Adaptation of ventricular repolarization to heart rate change in humans

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To my family

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ABSTRACT

Background: Life-threatening cardiac rhythm disturbances and sudden death are common manifestations of heart disease. Disturbances in electrical recovery (ventricular repolarization; VR) are important mechanisms behind ventricular arrhythmias, which often occur in relation to changes in heart rate (HR). It is therefore of both theoretical and clinical interest to study the adaptation of VR to changes in HR.
Aims: To investigate the adaptation of VR duration (QT and QT_{peak}) and VR heterogeneity (aka dispersion; T area, T amplitude and ventricular gradient) in response to changes in HR in subjects without structural heart disease and in patients with long QT syndrome type 1 (LQT1).
Methods: VR adaptation to HR changes was investigated in three clinical studies (four papers). In Paper I, patients scheduled for ablation of supraventricular tachycardia were incrementally paced in the atrium to an HR of 120–140 bpm, and the pacing was halted after 5 min. In Papers II and III, the HR increase was induced by sudden atrial or ventricular pacing, repeated at intervals comprising at least one month and was performed with the use of permanent pacemakers in patients with sick sinus disease. In Paper IV, an intravenous bolus of atropine was used to increase HR in patients with LQT1 and in healthy subjects. In all studies, vectorcardiography was used to record the electrical activity of the heart.
Results: Papers I and II: The adaptation of VR duration to a sustained HR change was mono-exponential, took 1.5–2.5 min and was longer following decreasing vs increasing HR. The intra-individual coefficient of variation for QT adaptation to increasing HR was ≤10%. Paper III: There were significant differences in the adaptation of global measures of electrical heterogeneity (dispersion) between HR increase induced by atrial vs ventricular pacing. For both pacing modes, the adaptation occurred in 2–3 rapidly changing phases. QT adaptation was faster in LQT1 patients vs healthy controls.

Conclusions: The adaptation of VR duration is gradual, takes longer in response to decreasing vs increasing HR and is intra-individually a stable process over time. The bi- or tri-phasic VR dispersion response possibly identifies a time period of electrical vulnerability. The atropine ‘stress test’ for VR adaptation is safe and feasible in LQT1 and could potentially be used as a future tool for risk assessment and prognosis.

Keywords: cardiac memory, hysteresis, long QT syndrome, QT adaptation, vectorcardiography, ventricular repolarization

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SAMMANFATTNING PÅ SVENSKA

Introduktion: Allvarliga hjärtrytmrubbningar från hjärtats kammare (kammartakykardi) kan ge upphov till svimning och plötslig hjärtdöd. Risken för sådana arytmier är ökad vid förekomst av tidigare hjärtsjukdom och uppstår ofta i samband med förändringar i hjärtfrekvens. Störningar i hjärtats elektriska återhämtningsfas förmodas vara av betydelse för uppkomst av arytmi. Det finns därför ett behov av att undersöka anpassningen av hjärtats elektriska återhämtningsfas (repolarisationen) till förändringar i hjärtfrekvens.

Syfte: Att kartlägga hur hjärtats repolarisation anpassar sig till plötsliga förändringar i hjärtfrekvens, hos patienter med lindrig störning i hjärtas elektriska funktion (godartade hjärtklappningsbesvär respektive pacemaker) och hos patienter med en potentiellt allvarlig rubbning i hjärtats repolarisation (långt QT-syndrom typ 1; LQT1).

Metod: Anpassningen av hjärtats repolarisation till hjärtfrekvensförändring undersöktes i tre kliniska studier (fyra delarbeten). Vektorkardiografi användes för att undersöka hjärtats repolarisation, metoden kan närmast beskrivas som en tredimensionell variant av EKG. I den första studien undersökte vi patienter i samband med s.k. ablationsbehandling av hjärtrytmrubbning, men utan övrig hjärtsjukdom. Förmaket stimulerades med successivt ökande frekvens upp till 120-140 slag/min, varvid den höga hjärtfrekvensen behölls under 5 min, och därefter avslutades stimuleringen och hjärtrytmen återgick till normal sinusrytm. I den andra studien inkluderade vi istället patienter med inopererad pacemaker p.g.a. låg puls. Patientens pacemaker användes för att öka hjärtfrekvensen till 120 slag/min. under 8 min, och sedan återgick vi till den ursprungliga rytmen. Undersökningen upprepades med minst en månads intervall. I den tredje studien undersöktes patienter med LQT1 och vi använde ett läkemedel (atropin) för att öka hjärtfrekvensen. Atropin lättar på ”hjärtats broms” och ökar därmed pulsen.

Resultat och slutsatser: Anpassningen av hjärtats repolarisation till en ihållande förändring i hjärtfrekvens tog 1.5-2.5 min., längre tid om hjärtfrekvensen minskade än om den ökade. Skillnaden kan möjligen bero på ett metabolt återhämtningsbehov i hjärtmuskeln efter en period av hög puls. Tiden för repolarisationens anpassning till förändring i hjärtfrekvens är inom individen tämligen stabil. Repolarisationen uppvisade också en betydande dispersion/heterogenitet under anpassning till förändrad hjärtfrekvens, vilket kan ha betydelse för uppkomst av arytmi. Patienter med LQT1 hade en snabbare anpassning av repolarisationen än friska försökspersoner. Den exakta mekanismen bakom detta är inte kartlagd. Förhoppningsvis kan undersökning av repolarisationens anpassning till förändrad hjärtfrekvens vara ett framtida hjälpmedel för bedömning av arytmirisk vid LQT1.

LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. Axelsson KJ, Brännlund A, Gransberg L, Lundahl G, Vahedi F, Bergfeldt L. **Adaptation of ventricular repolarization duration and dispersion during changes in heart rate induced by atrial stimulation.** Ann Noninvasive Electrocardiol. 2020;25(3):e12713.

II. Axelsson KJ, Gransberg L, Lundahl G, Vahedi F, Bergfeldt L. **Adaptation of ventricular repolarization time following abrupt changes in heart rate: comparisons and reproducibility of repeated atrial and ventricular pacing.** Am J Physiol Heart Circ Physiol. 2021;320(1):H381-H392.

III. Axelsson KJ, Gransberg L, Lundahl G, Bergfeldt L. **Adaptation of ventricular repolarization dispersion during heart rate increase in humans: A roller coaster process.** J Electrocardiol. 2021;68:90-100.

IV. Dahlberg P*, Axelsson KJ*, Jensen SM, Lundahl G, Vahedi F, Gransberg L, Bergfeldt L. **QT adaptation hysteresis during atropine induced heart rate increase: comparison between patients with long QT syndrome type 1 and healthy subjects**. In manuscript.

*Both authors contributed equally.

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ABBREVIATIONS AND DEFINITIONS

AP	Atrial pacing
APD	Action potential duration
CM	Cardiac memory
DAD	Delayed afterdepolarization
EAD	Early afterdepolarization
ECG	Electrocardiogram
HR	Heart rate
MAP	Monophasic action potential
ICD	Implantable cardiac defibrillator
I_{Kr}	Rapid delayed rectifier (I: current, K: potassium, r: rapid)
I_{Ks}	Slow delayed rectifier (I: current, K: potassium, s: slow)
LQTS	Long QT syndrome
LQT1-3	Long QT syndrome type 1-3
QRS	Ventricular depolarization on the ECG
QT	QT interval: the time from QRS start to T wave end
QTc	QT interval corrected for heart rate
QT_{peak}	Time from QRS start to T wave peak
SCD	Sudden cardiac death
T amplitude	The amplitude of the maximum T vector inscribed in the T-vector loop

T area	The vectorial sum of the area under the T wave curve during the JT interval in X, Y and Z leads
TdP	Torsades de Pointes
VCG	Vectorcardiography
VG	Ventricular gradient: the vectorial sum of the QRS area vector and the T area vector
VP	Ventricular pacing
VR	Ventricular repolarization

1 INTRODUCTION

1.1 ARRHYTHMIA AND SUDDEN CARDIAC DEATH

Sudden cardiac death (SCD) and life-threatening arrhythmias are common manifestations of cardiac disease (1-3). The most common cause of SCD is coronary heart disease (1, 3). In the young, however, other cardiac diseases are associated with SCD, such as cardiomyopathies (e.g. hypertrophic cardiomyopathy) or channelopathies (e.g. long QT syndrome; LQTS) (2, 4-7). Regardless of the underlying pathophysiological mechanism, the majority of SCD is caused by electrical disturbances that lead to ventricular tachycardia and fibrillation entailing hemodynamic collapse (1, 8).

There is a strong correlation between changes in heart rate (HR) and the occurrence of life-threatening ventricular arrhythmias (9-11). They can occur both in the setting of increasing HR (from exercise or stress), such as in LQTS type 1 (LQT1), and decreasing HR, such as in a complete atrioventricular block (5, 12, 13). Disturbances in ventricular repolarization (VR) are often the culprit in these situations (14-19).

It is therefore of clinical interest to further investigate the relation between changes in HR and possible substrates for cardiac arrhythmias. The aims of this thesis were to investigate the adaptation of VR duration (QT and QT_{peak} intervals) and electrical heterogeneity (VR dispersion) in response to changes in HR to clarify aspects of normal physiology and to identify differences in repolarization adaptation between healthy individuals and patients with a repolarization disorder (LQT1).

1.2 RECORDING THE ELECTRICAL ACTIVITY OF THE HEART

1.2.1 CARDIAC ACTION POTENTIAL AND THE ECG

In 1887, Waller recorded the first human electrocardiogram (ECG) (20). Einthoven developed the technique further and obtained, more than 100 years ago, ECGs of no less morphological quality compared with today's ECG tracings (20, 21). Since then, ECG has been of fundamental importance in medicine and cardiology for the diagnosis of common cardiac disorders (e.g. arrhythmias or ischemic heart disease).

The electrical activation and recovery of the heart can be recorded on the body surface as an ECG, wherein the differences in the electrical potentials in the ventricles during the cardiac cycle are reflected by the QRST complex (see Figure 1) (22, 23).

The ECG results from transmembrane action potentials at the cellular level (23, 24). The fast initial part of the action potential (Phase 0) depends on the rapid influx of sodium ions. Phase 1 is a partial repolarization due to a rapid efflux of potassium ions, which makes the transmembrane potential slightly more negative. Phase 2 is the plateau phase due to a balance between outward potassium currents and inward calcium and late sodium currents. The main repolarization (Phase 3) is mainly due to outward potassium currents, and it ends in the return to the resting potential (Phase 4). For a comprehensive review of the dynamics of the cardiac ion channels mentioned above, please see (25). In Figure 1, the relation between ionic currents and the action potential (AP) is overviewed.

