some frequent tachycardias
Patients undergoing ablation treatment
Compiled from ref. 78-94

Arrhythmia	No of patients	Gender proportion	
		Female	Male
AV nodal reentrant tachycardia	3.121	66%	34%
Atrioventricular reentrant tachycardia (WPW)	2.478	37%	63%
Isthmus dependant atrial flutter	1.054	20%	80%
Focal atrial tachycardia	717	62%	38%
Atrial fibrillation	1.184	33%	67%

lence of atrial fibrillation is higher in men compared with women in all ages. However, since there are twice as many women older than 75 years in the population, the total number of women with atrial fibrillation is higher than men in the higher ages. Among patients undergoing pulmonary vein ablation men usually constitute 70-80% [149-153].

AV nodal reentrant tachycardia (AVNRT)

Particularly in its most common form, the so called slow-fast variant, this is the most prevalent of all supraventricular tachycardias and is more common among women. AVNRT is probably an acquired arrhythmia [154]. In ablation studies women constitute up to 75% percent of referred patients [128,144,155,156].

Atrioventricular reentrant tachycardia (AVRT)

Accessory pathways may both be congenital and develop later in life. Preexcitation and AVRT during infancy may disappear during early life but may also recur later in life [154,158,159]. There does not seem do be any gender difference in the occurrence of preexcitation during childhood, although it is more common among men in the adult population [160]. Significantly more men are treated with ablation of accessory pathways than women; in most series, men constitute 60-65% of ablated patients [139,140].

Ventricular arrhythmias (VT, VF)

Gender prevalence in ventricular arrhythmias depends on mechanism. Right ventricular outflow tract tachycardias [161] are more common in women, while ischemic and certain idiopathic ventricular tachycardias are more common in men [122, 162]**.** Idiopathic ventricular fibrillation is 2.5-fold more common in men [163]. In patients with coronary artery disease who received ICD sustained VT/VF occurred in 52% of men and 34% of women. Men also experienced more VT/VF events and more electrical storms [164].

Sudden death

In the Framingham heart Study [165], more than 5,000 thousand individuals were followed up for 38 years: 325 sudden deaths occurred and of these 71% were male. However, with increasing age this gender difference decreased. Eighty percent of these deaths were attributed to coronary artery disease in men, but this was the cause in only 45% of the women. In women dilated cardiomyopathy and VOC were proportionally more common as was absence of any structural heart disease [166].

Long QT syndrome (LQTS)

In congenital LQTS there is a female prevalence [167]. However, men are at higher risk for cardiac events (syncope, cardiac arrest, or sudden cardiac death) before puberty, and women area at higher risk during adulthood [168]. This difference is probably related to the shortening of QT duration in men after puberty [169]. In acquired LQTS, most often seen with electrolyte abnormalities or drugs that prolong ventricular repolarization, women constitute 70% of reported cases of torsade de pointe [169, 170].

Gender differences in ablation results

The success rate of ablation in regard to gender has seldom been analyzed in published studies. Dagres et al. [156] analyzed 894 patients undergoing ablation of AVNRT or AVRT and found that women were more symptomatic, had tested more antiarrhythmic drugs and were referred for ablation later than men but the difference in ablation outcome was not significantly different.

Recently, a large Swedish study showed significant differences in ablation outcome between men and women [144]. This study presented 2,178 patients ablated for AVNRT, AVRT or AFL. Success rate was higher for women in ablation of AVNRT, and the success rate was lower in women in ablation of AFL. No difference was found regarding AVRT. The reason for these differences in ablation outcome is not clear. Electrophysiologic as well as anatomic differences may be important.

Forleo et al. [151] analyzed the impact that gender may have in referral patterns, complications and long-time outcome for catheter ablation of atrial fibrillation. Women referred for ablation were significantly older with a longer history of AF, they hade more comorbidities and larger left atria. There were no differences regarding complications or clinical outcome. Long-term overall success rate was over 80% regardless of gender.

ELECTROPHYSIOLOGIC TECHNIQUE

History

The electrophysiologic study, using electrodes in the heart to record intracardiac signals, programmed electrical stimulation and ablation of arrhythmia substrates with specially designed catheters, is based on the cardiac catheterization technique. This technique began with a historic self-experiment by Werner Forssmann. In 1929, Forssmann performed the first right heart catheterization on himself. Forssmann later received the Nobel Prize in medicine in 1956 for his pioneering work.

The His-bundle potential was first demonstrated by Puech in 1957 [171] during a catheterization of a patient with tetralogy of Fallots and later by Watson in 1967 [172]. Programmed electrical stimulation was introduced in 1967 by Durrer and Roos [173] and further developed by Wellens [174].

The first ablation of the His bundle was accidentally performed 1979 [175] when an

electrode catheter at the His bundle happened to come in contact with a defibrillating lead during defibrillation in a patient with atrial fibrillation. The first catheter ablations of accessory pathways with direct current technique were reported in 1985 [176,177], and with radiofrequency technique a few years later [178,179].

Catheterization technique

The standard diagnostic electrophysiologic study is usually performed as a right heart catheterization with electrode catheters inserted percutaneously into the right femoral vein and advanced under fluoroscopic guidance to the right atrium and right ventricle. Catheters are routinely positioned in the apex of the right ventricle, in the superior lateral region of the right atrium, and into the coronary sinus and across the tricuspid valve to record His bundle electrograms.

To assess cardiac electrophysiologic function, a number of standard parameters are evaluated: sinus node function by means of measuring the sinus node recovery time, SNRT, also corrected for sinus cycle length, CSNRT, conduction parameters in the right atrium, through the AV node to the His bundle, and distal to the His bundle, and refractoriness in the right atrium, the AV node and the right ventricle.

Measuring electrophysiologic parameters

Sinus node recovery time (SNRT) is a measure of sinus node automaticity. SNRT is determined by high right atrial pacing for a specified time, usually 10 seconds, 30 seconds, or 1 or 2 minutes, and measuring the interval from the last paced atrial beat to the first spontaneous sinus beat.

SNRT is a well-evaluated test to unmask sinus nodal abnormalities. It has also been used to evaluate effects of drugs and autonomic modulation on sinus node function [180-182].

Assessment of refractoriness is usually performed by scanning diastole with a single extra stimulus using 10 ms decrements after a train of beats with fixed cycle length. The refractoriness of the AV node is sometimes not possible to determine, since the atrial refractory period is encountered earlier than the AV nodal refractory period. This is particularly common at a low basic drive, which tends to shorten the AV nodal refractoriness while simultaneously lengthen the atrial refractoriness or during increased sympathetic tone [183]. In studies where repeated measurements are performed during various autonomic interventions, it is apparent that great caution should be taken to create a relaxed and quite laboratory environment without sudden disturbances causing changes in the autonomic tone that may influence the results.

Dual AV nodal physiology, presented as a discontinuity in the antegrade conduction curve, is a common finding and has been reported in 15-40% of patients undergoing electrophysiologic studies (excluding patients with AVNRT) [130,184-187]. The anatomic and electrophysiologic explanation for this finding is the presence of regions in the AV node, often simplified as pathways, with different refractoriness and conduction times. The most commonly used model describes an anterior fast pathway with short conduction time and long refractory period and a posterior pathway with long conduction time and short refractory period. An extrasystole with a short coupling interval will not conduct through the fast pathway, which is refractory, but instead conduct through the slow pathway which has a shorter refractoriness. The presence of dual AV nodal physiology partly depends on the autonomic tone.

Dual AV nodal physiology can make the evaluation of a presumed effect on the AV nodal conduction and refractoriness during an autonomic intervention difficult or impossible to interpret.

Reproducibility of electrophysiologic measurements

The reproducibility of electrophysiologic measurements at two different study occasions has been evaluated in several

studies. De Marneffe et al. [188] found no significant difference in the mean value of CSNRT when measured in patients without evidence of sinus node disease at two different occasions at least two days apart.

Bergfeldt et al. [189] performed electrophysiologic studies in healthy volunteers on two different occasions with a mean interval of 25 days between. They found a high reproducibility with coefficients of variation below 10% for the mean sinus cycle length, ventricular depolarization and repolarization, and atrial, AV nodal and infranodal conduction times, while it was slightly higher for AV nodal refractoriness. The mean sinus node recovery times varied considerably.

Lahay et al. [190] studied patients with permanent pacemakers and measured sinus nodal and AV nodal parameters and then repeated the study after two weeks. They found that the measurements of sinus and AV nodal functions were reproducible over two weeks.

AIMS OF THE THESIS

I. To investigate the electrophysiologic effects on different levels of the cardiac conduction system elicited by Stroop's Colour Word Conflict test (CWT) and to compare them with the effects of epinephrine infused to levels similar to the physiological elevations produced by mental stress and physical exercise.

II. To assess the overall electrophysiologic effects of β2-agonist infusion by administering salbutamol at different infusion rates, resulting in increases in heart rate similar to those attained during β2-agonist inhalation therapy. In order to distinguish β2-agonist effects comparisons were also made with the β1 selective agonist dobutamine.

III. To investigate gender differences in the electrophysiologic effects on different levels of the cardiac conduction system elicited by CWT or produced by autonomic tone inhibition.

IV. To evaluate whether the type of β-blocker, β1-selective or unselective, is of importance to inhibit the electrophysiologic effects of CWT.

V. To evaluate whether vagal withdrawal is of importance in the electrophysiologic response to mental stress.

MATERIALS AND METHODS

Introduction

In all studies, healthy volunteers were investigated by means of standard electrophysiologic catheterization procedures. Electrophysiologic and hemodynamic variables were measured under different settings of autonomic modulation with the primary goal to characterize the effects of mental stress on cardiac conductive tissues and the importance of β1 and β2 adrenergic receptors in cardiac tissues. To this mean infusion of adrenergic agonists (using epinephrine, dobutamine and salbutamol), a mental stress test (Stroop's Colour Word Conflict Test) and selective and unselective autonomic tone inhibition (using atenolol, propranolol and atropine) were used.

The studies were performed with the subjects fasting over night and without sedation. The subjects were not allowed to smoke or drink beverages containing caffeine on the day of the investigation.

The subjects were primarily recruited among health care personnel and students.

Stroop's Colour Word Conflict test (CWT)

This test was used in study I, II, and IV. It is a modified version of the original Stroop´s Colour Word Conflict test [191]. For 10-20 minutes (depending on the protocol), the subject was shown rapidly changing pictures (20 pictures/min) on a video screen. Each picture displays the name of one of four colours written in letters of a non-matching colour and simultaneously the name of another non-matching colour is read aloud. The subject is requested to mark on a paper the colour matching the meaning of the word on the screen.

CWT is a well-documented mental stress test that with good reproducibility evokes a cardiovascular response resembling the classical defence reaction: significant increases in the heart rate, blood pressure and cardiac output, and vasodilatation in peripheral muscle tissue [36-38].

CWT produces significant circulatory effects already after 2-3 minutes paralleled by elevations of epinephrine and norepinephrine plasma levels, which remain elevated during 20 minutes of CWT [36,37].

Selective and unselective adrenergic agonists

Epinephrine is a β1- and β2-adrenoceptor agonist as well as an α-adrenoceptor agonist. The two different rates of epinephrine infusion used in study I have previously [192] been shown to result in plasma levels corresponding to physiological elevations observed during mental stress [36,37] and physical exercise [193], respectively.

Dobutamine has β1-, β2-, and α-receptor agonist effects, although the β1 effect dominates at the low dosages used in study III. During infusion the effect has its onset within two minutes and the maximum effects are reached after 10 minutes [194].

Salbutamol has both β1- and β2-receptor agonist effects, but the β2 effect dominates

at the low dosages used in study III. An infusion of salbutamol at the dosages used has been reported to result in a 20% to 40% increase in heart rate reaching a steady plateau phase within 20 minutes [195].

Selective and unselective autonomic tone inhibition

Propranolol combined with atropine has routinely been used for autonomic tone inhibition in a large number of studies. In study 2 and 4, propranolol was administered intravenously with 0.15 mg/kg, which was considered adequate for β-receptor inhibition in these settings [196, 197], and atropine 0.02 mg/kg was given. The same dosage used for propranolol was also used for atenolol, aiming for equipotent dosages in regard to the effect on heart rate [198].

Catheterization and recording techniques

For electrophysiologic assessments, three electrode catheters were inserted percutaneously into the right femoral vein and advanced under fluoroscopic guidance to the lateral wall of the right atrium, to the apex of the right ventricle and across the tricuspid valve to record intracardiac electrograms and programmed electrical stimulation. ECG and intracardiac electrograms were recorded on a Mingograf (Siemens-Elema, Sweden) inkjet recorder (study I and III) and on a Recor Electrophysiology System (Siemens-Elema, Sweden) (study II and IV), at a paper speed of 100 mm/s.

A short teflon catheter was inserted into the left brachial artery to record arterial pressure and for arterial blood sampling. After introduction of hemodynamic and electrode catheters, the subjects rested for 30 minutes.

In study I and III, arterial pressure and systolic time intervals were measured on a separate ECG recorder with simultaneous recording of ECG, phonocardiogram, and arterial pulse at a paper speed of 100 mm/sec in accordance with established methodology [199].

In study II and IV, arterial pressures, surface ECG leads and intracardiac electro-grams were simultaneously displayed and recorded on the Recor Electrophysiology System.

In study I, calf blood flow was measured by venous occlusion plethysmography with a distal cuff occluding the circulation in the foot. Calf vascular resistance was determined by dividing mean arterial pressure by calf blood flow.

In study III, stroke volume was measured by the Doppler ultrasound technique (Vital Science Quantascope) [200].

Measured variables

- AV nodal (**AH**) and His-Purkinje and infranodal conduction times (**HV**) were determined during atrial pacing with a driving cycle length of 600 ms and are presented as a mean of three measurements.
- Atrial and AV nodal refractory measurements (**AERP** and **AVNERP**) were determined with extra stimuli scanning following atrial pacing with a driving cycle length of 600 ms.
- Ventricular refractoriness (**VERP**) was determined with extra stimuli scanning following ventricular pacing with a driving cycle length of 600 ms.
- The sinus node recovery time (**SNRT** (also corrected for sinus cycle length (**CSNRT**)) was determined after 30 seconds of atrial pacing with a driving cycle length of 600 ms; they are presented as the mean of four measurements.
- QT duration was calculated as the intersection of the down-slope's steepest point of the T wave and the isoelectric line and presented as the mean of three consecutive measurements at a driving cycle length of 600 ms.
- Arterial pressures, calf blood flow and systolic time intervals (electromechanical systole, left ventricular ejection time and pre-ejection period) were all determined during atrial pacing at the same cycle

length (600 ms), to avoid the need to apply rate adjustments to the non-invasive hemodynamic measurements.

- Measurements and calculations were performed with the investigator blinded as to the protocol step of the recording. However, CWT caused muscular interference, obvious on the ECG strips, which made blinded reading impossible for these particular recordings.
- All measurements were always performed in the same order during each protocol.

Studied subjects

Paper I

Ten healthy male volunteers (mean age 31 years, range 22-45) participated in the study.

Paper II and IV

For study IV, sixteen healthy volunteers, eight males and eight females (mean age 34 years, range 25-49), participated. The subjects were investigated on two different occasions with selective and unselective autonomic tone inhibition in random order.

For study II, seven further healthy volunteers were included for evaluation with unselective autonomic inhibition only. These seven subjects together with the sixteen subjects above comprise the subjects for study number II, which therefore included in all 23 healthy volunteers, 11 males (mean age 32 years, range 25-49) and 12 females (mean age 33 years, range 24-45).

Paper III

Ten healthy volunteers participated in the investigation, five males and five females (mean age 29 years, range 21-41).

Methods

Paper I, study protocol

Electrophysiologic and hemodynamic variables were measured at baseline, during a period of mental stress, again after one hour's rest as a second baseline reading, and during i.v. infusion of epinephrine at a low and a high rate.

To evoke mental stress, CWT was used. Epinephrine was infused at a rate of 0.025 μmol/kg/min and 0.3 μmol/kg/min.

The following variables were measured during each sequence as previously described: SCL, SNRT and CSNRT, AERP, VERP, AVNERP, Stim-A duration, AH duration, HV duration, QS duration, QT duration, arterial pressures, calf blood flow, and systolic time intervals.

Arterial blood samples for measurement of catecholamines and potassium were drawn at the end of each baseline period and 3 and 12 minutes after the start of each intervention. Catecholamine concentrations were assayed by high-performance cation exchange liquid chromatography with electrochemical detection [201].

PROTOCOL STUDY I

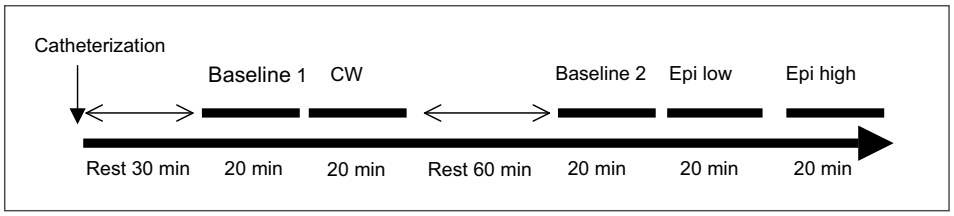


Figure 2. CWT = Stress test; Epi low = low infusion rate of epinephrine; Epi high = high infusion rate of epinephrine.

In this study, the described sequences of measurements were started after three minutes of CWT. Measurements during the baseline period and during epinephrine infusion were performed with a time schedule identical to that during CWT to keep the electrophysiologic and hemodynamic effects of pacing per se as similar as possible. To determine whether steady catecholamine levels were reached, blood samples were drawn after three and after 12 minutes of CWT and epinephrine infusion.

The degree of stress, irritation, tiredness, and strength of heart palpitations experienced by the participants was evaluated on visual analogue scales, as reported earlier [38], during each of the five protocol sequences.

Paper II, study protocol

In all subjects electrophysiological and hemodynamic variables were measured at baseline and during a period of mental stress. In fifteen subjects (7 men, 8 women), a second baseline recording was made again after one hour of rest and the measurements repeated after the administration of propranolol and atropine.

CWT was used for mental stress during periods of 10 minutes.

For autonomic tone inhibition propranolol was administered with 0.15 mg/kg, and atropine was administered with 0.02 mg/kg.

The following variables were measured during each sequence as previously described: SCL, SNRT and CSNRT, AERP, VERP, AVNERP, St-A, AH duration, HV-duration, QS duration, JT/QT duration, QT dispersion and arterial pressures.

All procedures were always performed in the same order. During the baseline period and during autonomic inhibition, measurements were performed with a time schedule identical to that during CWT to keep the electrophysiologic and hemodynamic effects of pacing per se as similar as possible. Arterial blood samples were drawn five minutes after the measurements started for determination of catecholamine and potassium concentrations.

PROTOCOL STUDY II

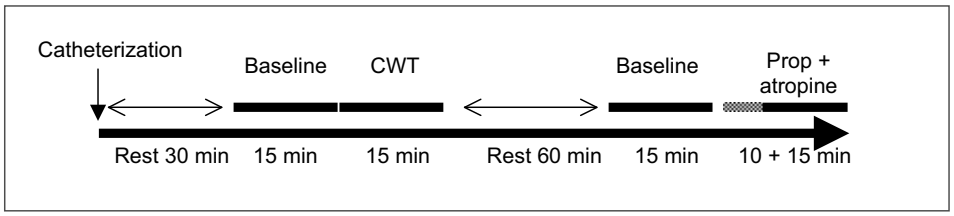


Figure 3. CWT = stress test; Prop = propranolol

PROTOCOL STUDY III

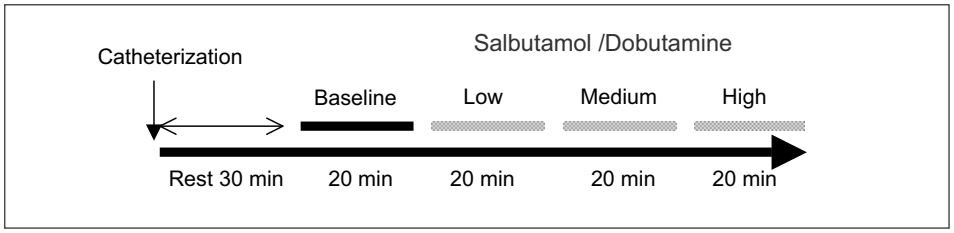


Figure 4. Low = low infusion rate of salbutamol respectively dobutamine; Medium = medium infusion rate of salbutamol respectively dobutamine; High = high infusion rate of salbutamol respectively dobutamine.

All procedures were always performed in the same order. During the baseline period and during autonomic inhibition, measurements were performed with a time schedule identical to that during CWT to keep the electrophysiologic and hemodynamic effects of pacing per se as similar as possible. Arterial blood samples were drawn five minutes after the measurements started for determination of catecholamine and potassium concentrations.

Paper III, study protocol

The subjects were studied on two occasions with identical protocols except for administration of salbutamol and dobutamine, which were used in random order, one on each occasion.

As infusion of salbutamol at a rate of 0.1 mg/kg/min has been reported to result in a 20% increase in heart rate reaching a steady plateau phase within 20 minutes, and infusion at a rate of 0.4 mg/kg/min resulting in an increase in heart rate of 40% [195], we first infused salbutamol at a rate of 0.1 mg/kg/min and after 30 minutes in the first subject we increased the rate to 0.4 mg/kg/min. This higher infusion rate resulted in pronounced changes in several electrophysiologic parameters before the steady state heart rate was reached. In the following subjects, a dosage of 0.2 mg/kg/min was therefore used as the high infusion rate. The first subject was excluded from the study. Electrophysiologic and hemodynamic measurements were performed and arterial blood samples taken after 20 minutes of infusion at the low rate and after 10 minutes and 20 minutes of infusion at the high rate.

Dobutamine was infused at three rates - 1.25, 2.5 and 5.0 mg/kg/min - with the intention to produce mainly β1 stimulation.

The electrophysiologic and hemodynamic measurements started after 15 minutes of infusion at each rate. All steps of the protocol were carried out in the same order and were completed within 8 minutes.

The following variables were measured during each sequence as previously described: SCL, SNRT and CSNRT, AERP, VERP, AVNERP, Stim-A duration, AH duration, HV duration, QS duration, QT duration, and arterial pressures.

Arterial blood samples were drawn 5 minutes after the start of the measurements for determination of catecholamine and potassium concentrations.

Proportional comparison of electrophysiologic parameters

The proportional changes in electrophysiologic parameters characterising different properties of the same cardiac tissue, particularly in the sinus node or in the AV node, have been observed to be very similar during manipulations of adrenergic tone and autonomic state. This parallelism of the proportional changes in AV nodal conduction and AV nodal effective refractory period, in sinus cycle length and sinus node recovery time, and in the refractoriness of atrial and of ventricular myocardium has been documented in several studies of the electrophysiologic effects of mental stress (Study I), epinephrine [182], dobutamine [202], propranolol and propranolol combined with atropine [180], and atenolol [203].

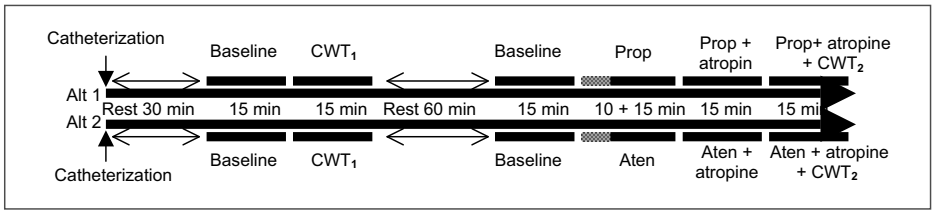
Thus, we considered it of interest to compare not only the proportional differences in electrophysiologic effects on different cardiac tissues, but also to compare the electrophysiologic responses of two adrenergic agents with different types of β selectivity.

Paper IV, study protocol

The subjects were divided in two groups, A and B, for two different protocols. The subjects in each group were studied on two occasions with identical protocols apart from the administration of atenolol and propranolol, which were used in random order – one on each occasion.

As shown in Figure 5, electrophysiologic and hemodynamic variables in group A were measured at baseline, during CWT and following 30 minutes of rest after administration of propranolol or atenolol (one on each study occasion). Measurements were then performed during ATI with atropine,

PROTOCOL STUDY IV A



PROTOCOL STUDY IV B

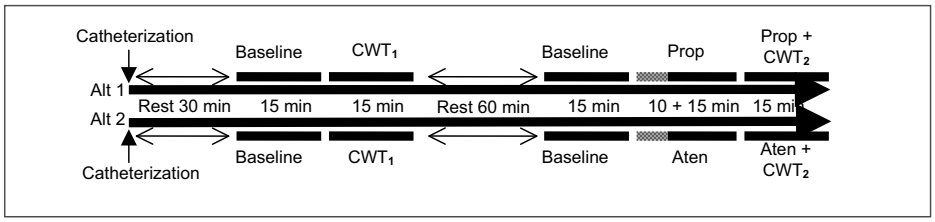


Figure 5. All subjects were studied on two occasions, alternative A and B, with identical protocols except for atenolol or propranolol, which were used in random order. 4A illustrates the protocol for group A, where ATI was administered before CWT. 4B illustrates the protocol for group B, where CWT was performed after only β-adrenergic inhibition.. CWT = stress test; Prop = propranolol; Aten = atenolol; ▩ = 10 min waiting period after atenolol or propranolol was administered before the measurement period started.

measured at baseline, during CWT and following 30 minutes of rest after administration of propranolol or atenolol (one on each study occasion). Measurements were then performed during ATI with atropine, combined with either propranolol or atenolol, directly followed by new measurements during CWT with ATI still active.

In the subjects of group B, electrophysiologic and hemodynamic variables were measured at baseline, during CWT and following 60 minutes of rest after administration of propranolol or atenolol (one on each occasion). Measurements were then performed during CWT with the β-blockade still active.

To evoke mental stress, CWT was used. Propranolol was administered intravenously with 0.15 mg/kg, which was considered adequate for β-receptor inhibition in this setting (18, 19). The same dosage was used for atenolol, aiming for equipotent dosages in regard to the effect on heart rate [20]. Atropine 0.02 mg/kg was given.

The following variables were measured during each sequence as previously described: SCL, SNRT and CSNRT, AERP, VERP, AVNERP, AH duration, QS duration, QT duration, and arterial pressures.

Statistical methods

Paper I

Results are given as mean values ± SD and as mean changes from baseline with 95% confidence intervals (CI). CWT was compared to the first baseline investigation and the two epinephrine infusions to the second baseline reading. Statistical evaluation of the effects of CWT was performed using Student's t -test for paired differences.

Analysis of variance (ANOVA) repeated measures was used to compare the effects of CWT and epinephrine infusions. When ANOVA demonstrated significant differences, further analyses were performed by Tukey's HSD post hoc test to evaluate separately whether CWT differed from each of the two epinephrine infusion rates. A $P < 0.05$ was considered significant.

Paper II

Results are presented as mean values ± SD. Comparison between groups and between proportional changes of different variables were analyzed using analysis of variance (ANOVA). A P-value < 0.05 was considered to indicate a significant change or difference.

Paper III

Results are presented as mean values ± SD and as mean changes from baseline with 95% confidence intervals. Analysis of variance (ANOVA) repeated measures were used to evaluate the effects of salbutamol and dobutamine infusion. Comparison between proportional changes in different variables were analysed by Factorial ANOVA. A p value < 0.05 was considered to indicate a significant change or difference.

Paper IV

Results are presented as mean values ± SD**.** Analysis of variance (ANOVA) repeated measures with two within factors was used to compare the *differences* in effects between propranolol and atenolol, differences in CWT-effects after inhibition with propranolol and atenolol, respectively, and between complete and incomplete ATI during CWT. A P value < 0.05 was chosen to indicate a significant change or difference.

Power

From clinical experience and from similarly designed studies [182] we assumed that a 20% shortening in nodal parameters and a 5% shortening in myocardial parameters would be relevant findings. With this assumption, with a power of 80% and a p value of 0.05, 8-10 subjects would be enough to show a difference. The results in study 1 supported this assumption. Furthermore, we assumed that the differences should be smaller comparing men and women and consequently these groups had to be larger.

RESULTS

Paper I

Electrophysiologic Effects of Mental Stress in Healthy Subject. A Comparison with Epinephrine Infusion

Aim

To investigate the electrophysiologic effects on different levels of the cardiac conduction system elicited by CWT and to compare them with the effects of epinephrine infused to levels similar to the physiological elevations produced by mental stress and physical exercise.

Results

Electrophysiologic measurements

No significant difference in any electrophysiologic parameter was seen between the two baseline readings, before and one hour after CWT, respectively.

All electrophysiologic variables shortened significantly during CWT with the exception of the atrial refractoriness and atrial conduction. The effect was most pronounced in the sinus node and in the AV node, with a parallel decrease in both AVNERP and AH interval. The mean reduction of the nodal parameters was in the order of 20%. The measurements of electrophysiologic properties of the right ventricle and the His-Purkinje system changed considerably less.

Although the plasma epinephrine concentration that was actually reached during the low-rate infusion was twice as high as the concentration produced by CWT, the electrophysiologic effects of the former were less pronounced for the AV nodal effective refractory period, the AH interval and the QT interval. The proportional changes in all nodal parameters during the high-rate infusion were similar to the response evoked by the CWT.

In addition to the findings during CWT, high-rate epinephrine infusion also caused significant shortening of the refractoriness of the atrial myocardium. In contrast to the effect of CWT, there was an *increase* in QS duration during infusion of epinephrine at the high rate, while the QT interval shortened to the same extent as during CWT.

Hemodynamic effects

The diastolic, mean, and systolic arterial pressures increased significantly during CWT as did the calf blood flow. The pre-ejection period decreased, but other

parameters were unaffected. During the infusions of epinephrine, the mean arterial pressure was unchanged, with an increase in systolic and a decrease in diastolic arterial pressure. Reductions in the length of the pre-ejection period and in calf blood flow as well as in calf vascular resistance were seen during the high-rate infusion.

Plasma epinephrine and norepinephrine concentrations

Neither epinephrine nor norepinephrine levels differed in the comparison of the two baseline periods, suggesting restoration of the basal state after one hour. Both the epinephrine and norepinephrine concentrations were significantly increased after 12 minutes of CWT.

The plasma concentration of epinephrine more than doubled during epinephrine infusion at the low-rate and increased twenty-fold during the high-rate infusion. The norepinephrine concentration increased significantly already during the low-rate infusion of epinephrine, an effect that was more pronounced during the infusion at the high-rate ($p<0.002$). The concentration of epinephrine during the low-rate infusion was well above that found during CWT.

Estimate of stress

The self-estimated scores of stress, irritation and palpitations increased both during CWT and during infusions of epinephrine compared to baseline. The scores were significantly higher during CWT than during either of the epinephrine infusions.

Conclusion

Mental stress elicited by CWT has pronounced effects on the electrophysiologic properties of the heart, most markedly in the sinus and AV nodes and to a lesser degree in the ventricle.

Although these electrophysiologic effects could be reproduced by epinephrine infusion, this was only reached at a much higher serum level of epinephrine and with a secondary release of norepinephrine as a confounding and probably contributory factor. Circulating epinephrine seems to play only a minor direct role, if any, as a mediator of mental stress effects on the heart, which to the greatest extent are neurogenically mediated.

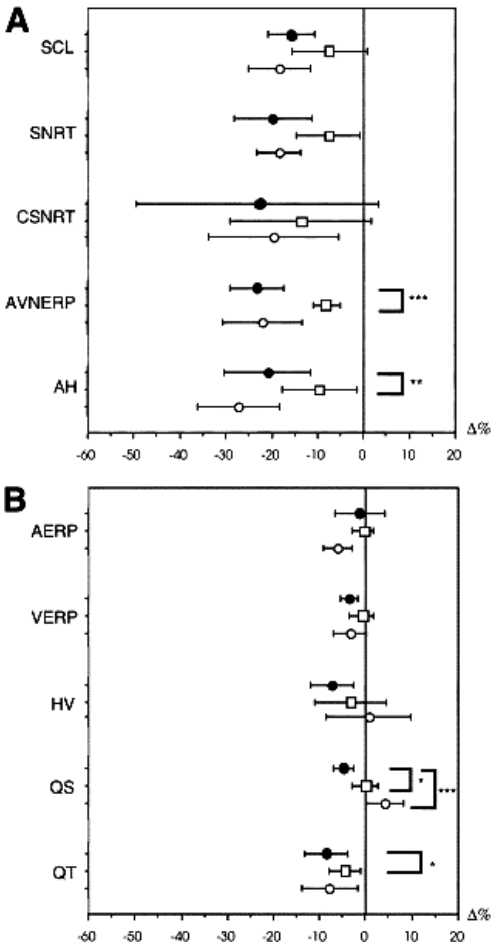


Figure 6. Results of electrophysiologic effects of Stroop´s colour word conflict test (CWT) and infusion of epinephrine; changes in percent from baseline (Δ%), with 95% confidence interval.
A. Effects on sinus node and AV node parameters.
B. Effects on atrial and ventricular parameters.
● = CWT; □ = E low, epinephrine infusion at 0.025 μmol/kg/min and ○ = E high, epinephrine infusion at 0.3 μmol/kg/min. * =$P<0.05$, ** $P<0.01$, *** $P<0.001$ CWT compared with E infusion.

Paper II

Gender Differences in Electrophysiologic Effects of Mental Stress and Autonomic Tone Inhibition: A Study in Healthy Individuals

Aim

To investigate gender differences in the electrophysiologic effects on different levels of the cardiac conduction system elicited by CWT and by autonomic tone inhibition (ATI).

Results

Electrophysiological effects of CWT

During mental stress, all electrophysiologic parameters except atrial conduction time and ventricular depolarization shortened significantly and with more pronounced decreases in the sinus and AV nodal parameters compared to the myocardial parameters. This pattern with proportionally more pronounced effect on nodal tissues was similar for both men and women.

Differences between men and women at baseline and during CWT

At baseline, women had shorter sinus cycle length than men while men had a tendency for shorter QT and JT durations. The latter differences were augmented by mental stress producing significantly shorter QT and JT durations in men. Women developed shorter refractoriness in the atrial tissue and the AV node.

Calculating the proportional changes produced by CWT women reacted with a more pronounced decrease in corrected sinus node recovery time and AV nodal refractoriness, while men showed significantly more pronounced shortening in JT duration. This difference did not reach significance in QT duration.

Electrophysiologic effects of ATI

During autonomic tone inhibition both AVNERP and AH duration were shorter in women while both QT and JT durations and VERP were shorter in men. There was no significant difference in the parameters of the sinus node.

Women had greater QT dispersion than men during baseline readings. After autonomic tone inhibition this difference decreased and was not significant, which was explained by a significant proportional increase in QT dispersion in men but not in women after autonomous tone inhibition (33%±27 vs. 12%±23, $p<0.05$).