Neither the action potential duration (APD) nor the morphology of the action potential is homogenous throughout the heart, and significant differences in duration exist between different areas of the heart (26-28). When the depolarization wave front travels through the ventricular conduction system (His–Purkinje network) and the myocardium, it can be recorded as a QRS complex on the surface ECG (29, 30). The combination of differences in APD and the sequence of activation (depolarization) gives rise to voltage differences in the myocardium during repolarization, which is seen as the T wave on the ECG (26, 28, 31, 32). The majority of depolarization and repolarization instants are, however, 'cancelled' (by opposite directed wave fronts) throughout the cardiac cycle, and the ECG is the recording of the residual voltage differences that reach and can be registered from the body surface (24). The duration of cardiac repolarization is measured as the QT interval ('QT'

hereafter), because the first activated cells start to repolarize during the QRS complex. Figure 1 shows the relation between the duration of action potentials throughout the heart and the resulting ECG on the body surface. The length of QT is strongly correlated with HR (33), and QT is therefore usually corrected for HR with the help of different formulas (Bazett, Fridericia, Hodges, etc.) (34). In this thesis, the heterogeneity in the repolarization instants is mostly referred to as dispersion in repolarization. VR dispersion is dynamic and changes according to variations in local APD and/or the activation sequence.

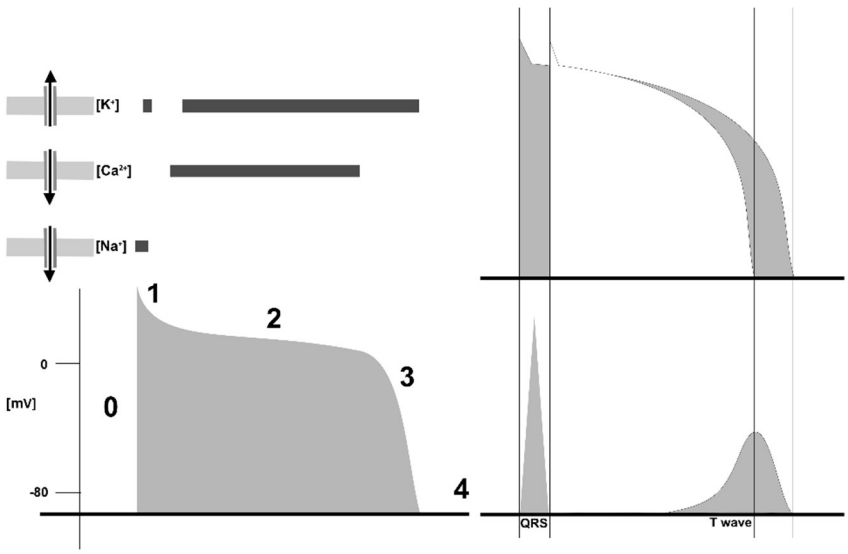


Figure 1. *Left panel: the different phases and ionic currents of the cardiac action potential in a ventricular myocyte (simplified for clarity). Right panel: the differences in activation time and action potential duration give rise to an ECG. (Right panel is adapted, with permission from the author, and modified from an original figure in Vahedi F: Vectorcardiographic evaluation of ventricular repolarization in healthy individuals and LQTS mutation carriers. Figure 5. PhD thesis, Sahlgrenska Academy, University of Gothenburg, Kompendiet, Gothenburg, Sweden. ISBN 978-91-628-8733-9.)*

1.2.2 VECTORCARDIOGRAPHY

The ECG is easy to acquire and offers great diagnostic opportunities for trained interpreters. However, the one-dimensional representation of the magnitude of voltage differences in the ECG omits information about the spatial direction of the depolarizing and repolarizing forces. This problem can be overcome by the use of vectorcardiography (VCG) (35, 36).

In the middle of the 20th century, the concept of modern VCG was introduced by Burger, and the method, including lead positioning, was developed further by Frank (37, 38). The VCG is similar to ECG but provides spatial measurements of depolarizing and repolarizing forces based on three orthogonal leads (X, Y, Z) introduced by Frank (37); see Figure 2. When recording VCG, a single dipole represents the electrical activity of the heart at any moment during the cardiac cycle. The magnitude (amplitude) and direction are displayed in the form of a spatial vector (35). This vector will inscribe loops during the cardiac cycle for atrial depolarization (P), ventricular depolarization (QRS) and ventricular repolarization (T). These loops are shown in three orthogonal planes (frontal, sagittal and transverse) (36, 37).

A PQRST complex can be derived from the loops for each of the three orthogonal leads, and from these, a global PQRST complex can be created. The global PQRST complex can then be used to obtain measures for different intervals (in this thesis, measures were based on QRST, e.g. QT). The regular 12-lead ECG provides measures of VR duration (QT and QTc intervals) but not the assessment of its dispersion. The VCG offers several measures of VR dispersion, but they are all global measures. That is, regional abnormalities can be detected by the VCG, but their location in the ventricles cannot be identified precisely (39). In this thesis, the VCG measures used to assess VR dispersion are further described in the ‘Methods’ section.

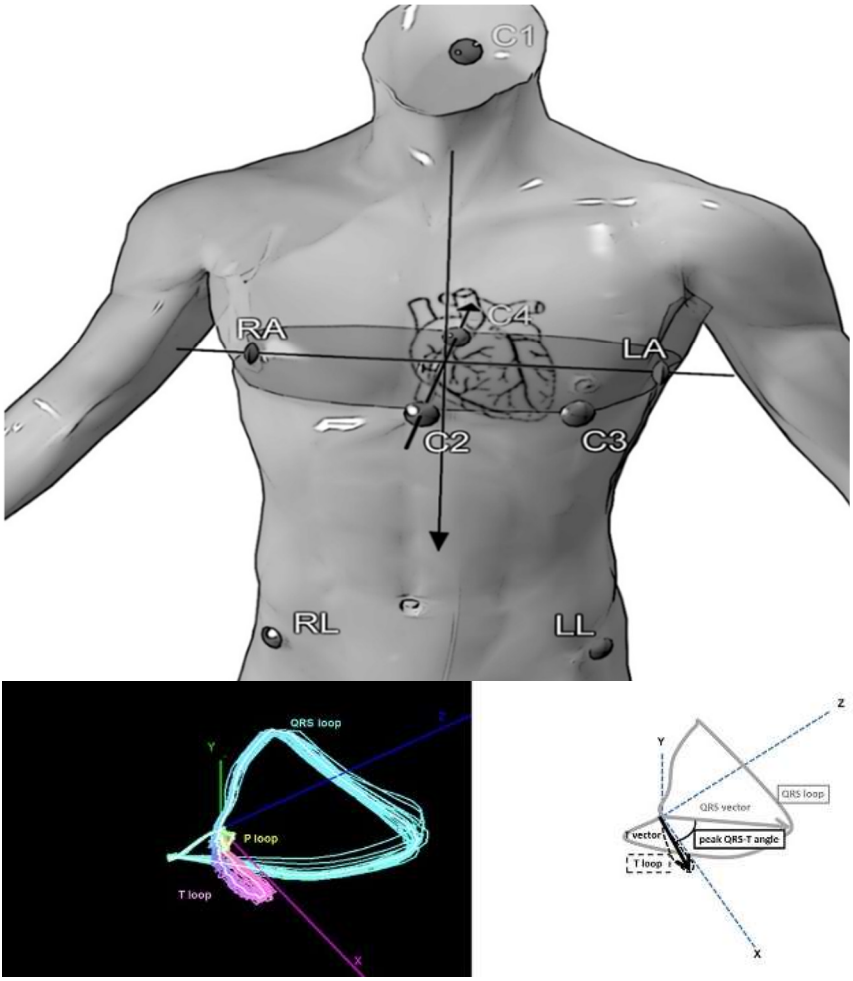


Figure 2. *The top panel shows the placement of VCG leads according to Frank. Lower panels display the P loop, QRS loop and T loop in space. Top panel is reproduced from Wecke L: Cardiac memory in two human models. PhD thesis, Karolinska Institutet, ReproPrint AB Stockholm, Sweden, 2006. ISBN 91-7140-614-X. Lower panels are reproduced from reference (40), with permission from Elsevier. URL: https://doi.org/10.1016/j.ijcard.2016.05.005*

1.3 ADAPTATION OF REPOLARIZATION DURATION AND HYSTERESIS

As discussed in the previous sections, the VR duration can be measured as QT on the ECG, where the T wave is the result of differences in the activation time and the duration of all ventricular action potentials (action potential duration, APD). The VR duration (i.e. QT) adapts to changes in HR; it decreases as HR increases.

In the following sections, the principles for this HR adaptation will be described. First, we discuss the response in VR duration to a single extra beat (QT or APD restitution), i.e. a premature supraventricular or ventricular beat. Then, in the following section, we focus on the adaptation of VR duration to a sustained change in HR, i.e. corresponding to a sudden tachycardia (QT or APD adaptation).

Finally, we consider an important component in these two types of cardiac adaptation to changes in HR: hysteresis, an ultra-rapid memory phenomenon (41). In Figure 3, the relationship between cardiac electrical activity and the mechanical pump function illustrates the importance of a fine-tuned adaptation to changes in HR. As the QT corresponds to mechanical systole, changes in QT influence the time for filling and emptying of the ventricles as well as coronary perfusion (in diastole) (42). An essential feature of QT adaptation to variations in HR is gradual change in QT, and this lag is referred to as hysteresis. Hysteresis is a common feature in both biological and electromechanical systems (43).