Comparison of baseline measurements

No difference was found in any electrophysiologic parameter or blood pressure when the two baseline readings were compared, verifying conclusion in paper 1 that one hour of rest after CWT is sufficient to restore the autonomic tone.

Conclusion

There are gender differences in cardiac electrophysiologic properties that appear intrinsic. After autonomic tone inhibition, women not only have higher heart rate and longer QT and JT intervals, they also have shorter AV nodal effective refractory

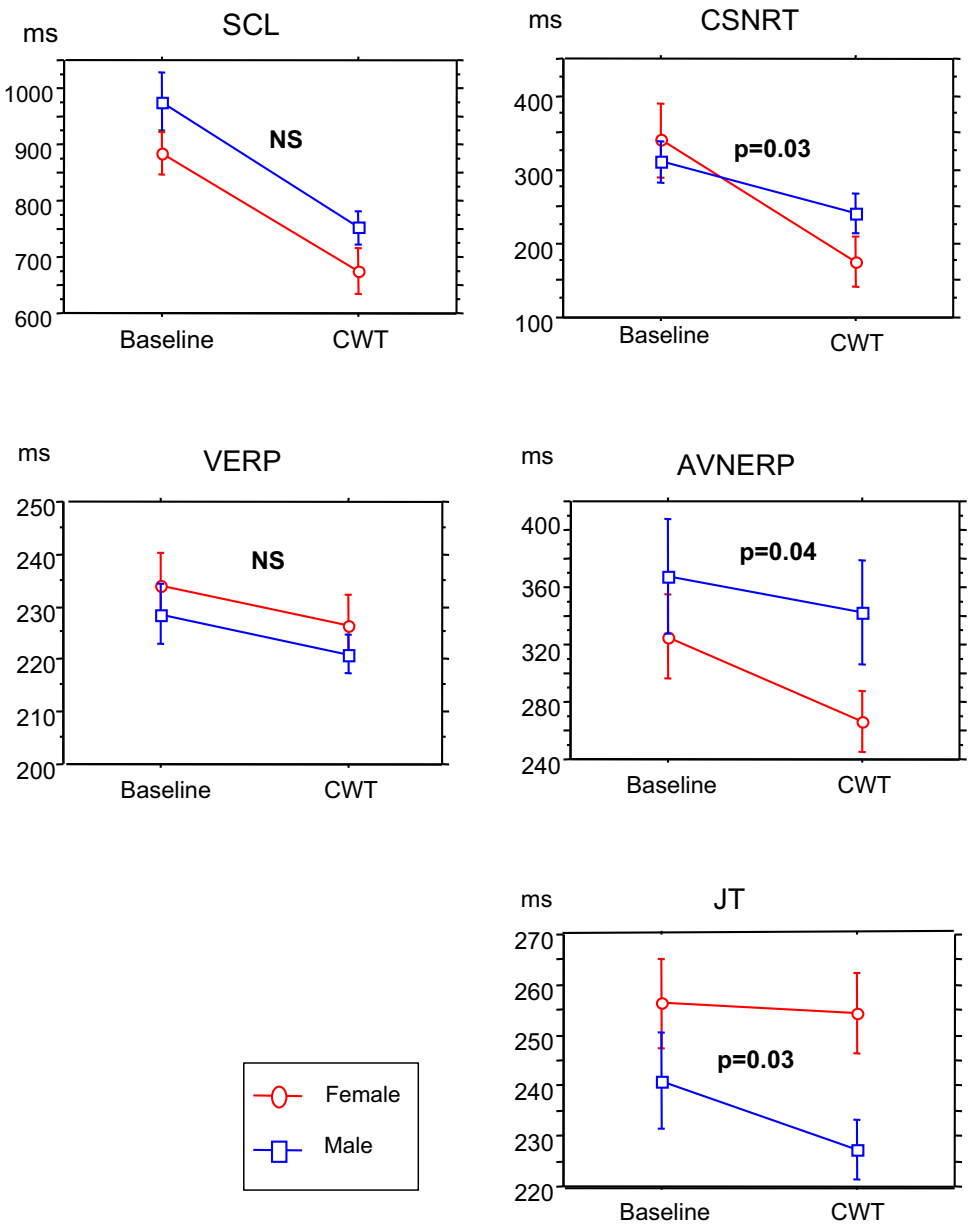


Figure 7. Examples of reaction patterns to mental stress in some electrophysiologic parameters and observed differences between men and women. Note the same shortening in SCL and VERP, a more pronounced shortening of CSNRT and AVNERP in women, and a more pronounced shortening of JT in men. CWT = mental stress; SCL = sinus cycle length; CSNRT = corrected sinus node recovery time; AVNERP = AV nodal effective refractory period; VERP ventricular effective refractory period; JT = JT duration.

period, faster AH conduction and longer effective refractory periods in the right ventricle. During mental stress, women exhibit a more pronounced reaction of the AV node and the sinus node, and men react with a more pronounced effect in the ventricular electrophysiologic properties.

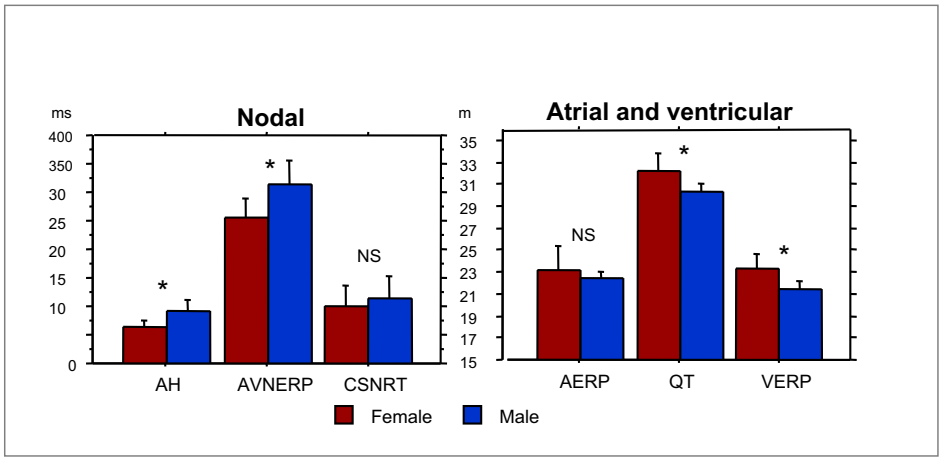


Figure 8. Comparison of electrophysiologic parameters after ATI. Women have shorter AV nodal conduction time and refractoriness, whereas men have shorter ventricular refractoriness and QT duration.

Paper III

Electrophysiologic Effects of Salbutamol, a β2 Selective Agonist

Aim

To assess the overall electrophysiologic effects of β2-agonist infusion, by administering salbutamol at different infusion rates, resulting in increases in heart rate similar to those attained during β2-agonist inhalation therapy. In order to distinguish β2-agonist effects a comparison was also made with the β1 selective agonist dobutamine.

Results

Electrophysiologic effects

Salbutamol produced significant changes in electrophysiologic properties both in the myocardium, the sinus node, and the AV node, the effects being significantly greater on nodal parameters. Similarly, proportionally more pronounced effects on nodal properties were also seen during dobutamine infusion.

Effects on the sinus node

A gradual shortening of the sinus cycle length and the sinus node recovery time was observed during infusion both of salbutamol and of dobutamine, reaching 35-40% during the higher dosages.

Effects on the AV node

The effects on the AV node were even more pronounced than those on the sinus node, with parallel shortening of AVNERP and

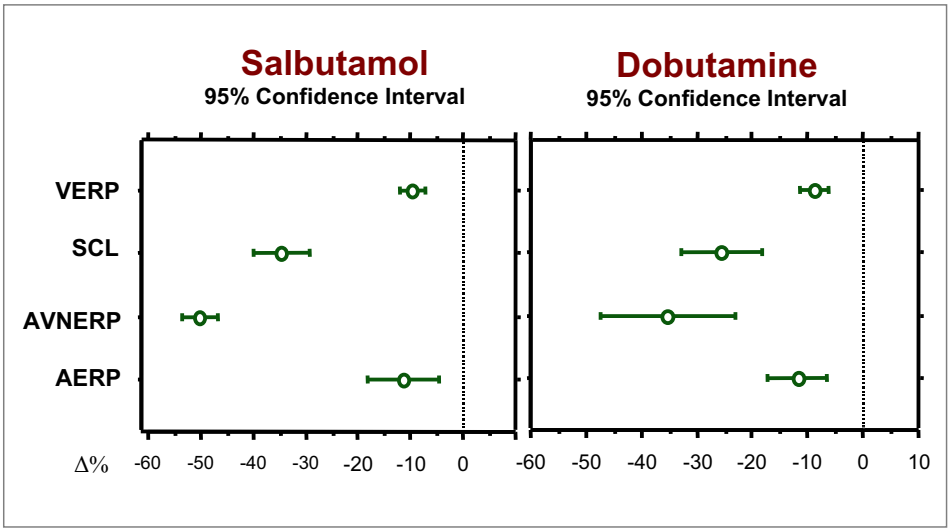


Figure 9. Proportional shortening of some electrophysiologic parameters during infusion of salbutamol and dobutamine. Note different effects in myocardial tissues (VERP and AERP) and in nodal tissues (SCL and AVNRP). VERP = ventricular effective refractory period; SCL = sinus cycle length; AVNERP = AV nodal effective refractory period; AERP atrial effective refractory period

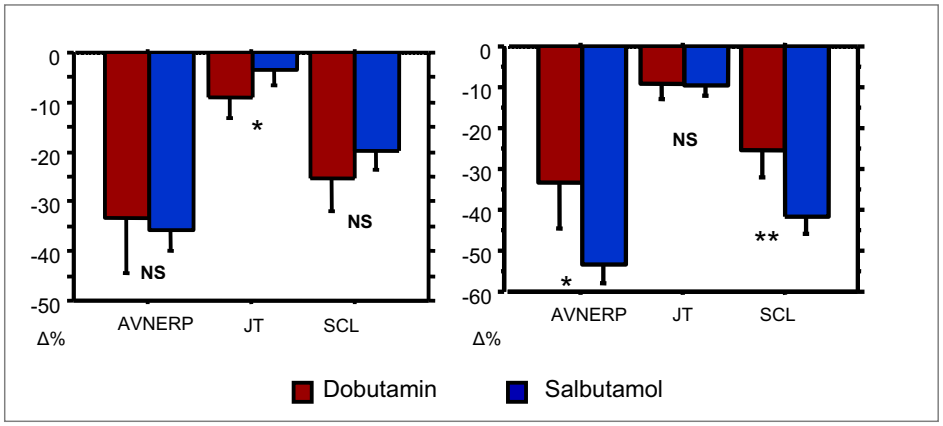


Figure 10. Comparison of effects (Δ%) between dobutamine and salbutamol at the same shortening in SCL (left) and at the same shortening in JT duration (right). AVNERP = AV nodal effective refractory period; JT = JT duration; SCL = sinus cycle length. Salbutamol has a proportionally stronger effect on nodal parameters.

AH duration of 36±6% and 40±10%, respectively, during infusion of salbutamol at the low rate and of 50±5% and 54±11%, respectively, during infusion at the high rate of salbutamol.

Similar effects were observed during infusion of dobutamine.

Effects on the atrial and ventricular myocardium

The changes in the atrial and ventricular refractory properties were moderate, although significant, during salbutamol infusion with a decrease of 5 to 10% in both the atrial and ventricular effective refractory periods. No effect on intra-atrial or His-Purkinje conduction was observed.

The ventricular depolarization interval *increased* significantly: the QS interval lengthened by 8 ms (95% confidence interval, 6 to 10). The QT and JT intervals shortened: the former to a lesser extent because of the lengthening of the QS interval. QT dispersion increased from 19 ms to 45 ms, p=0.009, with the heart rate kept constant by right atrial pacing.

During dobutamine infusion the effective refractory periods of the atrial and ventricular myocardium decreased, as did the intra-atrial and His-Purkinje conduction intervals, while the duration of ventricular depolarization was unchanged. The QT and JT intervals became shorter. There was no change in QT dispersion.

Comparison of proportional electrophysiologic effects

The proportional shortening of the sinus node parameters was clearly less pronounced than the effects on the AV node (a decrease in sinus cycle length of 20% vs. a decrease in AH time of 40%, $p<0.0001$) during salbutamol infusion at the low rate. This difference was of the same order of magnitude during the high-rate infusion (sinus cycle length 35% vs. AH time 54%, $p<0.0001$).

In contrast, no significant difference between the effects on the sinus cycle length and AV nodal parameters was found during infusion of dobutamine.

The effects on the atrial and ventricular refractory properties were proportionally weaker than those on the sinus node parameters both during infusion of salbutamol and dobutamine.

Hemodynamic effects:

Infusion of salbutamol caused a significant decrease both in mean blood pressure and in diastolic blood pressure, whereas the systolic blood pressure remained unchanged. Of the systolic time indices, both the pre-ejection period and the left ventricular ejection time shortened significantly, indicating a state of increased inotropy. An increase in stroke volume was seen already during the low-rate infusion. However, in contrast to the other inotropic parameters, this did not increase further during the infusion at a high dosage.

During dobutamine infusion the systolic blood pressure showed a gradual increase, while the diastolic blood pressure did not change. Stroke volume increased gradually, with a concomitant decrease in systolic time interval indices.

Effects on catecholamines and potassium concentrations

During infusion of salbutamol at the low rate, the plasma concentration of norepinephrine increased an increase that was not significantly augmented during the high-rate infusion. Simultaneously the potassium concentration decreased. The plasma concentration of epinephrine did not change.

No change in either the epinephrine, norepinephrine or potassium concentration was seen during dobutamine infusion.

Comparison of baseline measurements

No significant differences in any of the studied electrophysiologic or hemodynamic parameters were found between the baseline measurements before administration of dobutamine and salbutamol, except for stroke volume, which was higher at baseline before salbutamol infusion (62 ml vs. 54 ml, p=0.028).

Comparison of effects of salbutamol and dobutamine

With dosages at which salbutamol and dobutamine caused similar effects on the AV and sinus nodal parameters the reductions in the ventricular repolarization and atrial and ventricular refractoriness were less pronounced during salbutamol infusion.

When the above rate of dobutamine infusion was compared with the higher rate of salbutamol it caused similar reduction in JT duration, but the effects on the AV and sinus nodal parameters were significantly more pronounced during salbutamol infusion (Figure 10).

Conclusion

The positive chronotropic effect of β2-stimulating agents is associated with changes in electrophysiologic properties in other heart structures in addition to the sinus node, most markedly in the AV node.

A discordant effect on ventricular electrophysiology was found. Ventricular conduction slowed, while refractoriness shortened. QT dispersion increased.

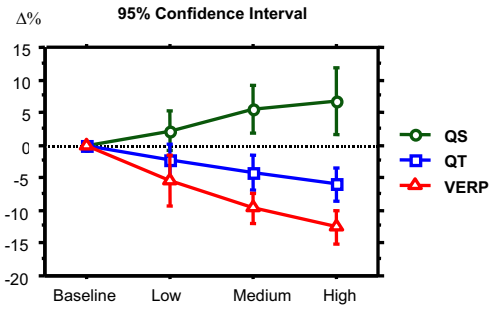


Figure 11. Different effects on QS (increasing) and QT and VERP (decreasing) during infusion of salbutamol at low, medium and high infusion rate. QS = QS duration; QT = QT duration; VERP = ventricular effective refractory period

Paper IV

Electrophysiologic Effects of Mental Stress after Autonomic Tone Inhibition. Different Effects of Selective and Unselective ß-Receptor Inhibition

Aim

To evaluate if the type of β-blocker, β1-selective or unselective, is of importance to inhibit the electrophysiologic effects of CWT. In order to prevent a possible compensating effect of the parasympathetic nervous system, CWT was also performed with each β-blocker combined with atropine, i.e., after ATI.

Results

Effects of Propranolol and Atenolol

The electrophysiologic effects of atenolol and propranolol on the function of the sinus node, the AV node and the ventricle were similar with a lengthening in SCL, SNRT, AVNERP, AH-interval, and in VERP. When the effects of propranolol and atenolol were compared no differences were found in any of the measured variable.

Effects of Mental Stress after Propranolol and Atenolol

Mental stress caused very similar electrophysiologic effects regardless of β-inhibiting agent used. SCL, SNRT, and VERP all decreased to the same extent.

Comparing the effects of CWT after inhibition with atenolol and propranolol, there were no differences except for the AERP, where the effect of stress after atenolol differed from that after propranolol.

Effects of Mental Stress after Selective and Unselective Autonomic Tone Inhibition

ATI with propranolol as the β-blocking agent resulted in a complete inhibition of the stress induced changes of all electrophysiologic parameters except the QT duration. In contrast, the combination of atenolol and atropine gave an incomplete inhibition of the stress mediated effects on the heart rate, AERP, AVNERP, VERP, and QT duration. The shortening of the refractoriness of ventricular myocardium were highly significant. A general tendency towards a shortening of most parameters during CWT after incomplete ATI was seen.

Systolic, mean, and diastolic blood pressure increased moderately and to the same degree during both types of ATI.

Comparing the effects of CWT after complete and incomplete ATI showed a significant more pronounced shortening of SCL and VERP during the latter and a trend towards significant differences in AERP. This is in contrast to comparison of the effects of CWT after unselective and selective betablockade but without the atropine inhibiting effect, when the shortening of these parameters were parallel as illustrated in Figure 12.

Effects of vagal withdrawal

Propranolol combined with atropine inhibits all effects of mental stress except on the QT duration. Mental stress after pretreatment with only propranolol, on the other hand, also shows a significant decrease in both SCL and SNRT, and a trend in

shortening of the VERP. Thus, after unselective β-blockade, there is still an effect on the sinus node and the ventricular myocardium during mental stress, which is caused by vagal withdrawal.

Comparison of Repeated Baseline and CWT Measurements

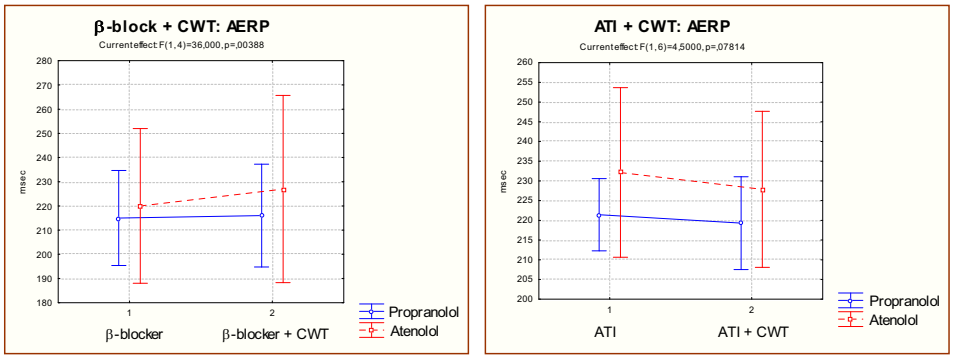
Comparison of the two repeated baseline recordings at the same investigation showed no differences. Furthermore, no differences were found when the results were compared between the two baseline and the two CWT readings at the two different investigations 4-6 weeks apart.

Conclusion

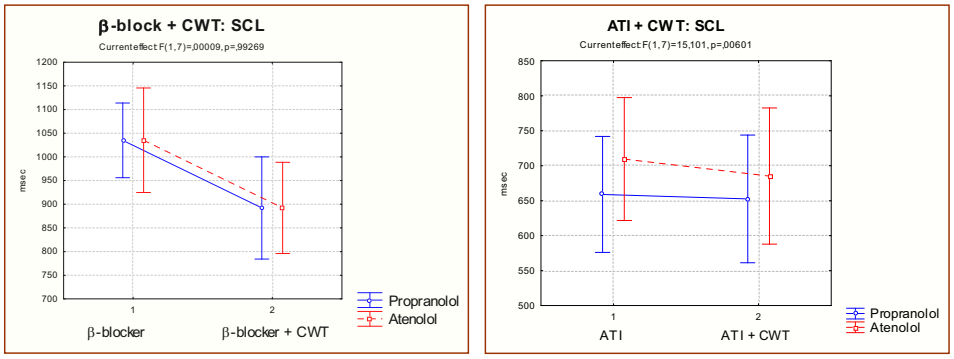
Mental stress as elicited by CWT had pronounced effects on the electrophysiologic properties of the heart.

Inhibition with the β1-selective antagonist atenolol does not eliminate the effects of mental stress on the sinus node and the ventricular myocardium, effects that at least partly appear mediated through uninhibited activation of β2 adrenoceptors. This finding may be of importance in the handling of patients with stress-mediated arrhythmias

A.



B.



C.

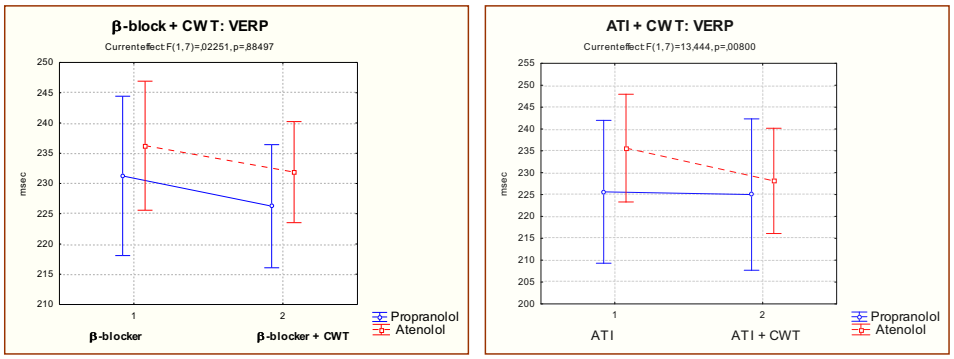


Figure 12. ANOVA repeated measures contrast analysis graphs comparing the effects of CWT after propranolol and atenolol, respectively, (left) and the effects of CWT after ATI using propranolol and atenolol, respectively, in combination with atropine (right). A. AERP = atrial effective refractory period; B. SCL= sinus cycle length; C. VERP = ventricular effective refractory period.

GENERAL DISCUSSION

Introduction

The main findings of our studies are:

- the demonstrated cardiac electrophysiologic effects of mental stress, which are most pronounced on nodal tissues
- the different electrophysiologic reaction patterns to stress for men and women
- the effects of β2 stimulation with its differing effects on AV nodal, sinus nodal and myocardial properties
- the importance of β2 adrenoceptor activation during mental stress; and
- the importance of vagal withdrawal in the electrophysiologic reaction to stress.

The following section discusses these main findings. More detailed discussions regarding methodology and limitations are given in each paper.

Electrophysiologic effects of mental stress

Our studies show that mental stress produced by Stroop's colour word conflict test causes widespread electrophysiologic effects in the heart. The effects are most pronounced on the parameters reflecting sinus node and AV node function, and lesser in the ventricular myocardium and conduction system, and on atrial refractoriness. No effect was seen on atrial conduction time. The shortenings of the sinus cycle length, the sinus node recovery time, the AV node effective refractory period and the AH conduction time were all in the magnitude of 20%. The effects on ventricular and atrial tissues were considerably less pronounced with a decrease below 10%.

Why are there proportionally different effects on different parts of the cardiac conduction system?

A possible explanation for the proportionally different effects on cardiac tissues is that the distribution and density of adrenoceptors and muscarinic receptors in nodal tissue differ from that in atrial and ventricular tissue. In addition, there may be differences in sympathetic and vagal innervation.

Using radioligand technique it has been shown that the β-adrenoceptor density in the nodal tissues is higher than in the surrounding atrial tissues. The proportion of β2-adrenoceptors also seems do be higher in both the sinus and the AV nodes [72,73,75]. Results from studies using quantitative autoradiography suggest that the density of both β1- and β2-adrenoceptors is higher in the AV node than in the right atrium and the right ventricle [76]. Radioligand studies of cardiac muscarinic receptor densities in the

human heart have shown regional differences, with higher concentrations of receptors in atrial and nodal tissues than in ventricular tissue [204]. In another animal study muscarinic receptors density was higher in the sinus node compared with surrounding atrial tissue [205].

Crick et al. [206, 207] have shown that nodal tissues are more densely innervated than the myocardium in other parts of the heart, the sinus node being most densely innervated. Furthermore, parasympathetic nerves represent 60% to 70% of the innervation in the nodal tissues and the atrioventricular bundle.

The effects of mental stress on atrial parameters are difficult to evaluate. Vagal stimulation and β-adrenergic stimulation have been reported as synergistic: they both shorten the atrial refractoriness. α-adrenergic stimulation seems to have an antagonistic effect increasing the refractoriness [208]. There was a small decrease in atrial refractoriness during mental stress, which was not significant in study I (10 subjects) but significant in study II (23 subjects). It is interesting that in the latter study a decrease in atrial refractoriness was observed in women but not in men.

Evaluating atrial conduction by means of measuring conduction time from an electrode positioned in high lateral right atrium to a His-electrode (Stim-A) is not unproblematic. On one hand the electrode should be positioned for stimulation near the sinus node, on the other hand the sinus node is located along the crista terminalis. Depending on whether one stimulates laterally or posteriorly in regard to crista terminalis, which is very difficult to determine using fluoroscopy, the conduction pathway to the AV node can be very different in length and also transverse regions with marked different conduction velocities, which in turn are differently influenced by autonomic tone [131, 209]. This makes the measurement of atrial conduction in the setting used in the present studies unreliable, particularly if the catheter has to be repositioned the

There was no significant effect of mental stress on conduction, which does not exclude an effect if a more accurate measurement had been performed.

We found a relatively modest effect on the ventricular variables we measured: the ventricular refractoriness and the QT interval. Other kinds of mental stress may have other effects on the ventricular myocardium than CWT used in this study and on parameters we did not evaluate. Studies in patients with ICD have shown that mental stress alters the VT cycle length and the termination characteristics [34] and that T-wave alternans, an indicator for myocardial vulnerability, increases during standardized mental stress tests [210].

A limitation of our study protocol is that it did not allow us to identify a possible α-adrenergic effect of CWT. The α- and β-adrenergic activities are known to interact. α-adrenergic stimulation has been shown to have a moderate positive chronotropic influence on the sinus node and to prolong repolarization although changes in conduction velocity are probably negligible [211,212].

An alternative or possible additional explanation for the disproportion of effects is a withdrawal of vagal activity, as parasympathetic predominance in the innervation of the sinus node, the AV node and the atrium has been reported. Vagal effects on refractoriness also differ between atrial and ventricular tissues, with vagal activation producing a shortening in the atrium and lengthening in the ventricle [213].

Comparison with other stress tests and real life stress

The endocrine response to CWT is characterized by elevated catecholamine levels, while increased cortisol levels very seldom have been reported in context with this test [64]. Other stress tests induce different endocrine responses. Mental arithmetics combined with public speaking is commonly used in the laboratory setting. This test evokes a response characterized by sympathetic, andrenomullary and adrenocortical activation [214]. Comparing the effects in subjects confronted with a number of different mental stress tests often show

different response patterns in regard to increases in excretion of epinephrine, norepinephrine, and cortisol [64].

The psychoendocrine reaction to real-life stressors has also been extensively studied. A great number of studies have evaluated the endocrine response to examination stress. Frankenhauser [61,62] described gender differences in the psychoendocrine reaction to examination stress and observed that epinephrine levels increased during this stress in women, while in men both epinephrine, norepinephrine, and cortisol levels increased.

Other investigators have reported contradictory results in regard to ACTH and cortisol. Another frequently studied stressful situation is waiting for surgical intervention. Summarizing these results, Bondi [64] found that the anticipation of a surgical intervention may induce a reaction involving increased levels of catecholamines and cortisol as well as opiate peptides and possibly prolactin.

Also parachute jumping has been studied. Richter et al. [215] performed continuous analysis at 10-min intervals in inexperienced tandem parachutists. They observed a significant increase in heart rate and epinephrine level during the jump itself. Norepinephrine, cortisol, growth hormone, prolactin, and TSH peaked 10-20 min after the jump. All endocrine parameters had normalized within 1 hour except for cortisol and TSH.

Thus, real life psychological stress often includes components of frustration, aggressiveness and/or fear, reactions that can only partially be reproduced in a test situation. CWT is dominated by the intellectual effort with a clear component of frustration, but it evokes a psychoendocrine response that probably only partly represents the response caused by most real-life stressors.

Effects of cardiac catheterization per se on electrophysiologic parameters

Evaluating the effects of stress tests by means of electrophysiological studies raises the question whether catheterization and the laboratory environment per se significantly influences the electrophysiologic parameters. The work by Jewell et al. [216] does not imply that this is necessarily the case. They showed that the sinus node parameters, the atrial effective refractory period, and the sinus cycle length do not differ when measured during the electrophysiologic study and again 24 hours later. For the latter measurements, they used a high right atrial catheter that had been introduced via the right antecubital vein, sutured and left.

In subjects not undergoing right heart catheterization, CWT causes an increase in heart rate of 10% to 23% and an increase in systolic blood pressure of 11% to 16% [232]. This compares well with the changes from the baseline observed in our studies.

The catheterization per se probably influences basic autonomic tone, but this seems to have only a minor effect on the response to CWT.

Gender differences

As outlined in the background, there are certain gender differences in electrophysiologic properties as well as in the prevalence of many arrhythmias. Study II shows that in women mental stress produces a pronounced effect on the AV node and on the sinus node, whereas men react with a more pronounced effect on the ventricular electrophysiologic properties.

The shortening of the AVNERP is more pronounced in women than in men. There are few studies evaluating gender differences in regard to the AV nodal functions. Taneja et al. [127] did not find any difference in AV nodal refractoriness between men and women in patients undergoing electrophysiologic evaluation.

Liu et al. [129] on the other hand found that the AVNERP, the AH and HV intervals were longer in men than in women in patients undergoing ablation of AV nodal reentry tachycardias and atrioventricular tachycardias but without structural heart disease. It is possible that the inclusion of patients with AVNRT in this latter study influenced the results. We did not find any differences between men and women in AV

nodal refractoriness during baseline measurements but observed a difference after autonomic inhibition.

There are reports describing gender differences in catecholamine response during mental stress tests. Frankenhauser [61,62] found that men responded with a larger increase in epinephrine level compared with women during stress and Davidson [63] observed higher norepinephrine levels in men during stress tests. We found that both epinephrine and norepinephrine increased significantly during CWT in men but not in women.

Thus, since both epinephrine and norepinephrine shortens AVNERP, this gender differences in catecholamine response would theoretically diminish the observed difference in AV nodal refractoriness during CWT.

In study II we found that during ATI healthy women both have shorter AH interval and shorter AVNERP, but longer effective refractory period in the right ventricle and QT duration during ATI (Figure 8).

QT dispersion was greater in women during baseline but not significantly so after autonomic blockade. These findings disagree with other reports that have found a greater QT dispersion in men or no difference between men and women [217,218]. Our measurements were made during atrial stimulation; however, when we analyzed QT dispersion during sinus rhythm, females had significantly greater QT dispersion than men did.

Challapalli [217] reported a decreased difference after ATI, a finding that agrees with our findings, indicating that the baseline difference is related to autonomic activity.

The gender difference in myocardial electrophysiologic function observed after autonomic inhibition and during stress may possibly be a contributing factor why women are less prone than men to develop certain ventricular arrhythmias and sudden death.

Sex steroids seem to influence autonomic tone which may partly explain gender differences in cardiac electrophysiology and the occurrence of arrhythmia [132, 219]. The conduction properties and the refractoriness of cardiac tissues are largely influenced by the adrenergic tone as we have shown in our studies. Hypo-estrogenic states and elevated levels of progesterone increase the levels of catecholamines [133].

After ATI we unmasked further differences between men and women in the electrophysiologic measurements of the AV node, in the refractoriness of the right ventricle, and in the QT and JT intervals. This suggests that although other mechanisms without doubt influence cardiac electrophysiologic properties, intrinsic differences exist between men and women.

Effects of adrenergic agonists

We found significant cardiac electrophysiologic effects during infusion of epinephrine (β1, β2 and α agonist), dobutamine (primarily β1 agonist), and salbutamol (primarily β2 agonist). As with the results during CWT, we found a proportional effect that was greater on nodal tissue compared with myocardial tissues for all three agonists. The argument made earlier, a higher density of both receptors and innervation in nodal tissues, is a reasonable explanation.

Compared with epinephrine infusion, mental stress has a greater influence on ventricular properties, with shortening of the infranodal conduction and a proportionately higher degree of shortening of the effective refractory period in the ventricle and the QT interval. This finding of more pronounced effect of neurally released catecholamines agrees with early experimental work by Vassalle et al. [220]. A higher proportion of β-1 adrenoceptors in the ventricle, combined with less influence of vagal withdrawal may be part of the explanation [72, 221].

In study III, infusion of dobutamine caused shortening of nodal electrophysiologic para-meters that was significantly more pro-nounced than the effects on atrial and ventricular refractoriness, but there was no significant difference in the proportional effects between the sinus node and the AV node.

These findings are in contrast with the results for salbutamol, which had significantly weaker effects on the electrophysiologic properties of the sinus node compared to those of the AV node. Reports observing [76] a higher β2-receptor density in the AV node compared with the sinus node and the myocardium may explain this finding.

Mechanisms of the electrophysiologic effects of salbutamol

The mechanism underlying the net electrophysiologic effects of salbutamol infusion is not clear, but might be explained either by a baroreceptor-mediated reflex due to peripheral vasodilatation with secondary release of norepinephrine, by vagal with-drawal, by a direct action on the cardiac β2-receptors, or by a combination of these fac-tors. Effects of induced hypokalemia should also be considered.

Reflex-mediated β1 stimulation

We found a moderate increase in norepinephrine levels during infusion of salbutamol; this is probably secondary to peripheral vasodilation generating norepinephrine release from the vascular tree and the heart.

Observed electrophysiologic effects of norepinephrine *infusion* are somewhat contradicting. Several authors report an anticipated positive chronotropic effect as a result of norepinephrine β1 agonist properties but also a net effect influenced by reflex mediated vagal activation [222,223]. Weiss et al. [224] reported the electrophysiologic effects of norepinephrine at an infusion rate that more than doubled the plasma concentration. They found a rise in systolic blood pressure, a positive inotropic effect; and a decrease in heart rate. The corrected sinus node recovery time and AV nodal, atrial, and ventricular refractoriness increased. The secondary increase in norepinephrine plasma levels we found during infusion of salbutamol was slightly less than the plasma concentration reached by Weiss et al.