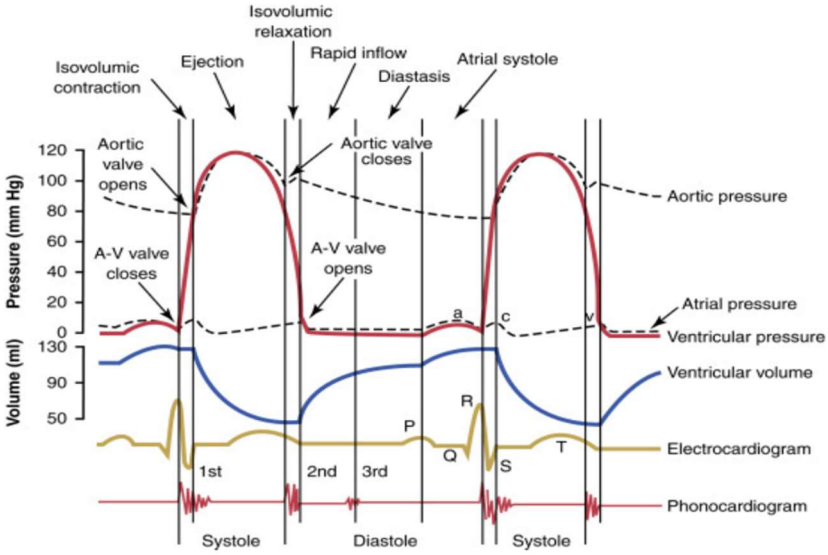


Figure 3. *The relation between systole/diastole and the ECG. The QT interval is normally slightly shorter than mechanical systole. Changes in QT and mechanical systole have implications for the time taken for filling and emptying of the ventricles as well as for coronary perfusion, which occurs in diastole. Reproduced from Guyton and Hall Textbook of Medical Physiology, 14th Edition, 2016, John E. Hall, Michael E. Hall, Chapter 9 "Cardiac Muscle; The Heart as a Pump and Function of the Heart Valves, Figure 8-8, page 118, with permission from Elsevier.*

1.3.1 CARDIAC RESTITUTION

QT depends on the previous RR interval, and a shorter preceding RR interval results in a shorter QT (or APD in experimental studies). This RR interval dependency is called cardiac restitution, and it is well described in human and animal studies (42, 44-47). In short, at a steady state HR, if a sudden extra beat occurs, QT is shortened immediately. The shorter the interval between the normal beat and the extra beat, the shorter the QT will be. However, the response is not linear, and the relation between the RR interval of the extra beat and QT becomes progressively stronger as the RR interval (of the extra beat) is shortened. In essence, cardiac restitution is the dependence of QT on the preceding RR (41, 44, 48). This relationship can be illustrated in a graph, a restitution curve, where an increased slope of the relation between RR and QT (or APD), in experimental studies, suggests increased arrhythmogenicity (42, 44, 45, 49, 50). In Figure 4, an example of a restitution plot is shown.

Cardiac restitution features a hysteresis function, where not only the immediately preceding RR determines the length of APD/QT. Instead, hundreds or even thousands of RR intervals are 'stored' in an ultra-rapid memory function (described in the section 'cardiac memory' below) (41, 48, 51). This is clearly visualised in Figure 4, where the properties of the restitution curve depend on the baseline cycle length (corresponding to the RR interval) to which the premature extra beat is coupled: the ultra-rapid cardiac memory (CM) is seen as the downward shift in the restitution curve following faster HR at baseline (51).

Cardiac restitution has been thoroughly investigated in animal studies, heart tissue preparations, in silico models and via global epicardial mapping (44, 47, 52-54). However, the relation between QT/RR in a restitution curve has not proven to be of particular clinical importance either as a prognostic marker or as a target for therapy. The reason for this is probably, as pointed out by Conrath and Opthof (28), the transient state of the individual QT/RR-relationships that creates the restitution curve. Since it shows the immediate reaction in QT or APD, the steepness does not reflect a steady state of repolarization duration in the myocardial tissue, but rather a response to single beat perturbations. This was clarified by Franz et al., who created restitution curves from continuous pacing to a steady state and found a difference between the first beat APD and the steady state value, a feature of QT hysteresis (55); this is also seen in Figure 4.

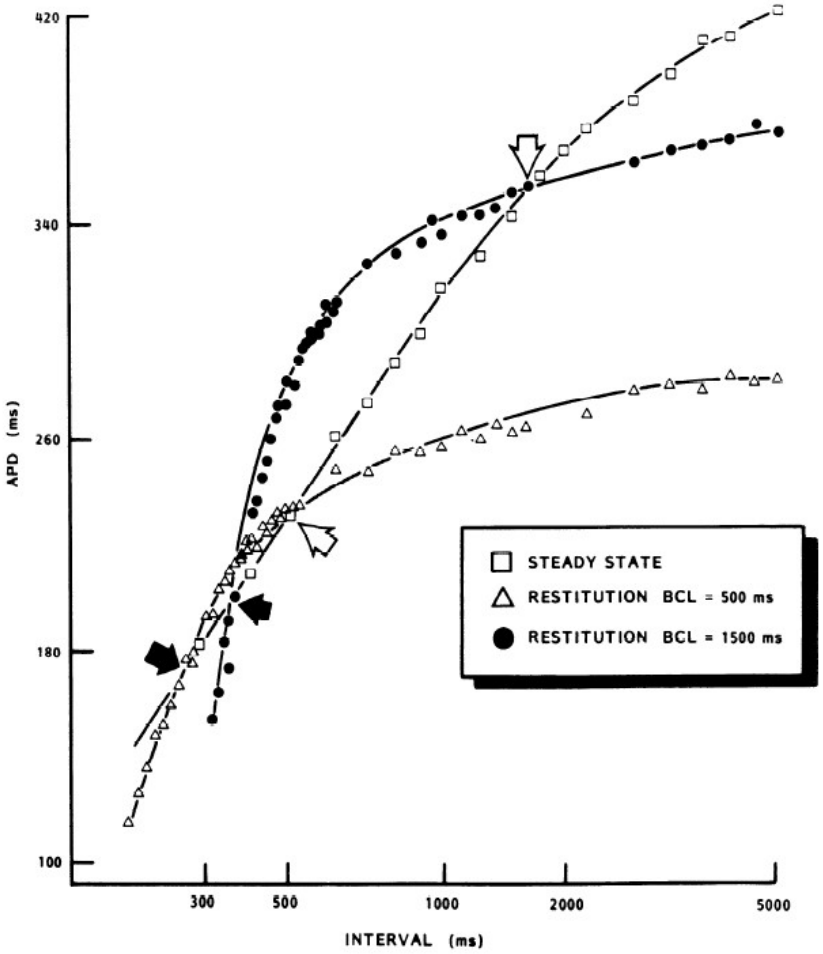


Figure 4. *Restitution curves in a dog papillary muscle. These curves are created by measuring the APD in the cardiac papillary muscle of dogs in response to an extra beat delivered at progressively shorter cycle lengths from a stimulus train at a basic cycle length (BCL). The restitution curves with a longer BCL (lower HR) are shown with filled circles, and restitution curves with a shorter BCL (higher HR) are shown with triangles. Clearly, the restitution curves are different depending on the baseline cycle length. Also, notice the difference between the steady state APD and APD on the restitution curve. This illustrates the difference between restitution and adaptation, as discussed in the section 'Adaptation of repolarization duration'. Reproduced from reference (51), with permission from The American Physiological Society. URL: https://doi.org/10.1152/ajpheart.1983.244.6.H782*

1.3.2 QT ADAPTATION

The QT interval, which corresponds to mechanical systole, depends on the preceding RR intervals, and following sustained changes in HR, QT changes with a certain delay (hysteresis) (56-61). The result of hysteresis in QT adaptation can be seen in the 'loop' shown in Figure 5 (59).

QT/APD adaptation has been investigated in animal studies and in humans (42, 55-57, 60, 62-68). From human data, we know that the adaptation of QT to a new sustained HR takes 2–3 minutes (up to 300s) (57, 58, 60, 67, 68). QT adaptation in response to changes in HR can be investigated by exercise testing, Holter recordings, chronotropic drugs and pacing (39, 69). The assessment of QT adaptation in response to HR change induced by temporary programming of permanent pacemakers has previously been described (61, 68). A functional benefit of hysteresis is gradual adaptation of the time for coronary perfusion and ventricular filling/emptying as the metabolic demands change (70). Hysteresis probably also provides electrical stabilisation, because too sudden changes in repolarization can lead to arrhythmias. In human physiology, there are several aspects of QT adaptation and its hysteresis that are not fully disclosed:

1) Is the QT adaptation time different following increasing vs decreasing HR?

2) Is the time for QT adaptation activation dependent? Are there differences in the adaptation time depending on whether the ventricles are activated through the normal conduction system (atrial pacing, AP) or by abnormal activation from ventricular pacing (VP)?

3) Is the time for QT adaptation similar in an individual, when measured repeatedly at long time-intervals (weeks or months)?

These questions were addressed in Papers I and II.

1.3.3 CARDIAC MEMORY

CM has for long mainly been associated with the T wave vector adaptation to the activation wave front vector, i.e. negative T waves in leads II, aVF and III after a prolonged period of apical ventricular activation pacing (71, 72). However, two additional and mechanistically different forms of cardiac

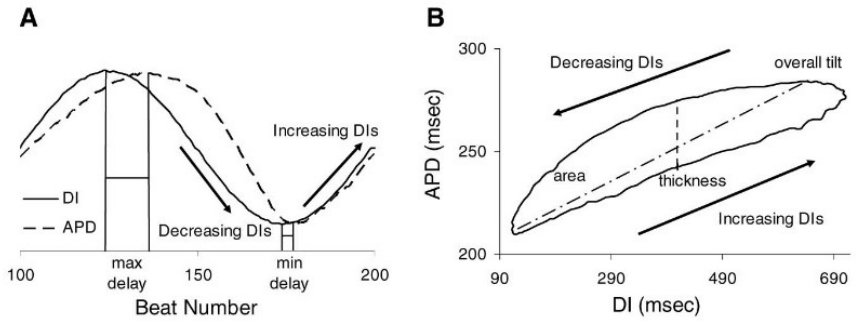


Figure 5. *Strips of ventricular tissue from pigs are paced in a sinusoidal pattern. A: there is a delay in changes in APD (dotted line) following changes in diastolic interval (DI). B: the values in panel A are given in a hysteresis loop, and it is evident that for each DI, the APD is different depending on whether DIs are increasing or decreasing. Reproduced from reference (59), with permission from John Wiley and Sons. URL: https://doi.org/10.1111/j.1540-8159.2009.02637.x*

memory operate to adapt ventricular function to changes in the activation pattern and HR. The reader is referred to reference (41) for a comprehensive review. In short, long-term and short-term memory are both activation dependent and a feature of cell signalling, effects on intracellular proteins and gene transcription. However, the term ultra-rapid CM is reserved for effects on APD or QT mediated by the rapid feedback mechanism from altered diastolic intervals on ion currents, ion concentrations, and transporters (41). However, in the literature, short-term CM is sometimes used synonymously with ultra-rapid CM (73). We prefer ultra-rapid CM since it allows for mechanistic differentiation on three different levels of memory (long-term, short-term, ultra-rapid).