Intravenous administration of trinitroglycerin during right atrial pacing in healthy volunteers resulted in a 20% decrease in mean arterial pressure and a similar shortening in the AV nodal conduction time and in sinus cycle length [225]. Alteration in baroreceptor activity was the presumed mechanism for these electrophysiologic effects. This may be compared with the 8% decrease in mean arterial pressure followed by 40% shortening of the AH interval and 20% shortening of the sinus cycle length observed in our study during salbutamol infusion at the low dosage.

Reflex-mediated β1 stimulation may influence the electrophysiologic response to salbutamol infusion, but it is not likely that this alone explains the pronounced change in the electrophysiologic properties of the AV node and the sinus node found in our study.

Vagal effects

Determining the influence of vagal withdrawal during infusion of salbutamol is difficult because of the complex nature of the vagal-sympathetic interaction on nodal properties and myocardial refractoriness.

Levine and Leenen [115] evaluated the role of vagal tone in response to β2-agonists using terbutaline infusion in dosages resulting in increases in heart rate very similar to the findings in study II. Comparing the effects of terbutaline alone and in combination with atenolol and atropine they concluded that infusion of β2-agonists in these dosages does not invoke a reduction in vagal tone.

Our protocol did not allow us to quantify the degree of vagal influence on the electrophysiologic effects caused by salbutamol. However, the vasodilation caused by salbutamol in our study was moderate, especially during the low dosage of salbutamol. Based on Levine and Leenen's findings, we think it is justified to consider the influence of vagal withdrawal as small.

Direct stimulation of cardiac β2-receptors

A direct stimulation of cardiac β2-receptors during infusion of salbutamol is the most

plausible explanation for the electrophysiologic response. This is supported by other studies having demonstrated a positive chronotropic effect of β2-receptor agonists. Infusion of terbutaline in subjects pretreated with atenolol and atropine still caused an increase in heart rate and blood pressure [114,115]. In another study injection of salbutamol into the right coronary artery resulted in an increase in heart rate that could be reproduced after pretreatment with selective β1 blockade but not after treatment with propranolol [157].

Electrophysiologic effects of hypokalemia

Moderate hypokalemia has been reported to slow repolarisation in myocardial tissue resulting in prolonged refractoriness. More severe hypokalemia may give rise to shortening of the refractoriness [226]. Hypokalemia usually decreases conduction velocity [227].

In our subjects we noted a moderate decrease in potassium serum concentration during the infusion of salbutamol (remaining within normal limits at the low infusion rate), making it reasonable to assume that it did not enhance the observed *decreases* in refractoriness and increases in conduction velocities.

Different effects of β1 and β2 stimulation on ventricular depolarization and repolarization

An interesting finding was an increase in the duration of the QS interval during infusion of salbutamol. This increase was not observed during infusion of dobutamine when QS duration did not change. A small increase in QS duration was also noted during infusion of epinephrine, which is a non-selective β-receptor agonist. In the absence of a change in the His-Purkinje conduction this increase represents a slower depolarization of the ventricles and may imply a decrease in conduction velocity in the ventricular myocardium.

CWT, on the other hand, causes a decrease in QS duration. In contrast to the effects by infusion of β-agonists, CWT induces a prominent vagal withdrawal.

We also noted a more than 2-fold increase in QT dispersion during infusion of salbutamol, a change that was absent during dobutamine infusion. In this context it should be emphasized that all QS and QT measurements were performed during atrial pacing at the same cycle length to avoid the need to apply rate adjustments to compensate for rate dependant effects on the QT interval, i.e., different algorithms for "correcting" the QT interval, the QTc.

Thus, the overall electrophysiologic effects of β2 stimulation on the ventricular myocardium include shortening in refractoriness, a shortening in paced QT duration but an increase in QT dispersion, and slowing of ventricular depolarisation, a combination that in predisposed individuals could facilitate the development of re-entry arrhythmias.

Lowe et al. [228] found a significant increase in QT dispersion after infusion of salbutamol, which was most pronounced in patients with coronary heart disease. Mettauer et al. [229] has reported that patients with congestive heart failure treated with salbutamol have an increased incidence of ventricular arrhythmias. Ventricular arrhythmias have also been reported to occur during treatment with fenoterol aerosols in patients with chronic airway obstruction [117,118].

The electrophysiologic effects demonstrated in our study may be a contributory explanation to these findings.

The importance of β2 receptors

The electrophysiologic effects of mental stress produced by CWT are incompletely inhibited by propranolol as well as by atenolol. In both settings, significant changes in heart rate, sinus node recovery time, and QT duration occur. Autonomic tone inhibition with atropine added to propranolol completely inhibits these effects except on the QT duration, suggesting that the electrophysiologic effects elicited by stress after administration of propranolol are mainly caused by vagal withdrawal.

In contrast, autonomic tone inhibition with atropine added to atenolol produces an inhibition that remains incomplete in the sinus node and the AV node, as well as in the atrial, and the ventricular myocardium, suggesting that the electrophysiologic effects elicited by stress after administration of this β1-selective inhibitor are caused not only by vagal withdrawal but also by direct β2-receptor stimulation

It is interesting to note that the changes of the sinus nodal, AV nodal and ventricular parameters caused by CWT after propranolol are similar in extent to those after atenolol. If we hypothesize that the changes seen after CWT combined with propranolol treatment are caused by vagal withdrawal and the corresponding changes during atenolol treatment are a combination of vagal withdrawal and β2-receptor stimulation, these latter effects do not seem to be additive. Furthermore, the magnitude of the changes caused by CWT after ATI with atenolol are smaller than those seen after CWT and atenolol ($p<0.001$) with the exception of VERP and QT, which change to the same extent, suggesting that β2-receptor stimulation is more important in this tissue.

This corresponds with the findings in study III where the effects of the β2 agonist salbutamol although most pronounced on nodal tissues, also significantly influenced ventricular refractoriness and QT-duration.

As has been mentioned earlier, several studies using radioligand-binding techniques and quantitative autoradiography have demonstrated that β2-adrenoceptors constitute 20% to 40% of the adrenergic receptors in atrial tissue and 10% to 40% of those in the ventricular myocardium [72-75], that the proportion of β2-adrenoceptors seems to be higher in the AV node than in the surrounding myocardium [76], and that the density of β-receptors found in the sinus node is three times higher compared with the density of the atrial myocardium, with the β2-subtype accounting for 30% of the total amount in sinus nodal tissue [77].

The effect of mental stress and autonomic tone inhibition on atrial refractoriness is complex. Vagal stimulation and β-adrenergic stimulation have been reported as synergistic shortening the atrial refractoriness, whereas α-adrenergic stimulation seems to have an antagonistic effect, increasing the refractoriness [208]. We found a small decrease in atrial refractoriness during mental stress performed after inhibition with atenolol and atropine, perhaps indicating a β2-mediated effect on this tissue, agreeing with the observed decrease in atrial refractoriness during infusion of salbutamol

β3- and α-adrenoceptors

Our protocols have not taken into account possible effects of α- and β3-receptor stimulation. α-1 adrenergic stimulation decreases heart rate, modulates ventricular automaticity, and shortens QT duration [87,230], results that agree with the finding of a decreased duration of the QT interval.

In vitro studies have demonstrated that β3 adrenoceptor agonists produce effects in myocardium [82]. Wheeldon et al. [84] showed that after β1 and β2 adrenoceptor inhibition with nadolol, an investigational β3 adrenoceptor agonist showed a small but significant chronotropic effect in healthy volunteers. I am not aware of any studies evaluating the effects of β3 stimulation on refractoriness and repolarization of the ventricular myocardium.

Clinical relevance of cardiac β2-adrenoceptors

As we have shown, it is reasonable to presume that β2-adrenoceptors influence the con-duction and refractoriness significantly in several cardiac structures. This may be particularly relevant in situations causing more pronounced stress**.**

However, the present results concern healthy young individuals. It should be noted that β-adrenoceptor density is influenced by age, cardiovascular disease and concomitant treatment with betablockers. Therefore, theoretically the importance of β-receptor inhibition differs during these circumstances compared with that in our healthy volunteers.

There is a decline in β-adrenoceptor density with age. This decline seems to affect β1-adrenoceptors in the ventricles, while β2-adrenoceptor density is unaltered and the density of both receptor subtypes in the atria is unaltered [71,78]. Treatment of arrhythmias with a stress-mediated component may not be as effectively achieved with a selective β1 antagonist as compared to a non-selective one and this may be particularly relevant in the elderly patient.

In addition, in the failing heart, there is a selective decrease in β1-adrenoceptor that leads to a shift in the proportions of β1 and β2 adrenoceptor density. This decrease is less pronounced in atrial tissues compared with ventricular. Patients suffering from biventricular failure show a down-regulation of β-adrenoceptors in both ventricles. In patients suffering from pulmonary hypertension with right ventricular failure the down-regulation is only in the right ventricle [79].

Chronic treatment with β1 receptor inhibiting agents results in a selective increase of cardiac β1-adrenoceptors. Treatment with selective β1 blockers seems to sensitize cardiac β2-adrenoceptors since the effect of β2 agonists are markedly enhanced [80]. This sensitizing effect has been observed both in patients with coronary artery disease and in healthy subjects. The mechanism is not understood.

The importance of β2 receptor inhibition in patients with chronic heart failure is not clear. In a meta-analysis of secondary and primary prevention trials of patients with chronic heart failure it seems that selective agents were inferior to non-selective agents in reducing overall mortality [110]. However, several of these studies evaluated the effect of carvedilol, which beside β1 and a β2 inhibiting effect also has a α1 inhibiting effect, which may have influenced the outcome. In addition, it is not clear to what degree other characteristics of β–blockers - such as lipophilia, intrinsic activity, and membrane stabilizing properties - are important.

In summary, the role played by 2-adrenoceptors in conductive and myocardial tissues during mental stress may be altered by age, heart failure, and treatment with selective betablockers.

Effects of vagal withdrawal

Comparing the effects caused by mental stress after treatment with propranolol and the effects of mental stress after autonomic inhibition with propranolol combined with atropine, we find that CWT does result in clear shortenings of sinus nodal parameters, AV nodal conduction time, and borderline significant reduction of ventricular refractoriness. However, there is a general shortening of all nodal parameters and ventricular myocardial parameters during CWT after pre-treatment with propranolol which we do not observe during CWT after unselective ATI.

This difference in reaction pattern is most obvious when plotting the effects for comparison as done in the diagrams seen in figures 12 A-C. Also for ventricular parameters the effects show similar patterns. The reason why a level of significance is not reached for e.g. ventricular refractoriness is with all pro-bability a matter of statistical power. Therefore, I consider it justified to conclude that a vagal withdrawal is of importance not only in nodal tissues but also in ventricular tissues.

The parasympathetic influence on nodal tissues is well established. The parasympathetic influence on ventricular tissues, though, is not as fully elucidated. It is unclear to what extent there is a direct vagal effect or to what extent the parasympathetic activation only counteracts the sympathetic effect.

Our results support a direct parasympathetic effect. However, an increase in ventricular refractoriness in response to vagal activation has been observed in human but not in isolated ventricular tissue. As was pointed out in the introductory background, this discrepancy may be explained by incomplete inhibition of adrenergic activity. This should certainly be taken into account judging our results

Reproducibility

The reproducibility of electrophysiologic measurements on two different study occasions has been evaluated in several studies. No significant difference in the mean value of CSNRT was observed when measured in patients without evidence of sinus node disease at two different occasions at least two days apart [188]. Bergfeldt [189] performed electrophysiological studies in healthy volunteers at two different occasions with a mean interval of 25 days and found that the mean sinus node recovery times varied considerably. However, they did find a high reproducibility for the mean sinus cycle length, ventricular depolarization, ventricular repolarization, and atrial, AV nodal and infranodal conduction times. Reproducibility was slightly lower for AV nodal refractoriness. Lahay [190] studying patients with permanent pacemakers found that the measurements of sinus and AV nodal functions were reproducible over two weeks.

Repeated measurements under various autonomic conditions were performed in all studies. In study I, II, and IV, repeated baseline measurements were performed in the same study. In study III and IV, repeated baseline measurements were also performed on two different study occasions 4-6 weeks apart. Furthermore, in study IV repeated measurements during CWT were performed on two different occasions.

When the two repeated baseline recordings at the same investigation were compared they showed no differences in any study, demonstrating that the electrophysiological state had restabilized 60 min after a period of CWT.

When the results were compared between the two baseline and the two CWT readings on the two different investigations 4-6 weeks apart, no differences were found. The conclusion is that the reproducibility of this test in this setting is good.

Mass significances

When performing repeated analysis on a large number of parameters, some caution is advisable in interpreting the results. Some p-values reaching our chosen level of significance ($p < 0,05$) may be a matter of chance and thus not necessarily relevant. Studying the effects on the cardiac electrophysiologic system in the repeated measures design we have used in all studies, a large number of different parameters have been measured.

In order to reduce the number of analyses performed, analysis of variance (ANOVA) repeated measures was applied and where relevant (Paper IV) ANOVA repeated measures with two within factors was used to compare the *differences* in effects between different treatments

Also, I think that there are some conditions, which reduce the problem of mass significances in the present studies.

The expected effect of adrenergic stimulation and inhibition on the different cardiac tissues is known [182]. Adrenergic stimulation decreases refractoriness and increases conduction velocities in all tissues, as do vagal withdrawal with the known exception of atrial refractoriness. Inhibition of adrenoceptors and of muscarinic receptors has an opposite effect. Our results are in accordance with these expected results.

Furthermore, scrutinizing the results of infusion of salbutamol or from mental stress after selective autonomic inhibition with atenolol and atropine, to take two examples, one finds that most parameters change in the same direction, although not every change reaches statistical significance.

I think it is justified to take these parallel effects of autonomic modulation in our studies into account when considering the possible importance of mass significance. Bearing this in mind, I think that the effects on different parameters that we have observed in fact constitute actual effects.

CONCLUSIONS

I. Mental stress has pronounced electrophysiologic effects, most markedly in the sinus and AV nodes and to a lesser degree in the ventricle.

II. Circulating epinephrine plays only a minor direct role, if any, as a mediator of mental stress effects on the heart, which thus is mainly neurogenically mediated

III. During mental stress women exhibit a more pronounced effect on the AV node and on the sinus node, and men react with a more pronounced effect on ventricular EP properties.

IV. After ATI, women have higher heart rate and longer QT and JT intervals, shorter AV nodal effective refractory period, faster AH conduction and longer effective refractory periods in the right ventricle.

V. β2-stimulation with salbutamol results in significant effects on cardiac electrophysiology, most pronounced in sinus and AV nodal nodal tissues and less pronounced on the atrial and ventricular refractoriness and on the ventricular repolarization

VI. A discordant effect by salbutamol on ventricular conduction, which slowed, and refractoriness of the ventricular myocardium, which shortened, was seen. QT dispersion increased.

VII. Inhibition with atenolol does not eliminate the effects of mental stress on the ventricular myocardium, effects that at least partly appears mediated through stimulation of β2 adrenoceptors.

VIII. Vagal withdrawal is part of the reaction to mental stress also in the ventricular myocardium

ACKNOWLEDGEMENTS

In particular I wish to express my sincere gratitude to:

Hans Vallin. Tutor, mentor, colleague, and friend. Who introduced me to the art of electrophysiology and the joy of analysing 100s of metres of intracardiac ECGs from the old Siemen's recorder and to calculate and graphically illustrate conduction curves for every single patient. They don't learn to appreciate this anymore, do they? I have immensely enjoyed this journey and, as you probably have suspected more than once, I have had no hurry in ending our collaboration and therefore, unconsciously of course, postponed this moment of mental stress (perhaps I should wear a Holter recorder and be the first subject in a forthcoming study?). Hans, thank you for all these years. Thank you for being generous with your time, for sharing your knowledge, for all fruitful discussions, and for being available at all times. And finally, which one of all the projects we have discussed lately would you like to start with now?

Göran Kennebäck. Friend and oldest colleague, who is doing an excellent although sometimes ungrateful job as the Head of the Arrhytmia Unit. You never give up in spite of all budgets cuts, and we are still growing. We have shared many memorable moments through the years learning/teaching electrophysiology, expanding and improving the electrophysiologic laboratories. And I am looking forward to many more (both memorable moments and laboratories). Thank you for your support and also for critically reading paper III.

Mats-Jensen Urstad. Room mate with a constant enthusiasm for scientific work (and ablations with a French touch). We have planned a lot of studies together (and even realized several of them), particularly after a bottle of wine (not in our office, please note!). It is a pleasure to work with you. Thank you also for constructive criticism of paper IV.

Present and past Heads of the Department of Cardiology Cecilia Linde, Eva Stråat, and Inger Hagerman for being very understanding for my somewhat unusual working conditions, for being supportive of electrophysiology and for maintaining an inspiring research environment.

My co-authors, Ulla Freyschuss and Anders Juhlin-Dannfelt, who introduced me to the methodology of Stroop's colour word conflict test, which in the beginning was even more stressful for all involved since the TV-monitor didn't always work.

Christer Sylvén, who was not directly involved in these studies, but who always is very helpful and generous with advice.

All other colleagues at the cath lab: Hamid Bastani, Frieder Braunschweig, Nikola Drca, Bita Sadigh, Jonas Schwieler, Fariborz Tabrizi, and Jari Tapanainen. Thank you all for generously letting me take time off to conclude this thesis.

All other associates at the cath lab and in particular

Christer Wredlert, not only for assisting but also for the versatile talent of being able to fix things and, of course, for being a soul mate in regard to true 60s music (and for volunteering to undergo several catheterizations);

Mats Andersson for his meticulous excellence in ECG recording and analysing;

Monica Borgren, Gittan Jonas, Ellen Bauge, and Anna Grahn for superb assistance (and not only these studies) and for including most of the healthy volunteers. I miss you (not you Anna, we still work together).

Other colleagues and associates who voluntarily underwent an electrophysiologic catheterization: Björn Eriksson, Karl-Erik Karlberg, Pia Olsson, and Helen Wimmerdahl.

Lennart Bergfeldt. Thank you for enjoyable collaboration during many years.

All other colleagues at the Department of Cardiology throughout the years. It is and has been a pleasure and privilege to work with you.

Anna Maria Bernstein for taking care of all practical matters.

Thank you Peter Tchaikovsky and Gustav Mahler for keeping me company when I was writing, especially during some recent long nights, while my computer was driving me nuts.

Last but definitely no least, thank you my beloved wife and daughters, Mona, Therese and Jessica, for your support and for being who you are.

This work was supported by grants from
Swedish Heart Lung Foundation

Financial support was also provided through
The Regional Agreement on Medical Training and Clinical Research (ALF)
between Stockholm County Council and the Karolinska Institute.

REFERENCES

1. Luke. Acts of the Apostles. *In: The Holy Bible, editor unknown.* Approx 80 AD.

2. Sextus Aurelius Victor (attr). *Epitome de Caesaribus.* Approx 380 AD.

3. *The New Encyclopaedia Britannica.* London: Encyclopaedia Britannica, Inc., vol. 6, 15th ed; 1998: 323.

4. Engel GL: Sudden and rapid death during psychological stress. Folklore or folk wisdom? *Ann Intern Med.* 1971;74:771-782.

5. Williams J, Edwards G: John Hunter's last pupil. *Ann Roy Coll Surg Eng.* 1968; 42:69-70.

6. Bove AA: The cardiovascular response to stress. *Psychosomatics*. 1977;10:13-17.

7. Eliot RS, Buell JC: Role of emotions and stress in the genesis of sudden death. *J Am Coll Cardiol*. 1985;6:95-98B.

8. Taggart P, Gibbons D, Somerville W: Some effects of motor-car driving on the normal and abnormal heart. *Br Med J*. 1969;4:130-134.

9. Ahern DK, Gorkin L, Anderson JL, Tierney C, Hallstrom A, Ewart C, Capone RJ, Schron E, Kornfeld D, Herd JA, Richardson DW, Follick MJ: Biobehavioral variables and mortality or cardiac arrest in the cardiac arrhythmia pilot study. *Am J Cardiol*. 1990; 66:59-62.

10. Brackett CD, Powell LH: Psychosocial and physiological predictors of sudden cardiac death after healing of acute myocardial infarction. *Am J Cardiol.* 1988; 61:979-983.

11. Meisel SR, Kutz I, Dayan KI, Pauzner H, Chetboun I, Arbel Y, David D: Effect of Iraqi missile war on incidence of acute myocardial infarction and sudden death in Israeli civilians. *Lancet*. 1991:338:660-661.

12. Follik MJ, Ahern DK, Gorkin L, Niaura RS, Herd JA, Ewart C, Schron EB, Kornfeld DS, Capone RJ: Relation of psychosocial and stress reactivity variables to ventricular arrhythmias in the Cardiac Arrhythmia Pilot Study. *Am J Cardiol.* 1990;66:63-67.

13. Lown B, Temte JV, Reich P, Gaughan C, Regestein Q, Hai H: Basis for recurring ventricular fibrillation in the absence of coronary heart disease and its management. *N Engl J Med*. 1976;294:623-629.

14. Stamler JS, Goldman ME, Gomes J, Matza D, Horowitz SF: The effect of stress and fatigue on cardiac rhythm in medical interns. *J Electrocardiol*. 1992; 25:333-338.

15. Cannon WB. *Bodily Changes in Pain, Hunger, Fear and Rage: An Account of Recent Research Into the Function of Emotional Excitement*, 2nd ed. New York:Appleton-Century-Crofts; 1929.

16. Cannon WB. "Voodoo" Death. *American Antropologist*. 1942; 44:169-181.

17. Sternberg EM. Walter B. Cannon and "Voodoo Death": a perspective from 60 years on. *Am J Public Health.* 2002; 92:1564-66.

18. Gradman AH, Bell PA, DeBusk RF. Sudden death during ambulatory monitoring. Clinical and electrocardiographic correlations. Report of a case. *Circulation.* 1977;55:210-211.

19. Trichopoulos D, Katsouyanni K, Zavit-sanos X, Tzonou A, Dalla-Vorgia P. Psychological stress and fatal heart attack: the Athens (1981) earthquake natural experiment. *Lancet.* 1983; 1(8322):441-444.

20. Leor J, Poole WK, Kloner RA. Sudden cardiac death triggered by an earthquake. *N Engl J Med.* 1996;334:413-419.

21. Seleye H. A syndrome produced by diverse nocuous agents. *Nature.* 1938; 138:32.

22. Selye H. The general adaptation syndrome and the diseases of adaptation. *J Clin Endocrinol.* 1946;6:117-130.

23. Selye H. *Stress. The physiology and pathology of exposure to stress*. Acta. Montreal, Inc. 1950.

24. Selye H. Stress and disease. *Science.* 1955; 122:625-631.

25. Selye H. *Stress in Health and Disease*. Boston: Butterworths. 1976.

26. Holmes SD, Krantz DS, Rogers H, Gottdiener J, Contrada RJ. Mental stress and coronary artery disease: a multi-disciplinary guide. *Prog Cardiovasc Dis.* 2006;49:106-122.

27. Lundberg U, Frankenhaeuser M. Pituitary-adrenal and sympathetic-adrenal correlates of distress and effort. *J Psychosom Res*. 1980;24: 125-130.

28. Hansson A, Madsen-Härdig B, Olsson SB. Arrhythmia-provoking factors and symptoms at the onset of paroxysmal atrial fibrillation: a study based on interviews with 100 patients seeking hospital assistance. *BMC Cardiovasc Disord.* 2004;4:13.

29. Shedd OL, Sears SF Jr, Harvill JL, Arshad A, Conti JB, Steinberg JS, Curtis AB. The World Trade Center attack: increased frequency of defibrillator shocks for ventricular arrhythmias in patients living remotely from New York City. *J Am Coll Cardiol.* 2004;44:1265-67.

30. Steinberg JS, Arshad A, Kowalski M, Kukar A, Suma V, Vloka M, Ehlert F, Herweg B, Donnelly J, Philip J, Reed G, Rozanski A. Increased incidence of life-threatening ventricular arrhythmias in implantable defibrillator patients after the World Trade Center attack. *J Am Coll Cardiol.* 2004; 44:1261-1264.

31. Wilbert-Lampen U, Leistner D, Greven S, Pohl T, Sper S, Völker C, Güthlin D, Plasse A, Knez A, Küchenhoff H, Stein-beck G. Cardiovascular events during World Cup soccer. *N Engl J Med.* 2008;-358:475-483.

32. Lampert R, Joska T, Burg MM, Batsford WP, McPherson CA, Jain D. Emotional and physical precipitants of ventricula arrhythmia**.** *Circulation*. 2002;106:1800-1805.

33. Stopper M, Joska T, Burg MM, Batsford WP, McPherson CA, Jain D, Lampert R. Electrophysiologic characteristics of anger-triggered arrhythmias. *Heart Rhythm.* 2007;4: 268-273.

34. Lampert R, Jain D, Burg MM, Batsford WP, McPherson CA. Destabilizing effects of mental stress on ventricular arrhythmias in patients with implantable cardioverterde-fibrillators. *Circulation*. 2000;101:158-164.

35. Lane RD, Laukes C, Marcus FI, Chesney MA, Sechrest L, Gear K, Fort CL, Priori SG, Schwartz PJ, Steptoe A. Psychological stress preceding idiopathic ventricular fibrillation. *Psychosom Med*. 2005; 67:359-365**.**

36. Hjemdahl P, Freyschuss U, Juhlin-Dannfelt A, Linde B. Differentiated sympathetic activation during mental stress evoked by the Stroop test. *Acta Physiol Scand;Suppl*. 1984;527:25-29.

37. Freyschuss U, Hjemdahl P, Juhlin-Dannfelt A, Linde B. Cardiovascular and sympathoadrenal responses to mental stress: influence of ß-blockade. *Am J Physiol*. 1988;255:H1443-1451.

38. Freyschuss U, Fagius J, Wallin BG, Bohlin G, Perski A, Hjemdahl P. Cardiovascular and sympathoadrenal responses to mental stress: a study of sensory intake and rejection reactions. *Acta Physiol Scand*. 1990;139:173-183.

39. Toivonen L, Helenius K, Viitasalo M. Electrocardiographic repolarization during stress from awakening on alarm call. *J Am Coll Cardiol.* 1997;30:774-779.

40. Kop WJ, Krantz DS, Nearing BD, Gottdiener JS, Quigley JF, O'Callahan M, DelNegro AA, Friehling TD, Karasik P, Suchday S, Levine J, Verrier RL. Effects of acute mental stress and exercise on T-wave alternans in patients with implantable cardioverter defibrillators and controls. *Circulation.* 2004;109:1864-1869.

41. Pagani M, Mazzuero G, Ferrari A, Liberati D, Cerutti S, Vaitl D, Tavazzi L, Malliani A. Sympathovagal interaction during mental stress. A study using spectral analysis of heart rate variability in healthy control subjects and patients with a prior myocardial infarction. *Circulation*. 1991;83(Suppl):II43-51.

42. Folino AF, Buja G, Turrini P, Oselladore L, Nava A. The effects of sympathetic stimulation induced by mental stress on signal-averaged electrocardiogram. *Int J Cardiol.* 1995;48:279-285.

43. Theorell T, Tsutsumi A, Hallquist J, Reuterwall C, Hogstedt C, Fredlund P, Emlund N, Johnson JV. Decision latitude, job strain, and myocardial infarction: a study of working men in Stockholm. The SHEEP Study Group. Stockholm Heart epidemiology Program. *Am J Public Health.* 1998;88:382-388.

44. Bosma H, Peter R, Siegrist J, Marmot M. Two alternative job stress models and the risk of coronary heart disease. *Am J Public Health*. 1998;88:68-74.

45. Wang HX, Leineweber C, Kirkeeide R, Svane B, Schenck-Gustafsson K, Theorell T, Orth-Gomér K. Psychosocial stress and atherosclerosis: family and work stress accelerate progression of coronary disease in women. The Stockholm Female Coronary Angiography Study. *J Intern Med.* 2007;261: 245-254.

46. Orth-Gomér K, Wamala SP, Horsten M, Schenck-Gustafsson K, Schneiderman N, Mittleman MA. Marital stress worsens prognosis in women with coronary heart disease: The Stockholm Female Coronary Risk Study. *JAMA*. 2000;284:3008-3014.

47. Kop WJ, Berman DS, Gransar H, Wong ND, Miranda-Peats R, White MD, Shin M, Bruce M, Krantz DS, Rozanski A. Social network and coronary artery calcification in asymptomatic individuals. *Psychosom Med.* 2005; 67:343-352.

48. Lacy CR, Contrada RJ, Robbins ML, Tannenbaum AK, Moreyra AE, Chelton S, Kostis JB. Coronary vasoconstriction induced by mental stress (simulated public speaking). *Am J Cardiol.* 1995;75:503-505.

49. Burg MM, Jain D, Soufer R, Kerns RD, Zaret BL. Role of behavioral and psychological factors in mental stress-induced silent left ventricular dysfunction in coronary artery disease. *J Am Coll Cardiol.* 1993;22:440-448.

50. Jain D, Burg M, Soufer R, Zaret BL. Prognostic implications of mental stress-induced silent left ventricular dysfunction in patients with stable angina pectoris. *Am J Cardiol.* 1995;76:31-35.

51. Donohue D, Movahed MR. Clinical characteristics, demographics and prognosis of transient left ventricular apical ballooning syndrome. *Heart Fail Rev.* 2005;10:311-316.

52. McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol*. 2008;583: 174-185.

53. McEwen BS, Stellar E. Stress and the individual. Mechanisms leading to disease. *Arch Intern Med.* 1993;153:2093-2101.

54. McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med.* 1998;338: 171-179.

55. Thayer JF, Lane RD. A model of neurovisceral integration in emotion regulation and dysregulation. *J Affect Disord.* 2000;61:201-216.

56. Coumel P, Leenhardt A. Mental activity, adrenergic modulation, and cardiac arrhythmias in patients with heart disease. *Circulation*. 1991;83(4 Suppl):II58-70.

57. Friedman M, Byers SO, Diamant J, Rosenman RH. Plasma catecholamine response of coronary-prone subjects (type A) to a specific challenge. *Metabolism.* 1975;24:205-210.

58. Lovallo WR, Pincomb GA, Wilson M. Predicting response to a reaction time task: heart rate reactivity compared with type A behavior. *Psychophysiology.* 1986; 23:648-656.

59. Bohnen N, Nicolson N, Sulon J, Jolles J. Coping style, trait anxiety and cortisol reactivity during mental stress. *J Psychosom Res.* 1991;35:141-147.

60. Arnetz BB, Brenner SO, Levi L, Hjelm R, Petterson IL, Wasserman J, Petrini B, Eneroth P, Kallner A, Kvetnansky R, et al. Neuroendocrine and immunologic effects of

unemployment and job insecurity. *Psychother Psychosom.* 1991;55:76-80.

61. Frankenhaeuser M, von Wright MR, Collins A, von Wright J, Sedvall G, Swahn CG. Sex differences in psychoneuroendocrine reactions to examination stress. *Psychosom Med.* 1978; 40:334-343.

62. Collins A, Frankenhaeuser M. Stress responses in male and female engineering students. *J Human Stress.* 1978;4:43-48.

63. Davidson L, Vandongen R, Rouse IL, Beilin LJ, Tunney A. Sex-related differences in resting and stimulated plasma noradrenaline and adrenaline. *Clin Sci (Lond).* 1984;67:347-352.

64. Biondi M, Picardi A. Psychological stress and neuroendocrine function in humans: the last two decades of research. *Psychother Psychosom.* 1999;68:114-150.

65. Rozanski A, Bairey CN, Krantz SK, Friedman J, Resser KJ, Morell M, Hilton-Chalfen S, Hestrin L, Bietendorf J, Berman DS. Mental stress and the induction of silent myocardial ischemia in patients with coronary artery disease. *N Engl J Med.* 1988;318:1005-1012.

66. Kawashima T. The autonomic nervous system of the human heart with special reference to its origin, course, and peripheral distribution. *Anat Embryol (Berl).* 2005;209:425-438.

67. Hutchins GD, Miller MA, Zipes DP. Neurocardiac imaging. *In: Zipes DP, Jalife J, eds. Cardiac Electrophysiology. From Cell to Bedside.* 4th ed. Philadelphia, PA:WB Saunders;2004: 848-849.

68. Maehle AH, Prüll CR, Halliwell RF. The emergence of the drug receptor theory. *Nat Rev Drug Discov.* 2002;1:637-641.

69. Ahlquist RP. A study of the adrenotropic receptors. *Am J Physiol.* 1948;153:586-600.

70. Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG Jr. Differentiation of receptor systems activated by sympathomimetic amines. *Nature.* 1967;214: 597-598.

71. Brodde OE, Michel MC. Adrenergic and muscarinic receptors in the human heart. *Pharmacol Rev*. 1999;51:651-690.

72. Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S, Stinson EB. β1- and β2-adrenergic receptor subpopulations in nonfailing and failing human ventricular myocardium. C*irc Res.* 1986;59:-297-309.

73. Brodde OE, Karad K, Zerkowski H-R, Rohm N, Reidemeister. Coexistence of β1- and β2-adrenoceptors in human right atrium. *Circ Res.* 1983;53:752-758.

74. Brodde OE, Zerkowski HR, Borst HG. Drug- and disease-induced changes of human cardiac β1- and β2-adrenoceptors. *Euro Heart J*. 1989;10 (Suppl B):38-44.

75. Brodde OE, Bruck H, Leineweber K, Syfarth T. Presence, distribution and physiological function of adrenergic and muscarinic receptor subtypes in the human heart. *Basic Res Cardiol*. 2001; 96:528-538.

76. Summers RJ, Molnaar P, Russel F, Elnatan J, Jones CR, Buxton BF, Chang V, Hambley J. Coexistence and localisation of β1- and β2-adrenoceptors in the human heart. *Euro Heart J.* 1989;10 (Suppl B):11-21.

77. Rodefeld MD, Beau SL, Schuessler RB, Boineau JP, Saffitz JE. β-adrenergic and muscarinic cholinergic receptor densities in the human sinoatrial node. *J Cardiovasc Electrophysiol*. 1996;7:1039-1049.

78. Schutzer WE, Mader SL. Age-related changes in vascular adrenergic signalling. clinical and mechanistic implications. *Ageing Res Rev.* 2003;2:169-190.

79. Brodde OE, Bruck H, Leineweber K, Seyfarth T. Presence, distribution and physiological function of adrenergic and muscarinic receptor subtypes in the human heart. *Basic Res Cardiol.* 2001; 96:528-538.

80. Hall JA, Petch MC, Brown MJ. In vivo demonstration of cardiac beta 2-adrenoreceptor sensitization by beta 1-antagonist treatment. *Circ Res*. 1991;69:959-964.

81. Hall JA, Ferro A, Dickerson JE, Brown MJ. Beta adrenoreceptor subtype cross regulation in the human heart. Br Heart J. 1993;69:332-337.