Ultra-rapid memory is thus a function of ion current kinetics and ion concentrations, in response to extra beats or to changes in HR (41, 72). Calcium (I_{CaL}) and potassium currents (I_{Kr}, I_{Ks}), and the handling of intracellular calcium are key components of this memory function, but other mechanisms are also important, especially for the slow adaptation phase to a sustained change in HR (described in more detail in Discussion) (48, 58). Furthermore, VR adaptation is moderated by several intrinsic and external factors in normal physiology and can be impacted by various cardiac diseases about which less is known (69, 74). The hysteresis in cardiac restitution is closely related to the hysteresis in the adaptation of QT to sustained or 'dynamic' changes in HR, and they are both functions of ultra-rapid CM. The ultra-rapid memory

function can thus also be visualised in the hysteresis loop of QT/APD-adaptation (see Figure 5). Panel A in Figure 5 shows how APD lags behind changes in RR, and Panel B shows the corresponding loop created by APD plotted against the diastolic interval (DI = CL or RR − APD or QT).

In summary, hysteresis, a function of ultra-rapid CM, adjusts the response in QT to short term changes in RR (i.e. an extra beat, restitution) and to sustained changes in HR (i.e. tachycardia, QT adaptation). The heart remembers the (recent) history of RR intervals. *QT adaptation hysteresis in response to HR change was investigated in Papers I, II and IV.*

1.4 DISPERSION IN REPOLARIZATION

Ventricular tachyarrhythmias are mainly initiated by extra beats but need a substrate for their sustenance. Examples of arrhythmia substrates can be either a scar after a myocardial infarction (structural heart disease) or a repolarization disturbance secondary to impaired ion channel function (a 'functional' substrate). Such substrates for arrhythmias can develop or become significant during the VR adaptation to HR changes. There are regional differences in the heart in terms of the repolarization time, which, to some extent, depends on differences in the distribution and function of ion channels (25, 75, 76). Regional differences in the repolarization duration are commonly depicted as gradients in repolarization (77). When measuring the time to local repolarization in perfused swine hearts, Meijborg et al. found dispersion in repolarization in four anatomical gradients (interventricular, apico-basal, anterior-posterior and transmural) that contribute to the T wave morphology (32). In humans, a negative correlation between activation time (depolarization) and APD exists (26, 78); however, the time to local repolarization is the sum of activation time and APD, and Yuan et al. reported that endocardial repolarization follows the activation sequence (26). In a study on isolated, perfused human hearts, despite a clear activation sequence, repolarization started at multiple sites and created gradients along various 'axes' in the heart (79). This leads to the conclusion that human 'in situ' repolarization is certainly activation-dependent, but it is probably wise to refrain from drawing conclusions regarding the importance of specific axes in local repolarization time as well as in global repolarization.

The heterogeneity—or dispersion—in repolarization is altered in response to changes in HR. That is, when the HR changes suddenly, the repolarization time reacts differently in different parts of the heart. When a large difference in repolarization time emerges between adjacent areas in the heart, the stage is set for ventricular tachycardia to occur. It is generally considered that

exaggerated dispersion is an important cause of malignant ventricular arrhythmias not only in the acute phase of ischemic heart disease but also in patients without structural abnormalities (e.g. LQTS) (80).

In a publication from 1964, Han and Moe described increased dispersion in the ventricular muscle of dogs in response to a premature extra beat (81). That is, when the heart tissue was paced at a steady state rate, the time to repolarization after every impulse ('heart beat') was the same on different recording sites; however, after a premature extra beat, large differences in repolarization time occurred. In another experiment on dogs, Kuo et al. used a combination of hypothermia and regional warm blood flow to increase dispersion in local repolarization time; ventricular arrhythmia could be induced by electrically stimulated extra beats when dispersion reached a critical value (82). These observations lead to the understanding that increased dispersion in repolarization time is a key element for the initiation of ventricular arrhythmias.

However, exaggerated differences in APD (repolarization duration) between adjacent areas are not enough to provoke arrhythmias; it also takes a trigger (83). This trigger is often an early extra beat (premature ventricular beat). Usually, these extra beats occur from early (EAD) or delayed afterdepolarizations (DAD) in the myocardial tissue (83, 84). These afterdepolarizations are often seen when repolarization is prolonged and can be due to excess calcium inside heart muscle cells (myocytes), which is seen in ischemic heart disease and heart failure (85-87). For a review on afterdepolarization and triggered activity, see (88). In LQTS, afterdepolarizations are the triggers for polymorphic arrhythmias (Torsades de Pointes, TdP). If the afterdepolarization comes early after the preceding beat, some cardiac tissue can be refractory, while other regions of the ventricle can be excited by the early impulse (as explained in the previous paragraph). The refractory tissue will then be an obstacle for the afterdepolarization, which can lead to polymorphic ventricular tachycardias. This is illustrated in Figure 6, where an extra beat encounters an area of refractory cardiac tissue, leading to a break up in the wave front which can further lead to re-entry.

Which methods have been used to study VR dispersion? VR dispersion can be studied invasively and non-invasively. For invasive measures, catheters can be used to record signals from the inside of the heart (endocardium) (78, 89, 90).

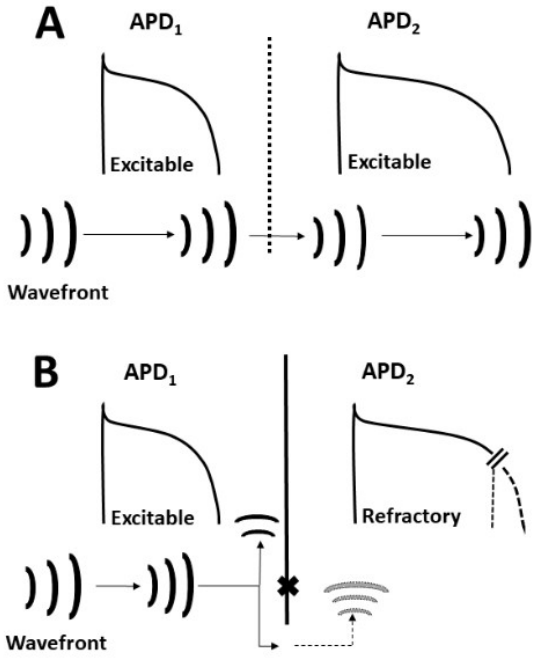


Figure 6. *VR dispersion and arrhythmia. This schematic and simplified picture illustrates how a sudden extra beat can give rise to arrhythmias based on differences in the repolarization time between adjacent areas in the heart. The differences in the repolarization time are illustrated as action potentials of different durations (APD_1 and APD_2). A: a depolarizing wave front traverses areas with different APDs, but since repolarization is completed in both areas, there is no conduction block. B: the difference between the adjacent areas in APD is larger, and the wave front encounters a functional block line. The wave front breaks up and travels along the area of refractoriness (block line) for a distance, which corresponds to the time it will take for the refractory area to recover. When the APD_2 area is depolarized with a delay, the APD_1 area is refractory, which leads to a new block line, and the stage is set for re-entry to occur.*

In animal research or during cardiac surgery in humans, a device ('sock-electrodes') can be applied on the outside of the heart to record multiple signals at the same time (91, 92). Non-invasive measures include ECG-derived measures of dispersion (i.e. QT dispersion), vectorcardiographic measures of VR dispersion and body surface mapping (93, 94).

HR change is a well-known arrhythmia trigger (9-11). From the seminal work of Han and Moe, we know that premature extra beats create heterogeneity in the refractoriness in the myocardial tissue (81), and this has been shown in

further studies, mainly on animals (18, 82, 95, 96). There is, however, a difference between cardiac adaptation to single beat perturbations versus adaptation of repolarization to a new, stable cycle length. As discussed above, the repolarization time adapts non-uniformly in response to a sudden, premature extra beat. The dispersion following a sustained increase (or decrease) in HR is less investigated. Using optical mapping and voltage-sensitive dye, Rosenbaum et al. discovered a biphasic pattern in the time-dependent change in the apico-basal gradient in guinea pigs (97). In 2012 and 2014, Bueno-Orovio et al. described a similar reaction pattern in response to HR increase from VP in both apico-basal and interventricular gradients (89, 90). They measured activation-recovery intervals (ARI) as a surrogate for local APD. In the 2014 paper, time constants were calculated for the slow phase of ARI adaptation and were larger in the left ventricle than in the right ventricle. In computer simulations, a difference (20 vs 80 s) in the time constants for ARI adaptation caused a partial block in wave front propagation, but a larger difference (10 vs 100 s) was needed to cause re-entry (e.g. from an EAD) (90).

It thus seems that the pre-existing gradients in cardiac repolarization (i.e. differences in time to repolarization between regions in the ventricles) show a complex adaptation pattern in response to HR changes. In the study by Rosenbaum et al., the gradient is transiently decreased and then increased, while Bueno-Orovio et al. demonstrated an early increase followed by a decrease (89, 90, 97). In a previous study from our research group, the VR dispersion in response to HR increase caused by a bolus dose of atropine was investigated, and an 'overshoot' reaction in response to HR increase by atropine (39) was observed. From previous studies, we know that there are regional differences in the APD adaptation time following HR change, but less is known about the adaptation pattern in global (whole heart) ventricular dispersion in humans. *The adaptation of VR dispersion in response to HR change was investigated in Papers I and III.*

1.5 ELECTRO-MECHANICAL FUNCTION AND ADAPTATION TO CHANGES IN HR

The heart is a pump that needs to rapidly adapt its mechanical function to the various demands caused by stress and exercise in everyday life. This remarkable ability is the result of an interplay between depolarization-repolarization and contraction-relaxation orchestrated by the autonomic nervous system. The hysteresis in QT adaptation allows for gradual accommodation of the time for ventricular filling/emptying and for coronary perfusion.

There is an inverse relation between APD and contractility in cardiac restitution as a function of increased HR (42). However, electro-mechanical coupling is a matter of more complexity than just the relation between APD or QT and mechanical systole. Despite its ominous connotation, dispersion (or heterogeneity) in repolarization and/or mechanical function is an essential feature of normal ventricular function (98, 99). In 1988, Katz and Katz used the example of ancient Greek warships (triremes) with three banks of oars, where the oars in each row were designed differently in order to optimise the overall rowing force and create 'homogeneity out of heterogeneity' (98). The authors use the example of optimised rowing force in triremes to suggest that structural heterogeneity in the heart walls is necessary for homogenous contraction. This paradigm has proven to hold up well in experimental studies on strips of connected heterogeneous cardiac muscles (Duplex) (99-102). A central finding is that when two elements of cardiac muscles are connected, they show substantial changes in mechanical behaviour (99). The results from extensive work on the Duplex model and simulations suggest that myocardial heterogeneity is necessary for proper regional electro-mechanical adaptation in order to optimise global heart function (99).