82. Gauthier C, Tavernier G, Charpentier F, Langin D, Le Marec H. Functional beta3-adrenoceptor in the human heart. *J Clin Invest.* 1996;98:556-562.

83. Moniotte S, Kobzik L, Feron O, Trochu JN, Gauthier C, Balligand JL Upregulation of beta(3)-adrenoceptors and altered contractile response to inotropic amines in human failing myocardium. *Circulation.* 2001;103:1649-1655.

84. Wheeldon NM, McDevitt DG, Lipworth BJ. Cardiac effects of the beta-3 adrenoceptor agonist BRL35135 in man. *Br J Pharmacol.* 1994;37:363-369.

85. Zhou S, Tan AY, Paz O, Ogawa M, Chou CC, Hayashi H, Nihei M, Fishbein MC, Chen LS, Lin SF, Chen PS. Antiarrhythmic effects of beta3-adrenergic receptor stimulation in a canine model of ventricular tachycardia. *Heart Rhythm*. 2008;5:289-297.

86. Steinberg SF, Robinson RB, Rosen MR. Molecular and cellular bases of β-adrenergic and α-adrenergic modulation of cardiac rhythm. *In: Zipes DP, Jalife J, eds. Cardiac Electrophysiology. From Cell to Bedside.* 4th ed. Philadelphia, PA:WB Saunders;2004: 293-294.

87. Chevalier P, Ruffy F, Danilo P Jr, Rosen MR. Interaction between alpha-1 adrenergic and vagal effects on cardiac rate and repolarization. *J Pharmacol Exp Ther.* 1998; 284:832-837.

88. Brodde OE, Konschak U, Becker K, Rüter F, Poller U, Jakubetz J, Radke J, Zerkowski HR. Cardiac muscarinic receptors decrease with age. In vitro and in vivo studies. *J Clin Invest.* 1998;101:471-478.

89. Warner MR, Zipes DP. Vagal control of myocardial refractoriness. *In:Levy MN, Schwartz PJ, eds. Vagal Control of the Heart.* Armonk, NY:Futura Publishing Co;1994:261-262.

90. Zipes DP, Mihalick MJ, Robbins GT. Effects of selective vagal and stellate ganglion stimulation of atrial refractoriness. *Cardiovasc Res.* 1974;8:647-655.

91. Morady F, Kou WH, Nelson SD, de Buitleir M, Schmaltz S, Kadish AH, Toivonen LK, Kushner JA. Accentuated antagonism between beta-adrenergic and vagal effects on ventricular refractoriness in humans. *Circulation.* 1988;77:289-297.

92. De Ferrari GM, Vanoli E, Schwartz PJ. Cardiac vagal activity, myocardial ischemia, and sudden death. *In: Zipes DP, Jalife J, eds. Cardiac Electrophysiology. From Cell to Bedside.* 3rd ed. Philadelphia, PA:WB Saunders;1999:422-433.

93. Morady F, Kadish AH, Schmaltz S, Rosenheck S, Summitt J. Effects of resting vagal tone on accessory atrioventricular connections. *Circulation*. 1990;81:86-90.

94. De Ferrari GM, Vanoli E, Curcuruto P, Tommasini G, Schwartz PJ. Prevention of life-threatening arrhythmias by pharmacologic stimulation of the muscarinic receptors with oxotremorine. *Am Heart J.* 1992;124:883-890.

95. De Ferrari GM, Salvati P, Grossoni M, Ukmar G, Vaga L, Patrono C, Schwartz PJ. Pharmacologic modulation of the autonomic nervous system in the prevention of sudden cardiac death. A study with propranolol, methacholine and oxotremorine in conscious dogs with a healed myocardial infarction. *J Am Coll Cardiol.* 1993;22:283-290.

96. Das G. Therapeutic review. Cardiac effects of atropine in man: an update. *Int J Clin Pharmacol Ther Toxicol.* 1989;27: 473-477.

97. De Ferrari GM, Mantica M, Vanoli E, Hull SS Jr, Schwartz PJ. Scopolamine increases vagal tone and vagal reflexes in patients after myocardial infarction. *J Am Coll Cardiol.* 1993;22:1327-1334.

98. Laitinen T, Niskanen L, Geelen G, Länsimies E, Hartikainen J. Age dependency of cardiovascular autonomic responses to head-up tilt in healthy subjects. *J Appl Physiol*. 2004;96: 2333-2340.

99. de Marneffe M, Jacobs P, Haardt R, Englert M. Variations of normal sinus node function in relation to age: role of autonomic influence. *Eur Heart J*. 1986;7:662-672.

100. Chow LT, Chow SS, Anderson RH, Gosling JA. Autonomic innervation of the human cardiac conduction system: changes from infancy to senility - an immunohistochemical and histochemical analysis. *Anat Rec.* 2001; 264:169-182.

101. Levi MN. Time dependency of the autonomic interactions that regulate heart rate and rhythm. *In: Zipes DP, Jalife J, eds. Cardiac Electrophysiology. From Cell to Bedside.* 3d ed. Philadelphia, PA:WB Saunders;1999:454-459.

102. Rosenblueth A, Simeone FA. The inter-relations of vagal and accelerator effects on the cardiac rate. *Am J Physiol.* 1934; 110:42-55.

103. Levy MN. Sympathetic-parasympathetic interactions in the heart. *Circ Res.* 1971;29:437-445.

104. Habermeier-Muth A, Muscholl E. Short- and long-latency muscarinic inhibition of noradrenaline release from rabbit atria induced by vagal stimulation. *J Physiol.* 1988; 401:277-293.

105. Wellstein A, Pitschner HF. Complex dose-response curves of atropine in man explained by different functions of M1- and M2-cholinoceptors. *Naunyn Schmiedebergs Arch Pharmacol.* 1988;338:19-27.

106. Buch AN, Coote JH, Townend JN. Mortality, cardiac vagal control and physical training-what's the link? *Exp Physiol*. 2002;87:423-435.

107. Sandercock GR, Bromley PD, Brodie DA. Effects of exercise on heart rate variability: inferences from meta-analysis. *Med Sci Sports Exerc.* 2005;37:433-439.

108. Genovesi S, Zaccaria D, Rossi E, Valsecchi MG, Stella A, Stramba-Badiale M. Effects of exercise training on heart rate and QT interval in healthy young individuals: are there gender differences? *Europace.* 2007;9:55-60.

109. Soriano JB, Hoes AW, Meems L, Grobbee DE: Increased survival with beta-blockers: importance of ancillary properties. *Progr Cardiovasc Dis* 1997; 39:445-456.

110. Lechat P, Packer M, Chalon S, Cucherat M, Arab T, Boissel JP. Clinical effects of beta-adrenergic blockade in chronic heart failure. *Circulation*. 1998;98:1184-1191.

111. Mifune J, Kuramoto K, Ueda K, Matsushita S, Kuwajima I, Sakai M, Iwasaki T, Moroki N, Murakami M. Hemodynamic effects of salbutamol, an oral long-acting beta-stimulant, in patients with congestive heart failure. *Am Heart J. 1982*;104:1011-1015.

112. Bourdillon PDV, Dawson JR, Foale RA, Timmis AD, Poole-Wilson PA, Sutton GC. Salbutamol in treatment of heart failure. *Br Heart J.* 1980;43:206-210.

113. Sharma B, Goodwin JF: Beneficial effects of salbutamol on cardiac function in severe congestive cardiomyopathy. *Circulation.* 1978;58:449-459.

114. Strauss MH, Reeves RA, Smith DL, Leene FHH. The role of cardiac beta-1 receptors in the hemodynamic response to beta-2 agonist. *Clin Pharmacol Ther.* 1986;40:108-115.

115. Levine MAH, Leenen FHH. Role of β1-receptors and vagal tone in cardiac inotropic and chronotropic responses to a β2-agonist in humans. *Circulation.* 1989;79:107-115.

116. Spitzer WO, Suissa S, Ernst P, Horwitz RI, Habbick B, Cockcroft D, Boivin JF, McNutt M, Buist AS, Rebuck AS. The use of beta-agonists and the risk of death and near death from asthma. *N Engl J Med.* 1992;32:501-506.

117. Tandon MK: Cardiopulmonary effects of fenoterol and salbutamol aerosols. *Chest*. 1980;77:429-443.

118. Williams AJ, Weiner C, Reiff D, Swenson ER, Fuller RW, Hughes JM. Comparison of the effect of inhaled selective and non-selective adrenergic agonists on cardiorespiratory parameters in chronic stable asthma. *Pulm Pharmacol.* 1994;7:235-241.

119. Salpeter SR. Cardiovascular safety of beta(2)-adrenoceptor agonist use in patients with obstructive airway disease: a systematic review. *Drugs Aging.* 2004;2: 405-414.

120. Bazett H. An analysis of the time relations of electrocardiograms. *Heart*. 1920;7:353-370.

121. Jose AD, Collison D. The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc Res.* 1970;4:160-167.

122. Sobotka PA, Mayer JH, Bauernfeind RA, Kanakis C Jr, Rosen KM. Arrhythmias documented by 24-hour monitoring in young women without apparent heart disease. *Am Heart J*. 1981;101:753-759.

123. Merri M, Benhorin J, Alberti M, Locati E, Moss AJ. Electrocardiographic quantitation of ventricular repolarization. *Circulation.* 1989; 80:1301-1308.

124. Burke JH, Goldberger JJ, Ehlert FA, Kruse JT, Parker MA, Kadish AH. Gender differences in heart rate before and after autonomic blockade: evidence against an intrinsic gender effect. *Am J Med.* 1996; 100:537-543.

125. Rautaharju PM, Zhou SH, Wong S, Calhoun HP, Berenson GS, Prineas R, Davignon A. Sex differences in the evolution of the electrocardiographic QT interval with age. *Can J Cardiol.* 1992; 8:690-695.

126. Nakagawa M, Ooie T, Ou B, Ichinose M, Takahashi N, Hara M, Yonemochi H, Saikawa T. Gender differences in autonomic modulation of ventricular repolarization in humans. *J Cardiovasc Electrophysiol.* 2005; 16:278-284.

127. Taneja T, Windhagen Mahnert B, Passman R, Goldberger J, Kadish A: Effects of sex and age on electrocardiographic and cardiac electrophysiological properties in adults. *Pacing Clin Electrophysiol.* 2001;24:16-21.

128. Liuba I, Jönsson A, Säfström K, Walfridsson H. Gender-related differences in patients with atrioventricular nodal reentry tachycardia. *Am J Cardiol*. 2006; 97:384-388.

129. Liu S, Yuan S, Kongstad O, Olsson SB. Gender differences in the electrophysiological characteristics of atrioven-tricular conduction system and their clinical implications. *Scand Cardiovasc J.* 2001;35: 313-317.

130. Insulander P, Kennebäck G, Strååt E, Jensen-Urstad M, Vallin H. Differences in dual AV nodal properties between men and women. *Eur Heart J.* 1999;20(Suppl):568.

131. Insulander P, Jensen-Urstad M, Kennebäck G, Tabrizi F, Wredlert C, Vallin H. Gender differences in conduction dispersion in the right atrium analysed with non-contact mapping. *Europace.* 2005;7 Suppl 1:S127.

132. Myerburg RJ, Cox MM, Interian A Jr, Mitrani R, Girgis I, Dylewski J, Castellanos A. Cycling of inducibility of paroxysmal supra-ventricular tachycardia in women and its implications for timing of electrophysiologic procedures. *Am J Cardiol*. 1999;83:1049-1054.

133. Davidson L, Rouse IL, Vandongen R, Beilin LJ. Plasma noradrenaline and its relationship to plasma oestradiol in normal women during the menstrual cycle. *Clin Exp Pharmacol Physiol.* 1985;12:489-493.

134. Rosano GM, Leonardo F, Sarrel PM, Beale CM, De Luca F, Collins P. Cyclical variation in paroxysmal supra-ventricular tachycardia in women. *Lancet.* 1996;347: 786-788.

135. Villareal RP, Woodruff AL, Massumi A. Gender and cardiac arrhythmias. *Tex Heart Inst J.* 2001;28:265-275.

136. Lee RJ, Shinbane JS. Inappropriate sinus tachycardia. Diagnosis and treatment. *Cardiol Clin.* 1997;15:599-605.

137. Ellenbogen KA, Wood MA. Atrial tachy-cardia. *In: Zipes DP, Jalife J, eds. Cardiac Electrophysiology. From Cell to Bedside.* 4th ed. Philadelphia, PA:WB Saunders;2004: 504-505.

138. Rodriguez LM, de Chillou C, Schläpfer J, Metzger J, Baiyan X, van den Dool A, Smeets JL, Wellens HJ. Age at onset and gender of patients with different types of supra-ventricular tachycardias. *Am J Cardiol.* 1992; 70:1213-1215.

139. Insulander P, Bastani H, Braunschweig F, Jensen-Urstad M, Schwieler J, Tabrizi F, Kennebäck G. Ablation of supraventricular tachycardias – higher success rate in women than in men. *Scand Cardiovasc J*. 2008 April; 42 (Suppl 56): 21-22.

140. Porter MJ, Morton JB, Denman R, Lin AC, Tierney S, Santucci PA, Cai JJ, Madsen N, Wilber DJ. Influence of age and gender on the mechanism of supra-ventricular tachycardia. *Heart Rhythm*. 2004;1:393-396.

141. Anguera I, Brugada J, Roba M, Mont L, Aguinaga L, Geelen P, Brugada P. Outcomes after radiofrequency catheter ablation of atrial tachycardia. *Am J Cardiol*. 2001;87:886-890.

142. Hoffmann E, Reithmann C, Nimmermann P, Elser F, Dorwarth U, Remp T, Steinbeck G. Clinical experience with electroanatomic mapping of ectopic atrial tachycardia. *Pacing Clin Electrophysiol.* 2002;25:49-56.

143. Granada J, Uribe W, Chyou PH, Maassen K, Vierkant R, Smith PN, Hayes J, Eaker E, Vidaillet H. Incidence and predictors of atrial flutter in the general population. *J Am Coll Cardiol.* 2000;36:2242-2246.

144. Insulander P, Schwieler J, Bastani H, Braunschweig F, Kennebäck G, Tabrizi F, Bergfeldt L, Andersson M, Wredlert C, Jensen-Urstad M. Ablationsbehandling som förstahandsval vid supraventrikulära taky-kardier. Goda resultat och få komplikationer i studie av 2 207 konsekutiva patienter. *Läkartidningen.* 2008;105:3644-3647.

145. Moreira W, Timmermans C, Wellens HJ, Mizusawa Y, Perez D, Philippens S, Rodriguez LM. Long term outcome of cavotricuspid isthmus cryoablation for the treatment of common atrial flutter in 180 patients: A single center experience. *J Interv Card Electrophysiol.* 2008;21:235-240.

146. Maury P, Raczka F, Gaty D, Duparc A, Couderc P, Hollington L, Celse D, Delay M, Fauvel JM, Puel J, Davy JM. Radio-Frequency Ablation of Atrial Flutter: Long-Term Results and Predictive Value of Cavo-Tricuspid Isthmus Bidirectional Block as Determined by a Simplified Technique. *Cardiology.* 2007; 110: 17-28.

147. Ellenbogen KA, Wood MA. Atrial tachycardia. *In: Zipes DP, Jalife J, eds. Cardiac Electrophysiology. From Cell to Bedside.* 4th ed. Philadelphia, PA: WB Saunders;2004:508-509.

148. Benjamin EJ. Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. *JAMA.* 1994;271:840-844.

149. Pappone C, Rosanio S, Augello G, Gallus G, Vicedomini G, Mazzone P, Gulletta S, Gugliotta F, Pappone A, Santinelli V, Tortoriello V, Sala S, Zangrillo A, Crescenzi G, Benussi S, Alfieri O. Mortality, morbidity, and quality of life after circumferential pulmonary vein ablation for atrial fibrillation: outcomes from a controlled nonrandomized long-term study. *J Am Coll Cardiol.* 2003;42: 185-197.

150. Oral H, Scharf C, Chugh A, Hall B, Cheung P, Good E, Veerareddy S, Pelosi F Jr. Morady F. Catheter ablation for paroxysmal atrial fibrillation: segmental pulmonary vein ostial ablation versus left atrial ablation. *Circulation.* 2003;108: 2355-2360.

151. Forleo GB, Tondo C, De Luca L, Dello Russo A, Casella M, De Sanctis V, Clementi F, Fagundes RL, Leo R, Romeo F, Mantica M Gender-related differences in catheter ablation of atrial fibrillation. *Europace.* 2007;9:613-620.

152. Shah DC, Haïssaguerre M, Jaïs P, Hocini M, Yamane T, Deisenhofer I, Garrigue S, Clémenty J. Curative catheter ablation of paroxysmal atrial fibrillation in 200 patients: strategy for presentations ranging from sustained atrial fibrillation to no arrhythmias. *Pacing Clin Electrophysiol.* 2001;24: 1541-1558.

153. Jaïs P, Cauchemez B, Macle L, Daoud E, Khairy P, Subbiah R, Hocini M, Extramiana F, Sacher F, Bordachar P, Klein G, Weerasooriya R, Clémenty J, Haïssaguerre M.. Catheter ablation versus antiarrhythmic drugs for atrial fibrillation: the A4 study. *Circulation*. 2008;118:2498-505.

154. Deal BJ. Supraventricular tachycardia – mechanisms and natural history. *In: Current Concepts in Diagnosis and Management of Arrhythmias in Infants and Children*. Deal BJ, Wolff GS, Gelband H, eds. Armonk, NY: Futura Publishing Co;1998:117-143.