1.6 CARDIAC REPOLARIZATION AND THE AUTONOMIC NERVOUS SYSTEM

The heart is under control of both the sympathetic and parasympathetic nervous systems. The parasympathetic system reaches the heart via the vagus nerve (from the medulla oblongata). The effects of the parasympathetic system are mediated via the vagus nerve through acetylcholine activation of nicotinic acetylcholinic receptors on postganglionic neurons. They, in turn, release acetylcholine, which binds to muscarinic receptors in the heart. The effects of parasympathetic stimulation are decreased HR and contractility. (103-105)

The sympathetic innervation of the heart originates from the thoracic segments of the spinal cord and synapse with postganglionic nerves in the paravertebral intrathoracic ganglia (stellate ganglia). From here, postganglionic neurons innervate the myocardium. The neurotransmitter between pre- and post-ganglionic neurons is acetylcholine, and postganglionic neurons release norepinephrine (NE). In cardiac tissue, NE binds to beta-adrenergic receptors, which are G-protein-coupled and transmit signals via cAMP to increase contractility and HR. (106-109)

In the healthy heart, sympathetic stimulation decreases APD duration and VR dispersion. However, in for example, LQTS sympathetic stimulation can

induce arrhythmias, possibly through exaggerated regional dispersion or the induction of EADs (105, 106, 110). This is also true for ischemic heart disease and heart failure. In the case of structural heart disease, heterogeneity in sympathetic nervous innervation can also precipitate arrhythmias (111).

HR can be influenced by pharmacological stimulation at different levels of the autonomic nervous system. Beta blockers lower HR by their antagonistic effect on beta-adrenergic receptors, while beta-adrenergic agonists (e.g. isoprenaline) increase HR. Since HR at rest is a balance between parasympathetic (decreases HR) and sympathetic input (increases HR) (112, 113), another way to increase HR is to inhibit the decreasing effect on HR by inhibiting parasympathetic stimulation (i.e. 'the brake'). This can be achieved via intravenous administration of atropine, a competitive antagonist of acetylcholine at muscarinic receptors (114). *In Paper IV, a bolus dose of atropine was used to induce a rapid increase in HR.*

1.7 LONG QT SYNDROME (LQTS)

LQTS is a prototypical disorder of VR and an autosomal dominantly inherited disease with variable expression and penetrance (115, 116). The main clinical manifestations of the disease are syncope, palpitations and cardiac arrest. In a study on Italian infants (ECG and genetic testing), the prevalence was 1:2534 (95% CI, 1:1583 to 1:4350). However, considering the number of infants with QTc > 450 ms who did not undergo genetic testing, the prevalence may be closer to 1:2000 (117). Approximately 90% of LQTS is explained by mutations in two potassium channels, KCNQ1 and KCNH2 (LQTS type 1 and 2), or in the rapidly depolarizing sodium current channel, SCN5A (LQTS type 3) (116). The phenotypes of these LQTS types differ in terms of QT abnormalities, responses to autonomic variation and prognosis (118).

When the relationship between inward (depolarizing) and outward (repolarizing) currents is impaired by ion channel mutations in LQTS, the stage is set for EADs to occur during repolarization. If enough myocytes are involved, this can trigger a propagating action potential (84) and possibly the formation of an unstable ventricular tachycardia (14, 15, 17). The typical ventricular arrhythmia in LQTS is the Torsades de Pointes (TdP) polymorphic ventricular tachycardia, characterised by rotation of the electrical axis around the ECG base line (15, 119). The tachycardia can be self-limiting and the reason for syncope in LQTS, but can also deteriorate into VF and cause SCD (119).

There are several well-known risk factors for adverse events, including SCD in LQTS. The most important variables to consider are sex, QTc, LQTS type (mutation), age and symptoms (i.e. syncope or survived cardiac arrest) (120, 121). A protocol for risk stratification based on genotype, sex and QTc in LQTS patients has been proposed. The risk groups were defined as low risk (<30%), intermediate risk (30-49%) and high risk (≥50%), based on the probability of a first cardiac event (syncope, cardiac arrest or sudden death) before 40 years of age and without therapy (120). The most common therapy is beta-receptor blockade (116, 118, 121). Implantation of an ICD is recommended in LQTS patients with previous cardiac arrest and should be considered as a prophylactic measure in patients at very high risk (2, 121). The risk is not high enough to motivate implantation of an ICD in all LQTS patients, and there are clinically significant complications associated with this therapy (120-122).

When an LQTS patient (proband) is identified, genotype screening is recommended in the family members (123, 124). This praxis, however, results in the identification of an increasing number of asymptomatic LQTS patients in whom the prognosis and most adequate management is difficult to decide on. Even in the presence of normal QTc, such patients have an increased risk for adverse events compared with unaffected family members. However, the risk is significantly lower compared with phenotypically affected patients (125). There is therefore a need for additional tools for personalised risk evaluation in LQTS. For example, in the low-risk category defined by asymptomatic LQT1 patients with normal QTc, further evaluation could perhaps identify mutation carriers with an arrhythmia risk level very close to that of normal individuals. This could have important implications for treatment choice and lifestyle recommendations. It is well-recognised that events in LQT1 occur primarily during exercise, while in LQT3, events occur more often during sleep/rest. In LQT2, events often occur in response to emotional stress (126). Therefore, and especially for LQT1, the QT response to increased HR could be of interest for improved risk assessment. *This issue was explored in Paper IV.*

2 AIMS

The general aims of this thesis were to investigate the adaptation of VR duration and dispersion in response to changes in HR, to clarify remaining gaps in the knowledge pertaining to normal physiology (Papers I–III) and to reveal differences in VR adaptation between healthy individuals and patients with LQTS (Paper IV).

Paper I We aimed to investigate QT adaptation (repolarization duration) and ventricular repolarization dispersion during incremental HR increase followed by a steady state and then an abrupt decrease in HR in healthy individuals who were 18–50 years old. The HR increase corresponds to the increase in sinus rhythm during moderate exercise but without active involvement of the autonomic nervous system.

Paper II The purpose of the study was to compare the VR duration (QT and QT_{peak}) adaptation to changes in HR induced by pacing in the right atrium versus the right ventricle. Ventricular activation is normal during AP and abnormal during VP (similar to the left bundle branch block). The HR increase by AP and VP thus simulates a sudden onset slow atrial (SVT) and ventricular (VT) tachycardia at 120 bpm. In addition, we also intended to investigate the intra-individual stability of the VR duration adaptation by defining its time-dependent variability through repeated measurements at ≥ 1 month's interval.

Paper III The aim of Paper III was to analyse the adaptation of global measures of dispersion in response to AP vs VP (corresponding to SVT vs VT, see Paper II above). The rationale was based on the observation that VR dispersion is an important contributor to the sustenance of ventricular tachycardia.

Paper IV The aim was to study VR duration (QT and QT_{peak}) adaptation to a sudden increase in HR induced by an atropine bolus in LQTS patients and healthy individuals. Our working hypothesis was that the QT and QT_{peak} response to HR increase would be different from that in healthy individuals. We also hypothesised that the adaptation (hysteresis) of QT and QT_{peak} could provide a better assessment of the degree of impairment of the mutated potassium channel proteins than QTc alone, resulting in better risk prediction for adverse events. The study was also a test of safety and feasibility.

3 METHODS

3.1 STUDY SUBJECTS

Study subjects are described in more detail in the specific papers; here is a brief description of the recruitment and selection process. Written informed consent was obtained from all participants, and the research projects were approved by the local ethics committee.

Paper I Patients scheduled for ablation of supraventricular tachycardia were asked for participation in the study. Initially, patients who were 18–40 years old were recruited, but due to the slow inclusion rate, the upper age limit was expanded to 50 years (after approval of an amendment submitted to the local ethics committee).

Papers II and III Patients from the pacemaker outpatient clinics at the Sahlgrenska University Hospital were identified with help from the site-specific part of the Swedish Pacemaker Registry. Patients (18–70 years old) with pacemakers due to sick sinus syndrome and no history of diabetes, coronary disease or bundle branch block were asked for participation.

Paper IV Patients (18–50 years old) with LQT1 seen at the outpatient clinics at Sahlgrenska University Hospital and the University Hospital of Umeå were recruited. For comparison, we used data from healthy individuals aged 20–36 years who participated in a previous study using atropine to study VR in response to pharmacologically induced HR increase (127).

3.2 HEART RATE MODIFICATION

Paper I A diagnostic electrophysiology catheter was placed in the coronary sinus, and the atrium was incrementally paced (RR was shortened by 10 ms for every beat) from just above baseline HR to just below the HR for the atrio-ventricular block (Wenckebach point), but not faster than 140 bpm, where the HR was held for 5 min. Thereafter, pacing was stopped and the SR returned.

Papers II and III Permanent pacemakers were used to increase HR abruptly from baseline SR or low-rate atrial pacing (AP) to AP or ventricular pacing (VP), aiming at an HR of 120 bpm for 8 min. Thereafter, pacing was stopped, and the HR was abruptly returned to baseline SR or low-rate AP.

Paper IV HR was increased via parasympathetic inhibition from intravenous administration over 30 s of an atropine bolus dose (0.04 mg/kg b.w.).

3.3 DATA COLLECTION USING VECTORCARDIOGRAPHY

The principles behind VCG are described in more detail in the introduction; here, we focus on the specific parameters used in this thesis. For VR duration, QT and QT_{peak} are calculated from a vector magnitude QRST complex derived from the three orthogonal leads (X, Y and Z, see Figure 7). The VCG system (CoroNet II, Ortivus AB, Danderyd, Sweden) automatically annotates the onset, offset and peak for QRS and T waves. The annotated points were adjusted manually when required.

There are several possible VCG measures of VR dispersion, and we used the following three: T amplitude [μV], T area [μVs] and the ventricular gradient (VG, μVs). The T amplitude is the maximal vector of the T loop (Figure 8), and the T area is a composite of the T area measures in the three orthogonal leads (X, Y, Z); both reflect global VR dispersion (128). The VG (or QRST-area) is the vectorial sum of the QRS and T area vectors and a measure of the dispersion of the action potential morphologies; see Figure 7 (129-131).

T area and T amplitude reflect differences in the action potential morphology and repolarization time. On the other hand, VG is a measure that displays global dispersion in repolarization but includes an activation sequence (QRS area vector) (130, 131). VG, as a measure of repolarization dispersion, is therefore theoretically independent of the myocardial activation sequence. However, since the activation sequence (depolarization) affects APD, it is questionable whether it is completely independent of the myocardial activation order (132).

It is important to remember that T amplitude, T area and VG are all global measures of dispersion. Therefore, observations in global VR dispersion cannot be attributed to repolarization characteristics in specific areas of the heart. In Figures 7 and 8, the principles for the recordings of these measures are illustrated.

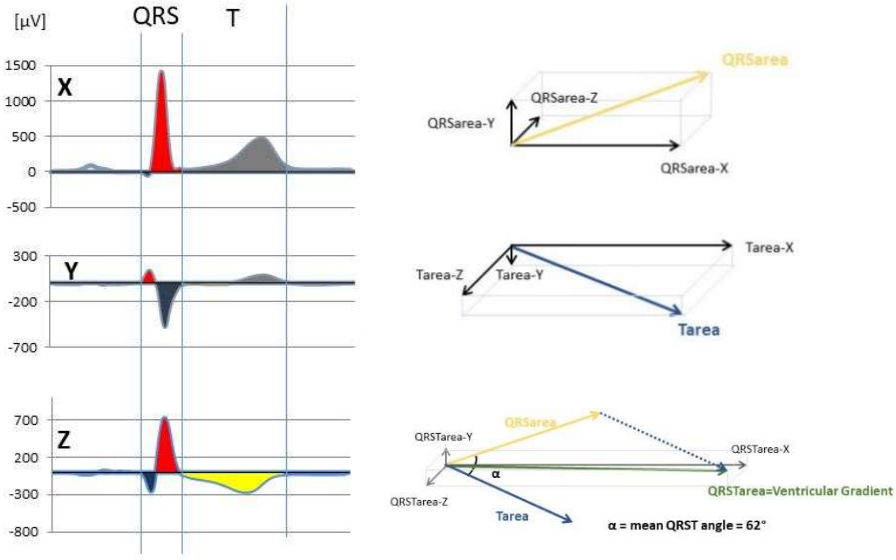


Figure 7. *To the left is the ECG derived from the X, Y and Z orthogonal leads. To the right: T area is the composite vector of the T areas in X, Y and Z, and the QRS area vector is the vectorial sum of the QRS area vectors in X, Y and Z. The VG (QRST area) is the vectorial sum of the QRS and T area vectors. The VG is therefore influenced by activation sequence (QRS area vector) and repolarization time (T area vector); hence, it is, theoretically, a global measure of the heterogeneity of APD. Adapted and modified under a Creative Commons license (CC BY 4.0; https://creativecommons.org/licenses/by/4.0/) from reference (129). URL: https://doi.org/10.1016/j.jelectrocard.2020.05.013 Copyright: Elsevier.*

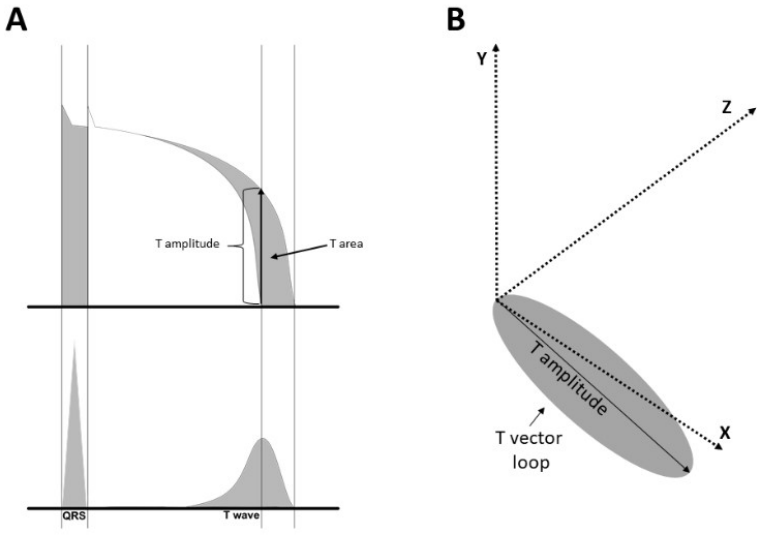


Figure 8. *A: A simplified illustration of how T amplitude and T area are created by differences in activation time and APD in the myocardium and how they relate to the T wave on the ECG. B: T amplitude is the maximum vector amplitude that can be inscribed in the T wave loop. See also Figures 1 and 7. Panel A is adapted and modified from an original figure in Vahedi F: Vectorcardiographic evaluation of ventricular repolarization in healthy individuals and LQTS mutation carriers. Figure 5, PhD thesis, Sahlgrenska Academy, University of Gothenburg, Kompendiet, Gothenburg, Sweden. ISBN 978-91-628-8733-9, with permission from author.*

3.4 CURVE FITTING PROCEDURES

A crucial part of the data processing was to obtain curves fitted to the individual raw data from the adaptation of both VR duration (QT and QT_{peak}) and dispersion measures (T amplitude, T area and VG). This was necessary to identify and, in a reproducible way, collect quantitative measures of the adaptation process, such as T90 End, immediate response (IR) and tau (τ). These measures are explained below and in Figure 9. In Paper I, we quantified QT and QT_{peak} adaptation by the use of T90 End. T90 End is the time to reach 90% of the difference between the baseline value and the end value. In Paper

II, we added IR and τ. These measures allowed us to further describe QT and QT_{peak} adaptation, since IR is the first step response in QT and in QT_{peak} at the sudden increase in HR, τ the time constant of an exponential function and T90 End 'covers' both IR and τ. This procedure followed the method described previously by Seethala et al. (60). In Paper III, a more complex curve fit procedure was developed based on a double exponential function with the addition of three different linear functions. This is explained in more detail in Paper III (see Figure 2 in Paper III). The QT and QT_{peak} adaptation after atropine differed slightly from that during incremental pacing in Paper I; Figure 9 C. The curve fitting procedure in Paper IV was therefore somewhat different from that described in Papers I and II.

3.5 STATISTICS

Mean with standard deviation (SD) and median with inter-quartile range (Q1–Q3) were used for descriptive purposes. Non-parametric statistical tests were used in all four papers.

In Paper I, the Wilcoxon signed rank test was used for testing differences between QT and dispersion measures. Spearman's rank correlation was used for the analysis of the correlation of adaptation time for RR vs QT and dispersion measures.

In Paper II, we used the Wilcoxon signed rank test for paired comparisons (QT and QT_{peak} adaptation in response to HR increase vs HR decrease) and the Mann-Whitney U test for non-paired analyses (QT and QT_{peak} adaptation following AP vs VP). We used the intra-individual standard deviation (s) to calculate the coefficient of variation (C_V) and to assess the time-dependent variability in QT and QT_{peak} adaptation (see Paper II for a detailed description).

In Paper III, Friedman's test with Dunn's test for post hoc analysis was used for comparison of quantitative measures of dispersion between T amplitude, T area and VG for AP and VP, respectively. The Mann-Whitney U test was used for comparison between AP and VP (unpaired comparison).

In Paper IV, the Mann-Whitney U test was used to test the differences in QT and QT_{peak} adaptation between LQT1 patients and healthy subjects.

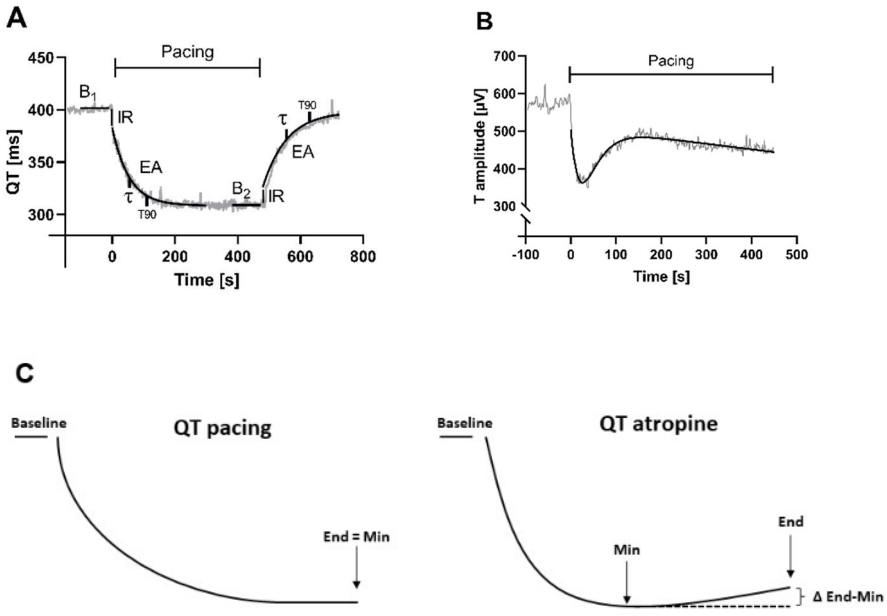


Figure 9. *Curve fitting procedures. In panel A, the fitted curves comprise an immediate reaction (IR) and an exponential adaptation (EA) following abrupt start of pacing. The time constant (τ) is the time constant of the exponential function, and T 90 End is the 'time constant' for 90% of the whole reaction from baseline to the end value. Panel B shows the curve fit to the tri-phasic adaptation response in T amplitude following abrupt atrial pacing. A more complex curve fit procedure was necessary to accommodate the tri-phasic response in global VR dispersion measures (described in detail in Paper III). In panel C, comparison of the reaction patterns in QT following incremental pacing vs. atropine injection are shown; a slight rebound is seen in response to atropine. The reason for this is a slight rebound in HR preceding the QT reaction. This was overcome by using the complex curve fit from panel B in order to identify the true min point with high precision. Thereafter, a mono-exponential function could be applied to the reaction from baseline to min. Panel A is reproduced from Paper II, with permission from the American Physiological Society.*

4 RESULTS AND CONCLUSIONS

Paper I We enrolled 27 subjects, and in nine (33%) of them, the study protocol was successfully completed, with recordings suitable for analysis. The high rate of failed protocols was mainly due to intermittent AV-block during steady state pacing at an HR close to the Wenckebach point at baseline. The HR, on average, increased from 74 to 122 bpm. The median (Q1–Q3) time for QT adaptation was 85 (51–104) s, in line with previous studies. The VR dispersion measures (T amplitude, T area and VG) showed a tri-phasic response pattern. When pacing was halted, VR dispersion returned suddenly towards baseline with a tendency to "overshoot" in T amplitude. This study confirmed hysteresis in QT adaptation in young study subjects without structural heart disease. The complex and tri-phasic adaptation in dispersion measures following incremental AP was a novel finding.