155. Liu S, Yuan S, Hertervig E, Kongstad O, Olsson SB. Gender and atrioventricular conduction properties of patients with symptomatic atrioventricular nodal reentrant tachycardia and Wolff-Parkinson-White syndrome. *J Electrocardiol.* 2001; 34:295-301.

156. Dagres N, Clague JR, Breithardt G, Borggrefe M. Significant gender-related differences in radiofrequency catheter ablation therapy. *J Am Coll Cardiol*. 2003; 42:1103-1107.

157. Hall JA, Petch MC, Brown MJ: Intra-coronary injections of salbutamol demonstrate the presence of functional β2-adrenoceptors in the human heart. *Circ Res*. 1989;65:546-553.

158. Lundberg A. Paroxysmal atrial tachycardia in infancy: long-term follow-up study of 49 subjects. *Pediatrics.* 1982; 70:638-642.

159. Perry JC, Garson A Jr. Supra-ventricular tachycardia due to Wolff-Parkinson-White syndrome in children: early disappearance and late recurrence. *J Am Coll Cardiol.* 1990;16: 1215-1220.

160. Sano S, Komori S, Amano T, Kohno I, Ishihara T, Sawanobori T, Ijiri H, Tamura K. Prevalence of ventricular preexcitation in Japanese school children. *Heart.* 1998; 79: 374-378.

161. Nakagawa M, Takahashi N, Nobe S, Ichinose M, Ooie T, Yufu F, Shige-matsu S, Hara M, Yonemochi H, Saikawa T. Gender differences in various types of idiopathic ventricular tachycardia. *J Cardiovasc Electrophysiol.* 2002;13:633-638.

162. Schatzkin A, Cupples LA, Heeren T, Morelock S, Kannel WB. Sudden death in the Framingham Heart Study. Differences in incidences and risk factors by sex and coronary disease status. *Am J Epidemiol.* 1984;120:888-899.

163. Viskin S, Belhassen B. Polymorphic ventricular tachyarrhythmias in the absence of organic heart disease: classification, differential diagnosis, and implications for therapy. *Prog Cardiovasc Dis.* 1998;4:17-34.

164. Lampert R, McPherson CA, Clancy JF, Caulin-Glaser TL, Rosenfeld LE, Batsford WP. Gender differences in ventricular arrhythmia recurrence in patients with coronary artery disease and implantable cardioverterdefibrillators. *J Am Coll Cardiol*. 2004;43: 2293-2299.

165. Kannel WB, Wilson PW, D'Agostino RB, Cobb J. Sudden coronary death in women. *Am Heart J.* 1998;136:205-212.

166. Albert CM, McGovern BA, Newell JB, Ruskin JN. Sex differences in cardiac arrest survivors. *Circulation.* 1996;93: 1170-1176.

167. Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, MacCluer J, Hall WJ, Weitkamp L, Vincent GM, Garson A Jr, et al. The long QT syndrome. Prospective longitudinal study of 328 families. *Circulation.* 1991;84: 1136-1144.

168. Locati EH, Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Lehmann MH, Towbin JA, Priori SG, Napolitano C, Robinson JL, Andrews M, Timothy K, Hall WJ. Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome: findings from the International LQTS Registry. *Circulation.* 1998;97: 2237-2244.

169. Yarnoz MJ, Curtis AB. More reasons why men and women are not the same (gender differences in electrophysiology and arrhythmias). *Am J Cardiol.* 2008;101:1291-1296.

170. Makkar RR, Fromm BS, Steinman RT, Meissner MD, Lehmann MH. Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs. *JAMA*. 1993;270:2590-2597.

171. Giraud G, Puech P, Latour H, Hertault J. Variations de potentiel lieés à l'activité du systéme de conduction auriculo-ventriculaire chez l'homme. *Arch Mal Coeur.* 1960;53:757-776.

172. Watson H, Emslie-Smith D, Lowe KG. The intracardiac electrocardiogram of human atrioventricular conducting tissue. *Am Heart J.* 1967;74:66-70.

173. Durrer D, Roos JP. Epicardial excitation of the ventricles in a patient with Wolff-Parkinson-White syndrome (type B). *Circulation.* 1967; 35:15-21.

174. Wellens HJ, Schuilenberg RM, Durrer D. Electrical stimulation of the heart in patients with Wolff-Parkinson-White syndrome, type A. *Circulation.* 1971; 43: 99-114.

175. Vedel J, Frank R, Fontaine G, Gros-gogeat Y. Bloc auricoloventriculaire intra-Hisien définitif induit au caours d'une exploration endocavitaire droite. 1979:*Arch Mal Coeur*; 72:107.

176. Morady F, Scheinman MM, Winston SA, DiCarlo LA Jr, Davis JC, Griffin JC, Ruder M, Abbott JA, Eldar M. Efficacy and safety of transcatheter ablation of posteroseptal accessory pathways. *Circulation.* 1985;72:170-177.

177. Critelli G, Gallagher JJ, Monda V, Scherillo M, Condorelli M. Catheter ablation of accessory pathway in the permanent form of junctional reciprocating tachycardia. *Arch Mal Coeur Vaiss*. 1985;78 Spec No:49-55.

178. Kuck KH, Kunze KP, Schlüter M, Geiger M, Jackman WM. Ablation of a left-sided free wall accessory pathway by percutaneous catheter application of radio-frequency current in a patient with the Wolff-Parkinson-White syndrome. *Pacing Clin Electrophysiol.* 1989; 12:1681-1690.

179. Jackman WM, Wang XZ, Friday KJ, Roman CA, Moulton KP, Beckman KJ, McClelland JH, Twidale N, Hazlitt HA, Prior MI. Catheter ablation of accessory atrioventricular pathways (Wolff-Parkinson-White syndrome) by radiofrequency current. *N Engl J Med*. 1991;324: 1660-1662.

180. Vallin HO. Autonomous influence on sinus node and AV node function in the elderly without significant heart disease: assessment with electrophysiological and autonomic tests. *Cardiovasc Res.* 1980;14:206-216.

181. Vallin HO, Edhag KO. Heat rate responses in patients with sinus node disease compared to controls: physiological implications and diagnostic possibilities. *Clin Cardiol.* 1980; 3:391-398.

182. Morady F, Nelson SD, Kou WH, Pratley R, Schmaltz S, De Buitleir M, Halter JB: Electrophysiologic effects of epinephrine in humans. *J Am Coll Cardiol.* 1988;11: 1235-1244

183. Josephson ME. *Clinical Cardiac Electrophysiology*. Philadelphia:Lea & Febiger, 2nd ed. 1993:52-53.

184. Denes P, Wu D, Dhingra R, Amat-y-Leon F, Wyndhamn C, Rosen KM. Dual atrioventricular nodal pathways, a common electrophysiological response. *Br Heart J*. 1975;37:1069-1076.

185. Thapar MK, Gillette PC. Dual atrioventricular nodal pathways: a common electrophysiologic response in children. *Circulation.* 1979; 60:1369-1374.

186. Casta A, Wolf GS, Mehta AV, Tamer D, Garcia OL, Pickoff AS, Ferrer PL, Sung RJ, Gelband H. Dual atrioventricular nodal pathways: a benign finding in arrhythmia-free children with heart disease. *Am J Cardiol.* 1980; 46:1013-1018.

187. Brooks R, GoldbergerJ, Kadish A. Extended protocol for demonstration of dual AV nodal physiology. *Pacing Clin Electrophysiol.* 1993;16:277-284.

188. de Marneffe M, Jacobs P, Englert M. Reproducibility of electrophysiologic parameters of extrinsic sinus node function in patients with and without sick sinus syndrome. *Pacing Clin Electrophysiol.* 1986;9:482-489.

189. Bergfeldt L, Melander H, Schenck-Gustafsson K. Time-dependent variation in the cardiac conduction system assessed in young healthy individuals at weeks' interval: implications for clinical trials. *J Am Coll Cardiol.* 1991;18: 792-800.

190. Lahaye S, Sheahan R, Darling D, Dorian P, Newman D. Serial measures of sinoatrial and atrioventricular nodal function in ambulatory

patients. *Pacing Clin Electrophysiol.* 1997;20 (Pt 1): 2219-2226.

191. Frankenhaeuser M, Mellis I, Rissler A, Björkvall C, Patkai P: Catecholamine excretion as related to cognitive and emotional reaction patterns. *Psychosom Med.* 1968;30: 109-120.

192. Freyschuss U, Hjemdahl P, Juhlin-Dannfelt A, Linde B: Cardiovascular and metabolic responses to low dose adrenaline infusion: an invasive study in humans. *Clin Sci.* 1986: 70:199-206.

193. Cryer PE: Physiology and Pathophysiology of the Human Sympathoadrenal Neuroendocrine System. *N Engl J Med*. 1980;303:436-444.

194. Leier CV, Webel J, Bush CA: The cardiovascular effects of continous infusion of dobutamine in patients with severe cardiac failure. *Circulation*. 1977;56:468-472.

195. Phillips PJ, Vedig AE, Jones PL, Chapman MG, Collins M, Edwards JB, Smeaton TC, Duncan BMcL. Metabolic and cardiovascular side effects of the β2-adrenoceptor agonists salbutamol and rimeterol. *Br J Clin Pharmac*. 1980;9:483-491.

196. Vallin H, Edhag O, Sowton E. Diagnostic capacity of sinus node recovery time after inhibition of autonomous neural tone. *Eur J Cardiol*. 1980;12:81-93.

197. Bergfeldt L, Vallin H, Rosenquist M, Insulander P, Nordlander R, Åström H. Sinus node recovery time assessment revisited: Role of pharmacologic blockade of the autonomic nervous system. *J Electrocardiovasc Electrophysiol.* 1996;7: 95-101

198. Gullestad L, Forfang K, Simonsen S. Differential effect of selective beta 1 and nonselective beta-adrenoceptor blockade on epinephrine and atropine response in normal humans. *J Cardiovasc Pharmacol.* 1992;20: 976-981.

199. Weissler AM, Harris WS, Schoenfeld CD: Systolic time intervals in heart failure in man. *Circulation.* 1968;37: 149-159.

200. Coats AJS: Doppler ultrasonic measurement of cardiac output: reproducibility and validation. *Eur Heart J.* 1990;11(Suppl):49-61.

201. Hjemdahl P: Catecholamine measurements by high performance liquid chromatography with electromechanical detection. *Methods Enzymol.* 1987;142:521-536.

202. Loeb HS, Sinno MZ, Saudye A, Towne DT, Gunnar RM. Electrophysiologic properties of dobutamine. *Circulatory shock*. 1974;1:217-220.

203. Robinson C, Birkhead J, Crook B, Jennings K, Jewitt D. Clinical electrophysiological effects of atenolol - a new cardioselective beta-blocking agent. *Br Heart J.* 1978;40:14-21.

204. Roeske WR, Yamamura HI: Biochemistry and Pharmacology of the Cardiac Muscarinic Receptors. *In: Levy MN, Schwartz PJ, eds: Vagal Control of the Heart*. Armont, NY: Futura Publishing Co;1994:91-118.

205. Sutyagin PV, Kalinina EE, Pylaev AS. Relative distribution densities of cholinergic and adrenoceptor structures in the central part of the sinoatrial node in rat heart. *Bull Exp Biol Med.* 2005;140:92-95.

206. Crick SJ, Wharton J, Sheppard MN, Royston D, Yacoub MH, Anderson RH, Polak JM. Innervation of the human cardiac conduction system. A quantitative immunohistochemical and histochemical study. *Circulation.* 1994; 89:1697-1708

207. Marron K, Wharton J, Sheppard MN, Fagan D, Royston D, Kuhn DM, de Leval MR, Whitehead BF, Anderson RH, Polak JM. Distribution, morphology, and neurochemistry of endocardial and epicardial nerve terminal arborizations in the human heart. *Circulation.* 1995;92: 2343-2351.

208. Shimizu W, Tsuchioka Y, Karakawa S, Nagata K, Mukai J, Yamagata T, Matsuura H, Kajiyama G, Matsuura Y. Differential effect of pharmacological autonomic blockade on some electrophysiological properties of the human ventricle and atrium. *Br Heart J.* 1994; 71:34-37.

209. Insulander P, Jensen-Urstad M, Kennebäck G, Tabrizi F, Wredlert C, Vallin H. Isoproterenol Increases Right Atrial Conduction Dispersion In Patients With Paroxysmal Atrial Fibrillation. *Europace.* 2004;6(S1):103.

210. Kop JW, Nearing BD, Krantz DS, Gottdiener JS, Franz M, Quigley JF, Tucker D, Baker D, Suchday S, Verrier RL. Increased T-wave alternans with mental stress and exercise in implantable cardioverter defibrillator patients. *Circulation.* 1999;100:I-581.

211. Talajic M, Villemaire C, Nattel S: Electrophysiological effects of alpha-adrenergic stimulation. *Pacing Clin Electrophysiol.* 1990;13: 578-582.

212. Cua M, Shvilkin A, Danilo Jr P, Rosen MR: Developmental changes in modulation of cardiac repolarization by sympathetic stimulation: the role of beta- and alpha-adrenergic receptors. *J Cardiovasc Electrophysiol*. 1997; 8:865-871.

213. Zipes DP, Mihalick MJ, Robbins GT: Effect of selective vagal and stellate ganglion stimulation on atrial refractoriness. *Cardiovasc Res.* 1974;8:647-655.

214. Kemmer FW, Bisping R, Steingrüber HJ, Baar H, Hardtmann F, Schlaghecke R, Berger M. Psychological stress and metabolic control in patients with type I diabetes mellitus. *N Engl J Med.* 1986;314:1078-1084.

215. Richter SD, Schürmeyer TH, Schedlowski M, Hädicke A, Tewes U, Schmidt RE, Wagner TO. Time kinetics of the endocrine response to acute psychological stress. *J Clin Endocrinol Metab.* 1996;8:1956-1960.

216. Jewel GM, Magorien RD, Schaal SF, Leier CV. Autonomic tone of patients during an electrophysiological catheterization. *Am Heart J.* 1980;99:51-57.

217. Challapalli S, Lingamnemi R, Horvath B, et al. 12-lead QT dispersion is smaller in women than in men. *Ann Non-invasive Electrocardiol.* 1998;3:25-31.

218. Fei L, Statters DJ, Camm AJ. QT-interval dispersion on 12-lead electrocardiogram in normal subjects. *Am Heart J*. 1994;127:1654-1655.

219. Larsen JA, Kadish AH. Effects of gender on cardiac arrhythmias. *J Cardiovasc Electrophysiol.* 1998;9:655-664.

220. Vassalle M, Stuckey JH, Levine MJ: Sympathetic control of ventricular automaticity: role of the adrenal medulla. *Am J Physiol.* 1969;217:930-937.

221. Beau SL, Tolley TK, Saffitz JE: Heterogeneous transmural distribution of ß-adrenergic receptor subtypes in failing human hearts. *Circulation.* 1993;88:2501-2509.

222. Cannon DS, Rider AK, Stinson EB, Harrison DC: Electrophysiologic studies in the denervated transplanted human heart. Respones to norepinephrine, isopreterenol and propranolol. *Am J Cardiol*. 1975;36: 859-866.

223. Leenan FH, Davies RA, Fourney A. Catecholamines and heart function in heart transplant patients: effects of beta1- versus nonselective beta-blockade. *Clin Pharmacol Ther.* 1998;64: 522-535.

224. Weiss R, Knight BP, Bahu M, Zivin A, Souza J, Goyal R, Daoud E, Man KC, Strickberger SA, Halter JB, Morady F. Cardiac electrophysiologic effects of nor-epinephrine in human beings. *Am Heart J.* 1998;135(Pt1): 945-951.

225. Mancia G, Bonazzi O, Pozzoni L, Ferrari A, Gardumi M, Gregorini L, Perondi R. Baroreceptor control of atrioventricular conduction in man. Circ Res 1979;44:752-758

226. Gettes LS, Surawicz B, Shiue JC. Effect of high K, low K, and quinidine on QRS duration and ventricular action potential. *Am J Physiol.* 1962;203: 1135-1140.

227. Surawicz B, Lepeschkin E, Herrlich HC, Hoffman BF. Effect of potassium and calcium deficiency on the monophasic action potential, electrocardiogram and contractility of isolated rabbit hearts. *Am J Physiol*. 1959; 196:1302-1307.

228. Lowe MD, Rowland E, Brown MJ, Grace AA. Beta(2) adrenergic receptors mediate impor-

tant electrophysiologic effects in human ventricular myocardium. *Heart.* 2001;86:45-51.

229. Mettauer B, Rouleau JL, Burgess TH. Detrimental arrythmogenic and sustained beneficial hemodynamic effects of oral salbutamol in patients with chronic congestive heart failure. *Am Heart J.* 1985;109:840-847.

230. Kurtz T, Yamada KA, Da Torre SD, Corr PB: Alpha 1-adrenergic system and arrhythmias in ischaemic heart disease. *Eur Heart J.* 1991;12 (SupplF): 88-108.

231. Soufer R, Arrighi JA, Burg M. Brain, behaviour, mental stress, and the neurocardiac interaction. *J Nucl Cardiol.* 2002;9: 650-662.

232. Grillot M, Fauvel JP, Cottet-Emard JM, Laville M, Peyrin L, Pozet N, Zech P Spectral analysis of stress-induced change in blood pressure and heart rate in normotensive subjects. *J Cardiovasc Pharmacol.* 1995;25: 448-455.