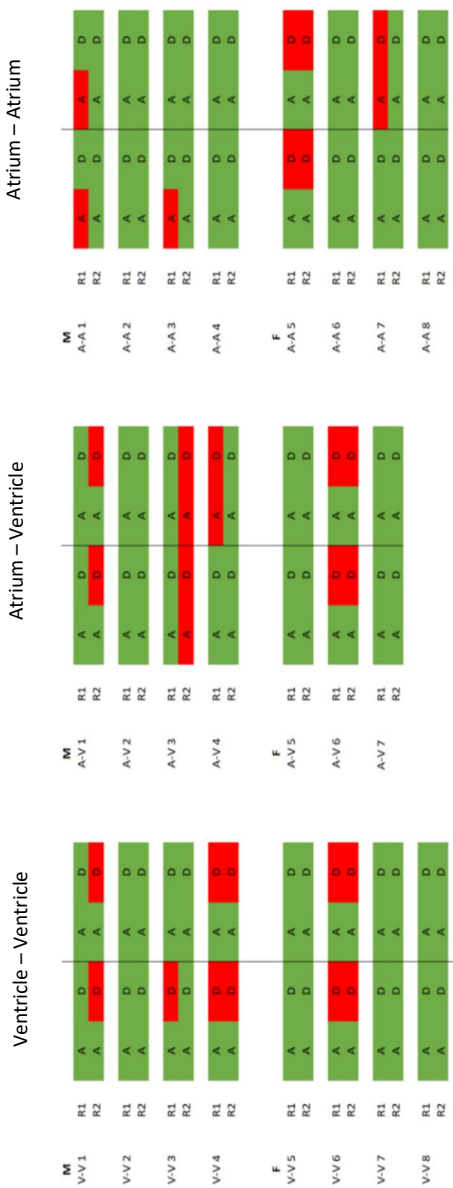
Paper II In the pacemaker study, 32 subjects were included, and in 25 (78%) subjects, sufficient quality was obtained in both registrations. The study experiments were performed with an average interval of 143 days. In Figure 10, the selection of study registrations is shown. We found that during AP, QT and QT_{peak} took longer to adapt following HR decrease compared with HR increase, which is a novel finding. For QT (T90 End), the difference was mean (SD) 24 (27) s (p=.006). We also found that the adaptation of QT and QT_{peak} to changes in HR is a fairly reproducible and robust process within individuals but varies between them. In response to increasing HR the intra-individual time-dependent coefficient of variation for QT adaptation (T90 End) was 8% for AP and 10% for VP.

Paper III This is the second paper in the pacemaker project (together with Paper II), and from a total of 32 registrations described above, 21 were selected for analysis of VR dispersion measures (T amplitude, T area and VG). We found that VR dispersion adapts to changes in HR in a tri-phasic pattern, and that there are profound differences in the adaptation between AP and VP, that is, activation-dependent differences. Our results confirm and extend previous knowledge from animal and human studies. The initial rapidly undulating phase in VR dispersion adaptation possibly reflects a time period of increased vulnerability to ventricular arrhythmias.

Paper IV We investigated the adaptation of RR, QT and QT_{peak} in 18 LQT1 patients and 28 healthy subjects (controls) from a previous study. The study procedures were performed as outpatient visits without complications but with expected side effects from atropine. The time for QT adaptation was

significantly shorter in patients with LQT1. We believe that the shorter adaptation time in LQT1 reflects the impaired function in the ion channel protein for I_{Ks} and that a stress test based on atropine response could help identify patients with a low-risk phenotype close to that of healthy individuals.

Figure 10.



The figure shows the selection of study registrations in Paper II for each study subject. For each study participant, there are two registrations (first and second study visit: R1 and R2) for QT & QT_{peak} and for acceleration (A) & deceleration (D). Green fields show registrations of good or acceptable quality, and red fields show registrations discarded for quality reasons. Atrium-Atrium: pacing in the atrium at both study visits; Atrium-Ventricle: pacing in the atrium at the first visit and in the ventricle at the second visit; Ventricle-Ventricle: pacing in the ventricle at both occasions. F: female, M: male.

5 DISCUSSION

In Papers I–III, we investigated the normal physiology in cardiac electrophysiological adaptation to rate change by electrical stimulation (incremental and abrupt). We confirmed previous findings of prolonged adaptation in response to decreasing vs increasing HR in VP and showed that this difference is also seen with AP. Additional novel findings are that the intraindividual variability in repolarization adaptation is relatively robust and that the dispersion in repolarization adapts in two or three distinct phases in response to HR increase. In Paper IV, the main finding was a faster QT adaptation time in subjects with LQT1 compared with healthy adults, following HR increase via atropine injection.

5.1 RATE ADAPTATION OF VR DURATION

In this thesis, three models for HR acceleration were used: incremental pacing, abrupt start of pacing and atropine bolus injection. In Papers I and II, we found that the adaptation of QT and QT_{peak} to a fast incremental or an abrupt increase in HR takes approximately 100 s in humans. These findings are consistent with previous human studies and simulations, but we investigated a larger sample size than earlier studies. Interestingly, in Paper I, the median (Q1–Q3) T90 End for incremental pacing was 18 (12–25) s for RR change and 85 (51–104) s for QT, and in Paper II, where HR change was abrupt, the mean (SD) for the T90 End QT was 103 (22) s. The change in HR was comparable (from 74–122 bpm in Paper I vs from 64–116 bpm in Paper II), suggesting that a brief incremental HR increase does not prolong QT adaptation compared with an abrupt HR increase. The median T90 End QT following incremental pacing was actually shorter than the mean T90 End QT in response to an abrupt change in HR, possibly because the adaptation process comprises two different phases: the immediate response and the 'slow' adaptation (10s–100s of seconds) (60). The immediate response in QT adaptation is very closely linked to cardiac restitution, and restitution is a response to two inputs: the immediately preceding diastolic interval and the short-term history of preceding diastolic intervals (51). An incremental increase in HR can therefore be regarded as a summation of restitution instants until a steady state HR is reached, but seemingly the slow phase of the VR duration is 'initiated' already at the start of incremental pacing.

The average T90 End for QT in healthy controls was longer following atropine—mean (SD) 149 (28) s—compared with both abrupt and incremental pacing. T90 End RR was 22 (9) s following atropine bolus, which is only

marginally longer than that in Paper I. The prolonged adaptation time in response to atropine is therefore suggestive of an effect of parasympathetic withdrawal on QT adaptation or hysteresis, since, in response to pacing, the balance between sympathetic and parasympathetic input is (in theory) not affected.

Further, in Paper II, we found that adaptation to decreasing HR was significantly longer than that for increasing HR. This was also the case in Paper I, but the difference was not significant. These findings corroborate earlier data on QT adaptation; the same pattern is apparent in human tissue in vivo and in silico (57, 58). There are studies that report increased QT hysteresis in ischemic heart disease (133, 134). Significant coronary disease is unlikely to affect the results in our study groups, and we have instead conveyed the theory of a physiologically prolonged recovery phase after increased HR that involves accommodation of ion concentrations, ion channel function and 'metabolic demands'. In Paper I, the participants were younger, and it is possible that the longer adaptation time following HR decrease reflects a 'metabolic debt' that becomes more pronounced at a higher age.

In a comprehensive review by Gravel et al., studies on QT hysteresis (i.e. QT adaptation) were categorised by four definitions of models based on the experimental design (135). The definitions were mostly based on how QT hysteresis was measured and how the history of RR intervals was taken into account in computing models for QT hysteresis. While categories IIa, IIb and III deal mainly with Holter recordings and exercise tests, category I describes studies where the preceding RR interval is controlled in the protocol and therefore not included in the function describing QT adaptation. This is the case in our studies, and the six papers in the review all include, at least as a part of the protocol, pacing (46, 57, 60, 67, 68, 135, 136). The benefit of pacing, or pharmacologically increasing HR, when assessing a QT/RR-relationship is that the baseline RR can be strictly controlled and the QT response can be described as a mono-exponential function without the need for weighted input from RR history. This is of importance since ultra-rapid CM affects QT adaptation (41). The models used to evaluate QT adaptation based on, for example, Holter recordings therefore need to take into account previous RR history and its influence on QT adaptation (62, 137).

Pacing and/or pharmacological protocol studies are difficult to set up compared with Holter recordings. The main difference is that in experimental studies, the baseline and change in HR is controlled, but in Holter-based assessments, the HR history has to be accounted for in a mathematical model. If QT/RR studies are supposed to be utilised as a clinical tool, would it be

possible to use information from pacing/pharmacological protocols to evaluate results from Holter-based studies? If QT adaptation should be used as a prognostic marker, assessment in larger groups may be more easily achieved by Holter registrations. However, we believe the most accurate assessment of QT adaptation would be achieved when the preceding RR is controlled at steady state for several minutes and HR is changed stepwise.

In conclusion, and regarding the adaptation of VR duration, our results corroborate previous findings and add to the existing knowledge. A new finding comes from the comparison between AP and VP, where we found the tendency for QT adaptation to be longer in response to VP; however, this finding was not statistically significant. The time-dependent variability in the adaptation of QT and QT_{peak} is also a novel finding, discussed in the following section.

5.2 TIME-DEPENDENT VARIABILITY IN VR DURATION

The time-dependent variation in electrophysiological variables has been previously investigated in healthy individuals. For example, QTc showed a low coefficient of variation (C_V, 2%, comparable to our results in Paper II) (138). 'Dynamic' measures, such as the effective refractory periods of the AV node and the Wenckebach point, showed higher variability (12% and 7%, respectively). Lecoq et al. investigated the QT/RR relationship in healthy subjects, at baseline and exercise, and in response to pharmacological HR increase by isoproterenol and atropine. In 4 study subjects, repeated measures of QT/RR at baseline and exercise were made after 10 months, and there were no significant differences between the exponential equations fitted to the QT/RR plots in these subjects (139). In Paper II, the C_V for QT adaptation (T90 End) following an increase in HR was 8% for AP (n=6) and 10% for VP (n=9); following a decrease in HR, the corresponding values were 8% (AP, n=7) and 15% (VP, n=4). Due to a lower T amplitude and difficulties with the correct annotation of T end following cessation of VP a lower number of registrations were suitable for variability analysis in the VP group, for decreasing HR. However, the results for QT in response to an abrupt increase in HR are in the same range as the variability in the conduction and refractoriness of the conduction system (138), which are more 'robust' measures but under the same influence as QT and QT_{peak} (autonomic nervous system tonus and electrolyte concentrations).

How can these findings be used in the clinic and in research? There are several situations wherein the assessment of QT adaptation (or VR duration) could possibly be useful: 1) for prognostic evaluation in individuals with a disturbance in VR (e.g. LQTS), 2) to assess the variability of measured QT adaptation in a clinical trial or in academic research, etc.

5.3 VR DISPERSION IN ADAPTATION TO INCREASED HR

The investigation of QT adaptation and its time-dependent variability was an important target for this thesis, but another equally important objective was to characterise VR dispersion by global measures in response to changes in HR. In a previous study from our research group, a complex, biphasic response in VR dispersion was seen when HR was increased by the use of atropine in healthy individuals (39). In Paper I, we attempted to reproduce the pharmacological HR increase with incremental pacing, and the response in VR dispersion was similar. In Paper III, the reaction pattern following both AP (normal ventricular activation) and VP (abnormal ventricular activation) was explored in more detail, a comparison which has not been published before.

The concept of VR dispersion is complex, but it refers to the time differences in repolarization in different regions in the ventricles of the heart. As discussed in the introduction, the time for local repolarization depends not only on the actual duration and morphology of the action potential but also on the sequence of activation (27, 28). This can be illustrated by the results from a seminal experiment on a dog by van Dam and Durrer in 1964. When the dog's heart was activated simultaneously by a field impulse, the following T wave was morphologically very different compared with the T wave in normal activation (31). That is, the voltage differences that give rise to the T wave (i.e. repolarization heterogeneity) are the result of 1) differences in activation time and 2) differences in repolarization duration.

It then follows that VR dispersion can also be affected by changes in: 1) the sequence of activation as well as 2) local APD and morphology. Changes in the sequence of activation are seen, for example, in bundle branch block, VP or activation from a focus in the ventricles (VT or ventricular extra beat). APD can be influenced by HR, electrolyte imbalance, ischemia, drugs and ion channel impairment. Further, the influence of HR on VR dispersion should be differentiated into two different entities: 1) single-beat perturbations, which create immediate effects in APD or QT from the shortened CL, and 2) the VR adaptation to a sustained change in HR.

From the viewpoint of VR dispersion, the difference between restitution and adaptation (in QT or APD) is that a single RR change causes immediate differences in tissue refractoriness (81), while regional differences in the APD adaptation time cause dynamic changes in repolarization dispersion during the VR adaptation to a new HR (89, 90, 97).

In this thesis, we are primarily concerned with the influence of HR change (Papers I and III) and the activation site (Paper III). In the literature, many experiments support the potential arrhythmogenicity of single beat perturbations, as different restitution properties create dispersion. Fewer studies have investigated the mechanistic role of VR adaptation in arrhythmias. Bueno-Orovio et al. investigated dispersion in response to a new HR, and via simulation of the data, the differences in repolarization time achieved during the study were enough to induce ventricular arrhythmia (89, 90).

In humans, there is evidence that the activation pattern from pacing alters VR in a way that increases dispersion and can be arrhythmogenic (140, 141). In dogs, endocardial mapping of MAP has revealed that VP causes increased dispersion and that it is a function of differences in the activation time (96, 142). Endocardial mapping studies in humans (MAP) show that the repolarization process is more complex than 'last to depolarize – first to repolarize', but that there is an inverse relation between activation time and APD (26, 78, 143). It would thus be expected that a difference in the activation of the ventricles would cause increased dispersion; this was also a finding in Paper III. However, even though there were pronounced differences in VR dispersion in response to HR increase by AP vs VP in global measures, an adaptation in three different phases was observed for both.

As described in detail in the introduction, in animal studies and in invasive human studies, a bi-phasic adaptation pattern is seen following an abrupt increase in HR, measured as APD or activation-recovery interval (ARI: the duration of a unipolar electrogram) (89, 90, 97). Following HR increase by atropine, a similar pattern was seen in the T amplitude in a previous study by our research group (39). In Papers I and III, this pattern is seen for most registrations; it is the most pronounced for T amplitude but is also evident in T area and VG. In Paper III, we convey the theory that the biphasic response seen in invasive measures is translated to a global 'scale' in our global measures of dispersion. The different repolarization adaptation (e.g. local APD) times between different areas of the heart give rise to a bi- or even tri-phasic adaptation pattern, described in detail in Paper III. In Figure 11, we describe the similarities between invasive and non-invasive measures of VR dispersion following a sudden increase in HR.

In conclusion, our results show increased dispersion in response to increased HR by VP compared with AP, but a complex bi- or tri-phasic adaptation pattern reflecting local heterogeneity in AP adaptation is evident both for 'normal' (AP) and 'abnormal' (VP) ventricular activation.

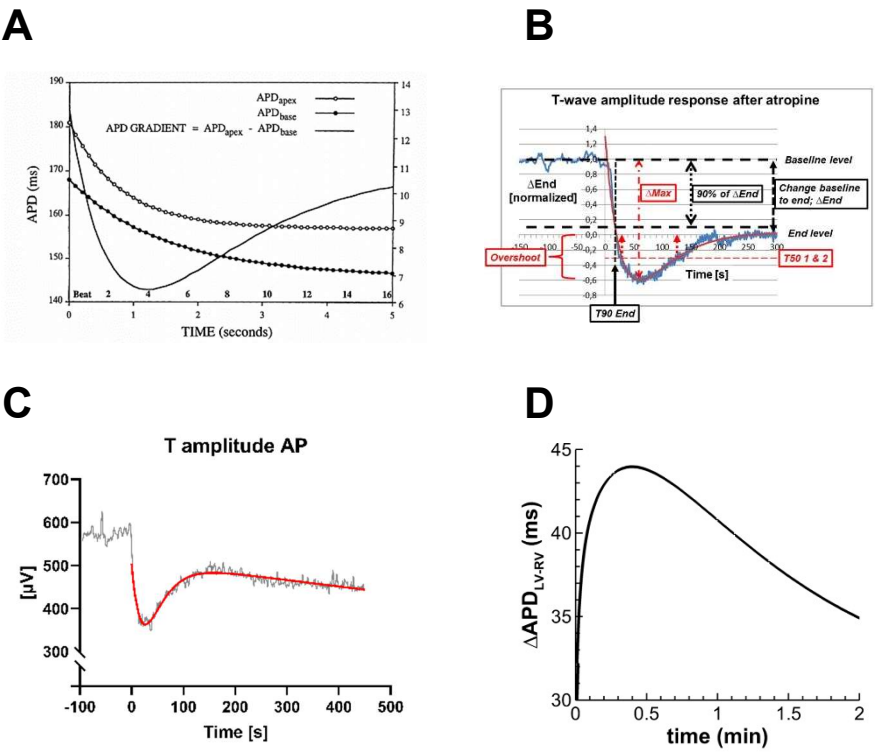


Figure 11. *Bi- or tri-phasic adaptation in VR dispersion in different 'scales' and models.* ***A****: The time-dependent change in the difference in APD between the apex and base in a Langendorff-perfused guinea pig heart.* ***B****: The reaction in T amplitude in response to HR increase from a bolus dose of atropine in a healthy human research subject.* ***C****: The reaction in T amplitude following an abrupt start of atrial pacing (HR increase to 120 bpm).* ***D****: Interventricular differences in the activation-recovery interval in a human research subject in response to an abrupt increase in HR (to 120 bpm). Thus, a pattern of bi- or tri-phasic adaptation is evident in regional invasive measures as well as on a global dispersion 'scale'. Presumably, the tri-phasic response in the global VR dispersion in Papers I and III reflects regional dispersion in repolarization adaptation times throughout the heart.* ***Panel A*** *is reproduced from reference (97), with permission from Wolters Kluwer Health, Inc. URL: https://doi.org/10.1161/01.CIR.84.3.1333* ***Panel B*** *is reproduced from reference (39), with permission from Elsevier. URL: https://doi.org/10.1016/j.jelectrocard.2017.03.014.* ***Panel D*** *is reproduced and modified under the terms of the Creative Commons Attribution License from Figure 3, Panel C in (89). URL: https://doi.org/10.1371/journal.pone.0052234*

5.4 ELECTRO-MECHANICAL ASPECTS OF VR ADAPTATION

The adaptation of cardiac repolarization should not be regarded as an isolated bioelectrical process. Instead, slow adaptation of the VR duration (QT-hysteresis) entails smooth accommodation of the filling and emptying of the ventricles as well as the time for coronary perfusion (in diastole). As considered briefly in Paper III, the relation between the complex adaptation patterns for VR dispersion and the adaptation in mechanical function (i.e. the relation between mechanical systole and diastole) has not been investigated. However, if we consider the initial bi-phasic response in T amplitude, T area and VG as a result of differences in regional adaptation time, the same regional differences should also be manifested as heterogeneity in contraction-relaxation.

The close relationship between electrical and mechanical restitution has been shown using invasive measures in humans (42). However, dispersion, or heterogeneity, in electro-mechanical functions has been investigated mainly in experiments on cardiac tissue and via computer modelling. From the early work on cat papillary muscle by Tyberg et al. (144), the concept of the cardiac muscle duplex approach to investigate regional heterogeneity in mechanical function has been thoroughly investigated by Markhasin et al. (99-102, 145). In their work, a major issue is the interaction between coupled muscle elements with temporal differences (heterogeneity) in their mechanical activity (101, 102). A pivotal observation from their vast experimental experience is that the biomechanical properties of the contracting elements are changed by mechanical coupling (100, 146). They also found that the electro-mechanical function is modulated by the activation sequence, and an important conclusion is that electro-mechanical heterogeneity is a necessary feature of normal heart function (100), as suggested by Katz and Katz in 1989 (98).

The clinical importance of proper electro-mechanical coupling is illustrated by its malfunction in LQTS, a disease often regarded as an ‘electrical disorder’. However, several studies have shown increased mechanical dispersion and impaired diastolic function in LQTS (147-150). In healthy individuals, QT is slightly shorter than mechanical systole (151). The relation between mechanical systole and QT is referred to as the electro-mechanical window (EMW=mechanical systole minus the QT interval), and a negative EMW is suggested to be a marker of arrhythmia risk in animal studies (152, 153). In a multicentre study on 244 patients with LQTS, a negative EMW was an independent predictor of arrhythmic events (154). Further, in a large cohort of healthy individuals (n=1140), longer QTc was associated with increased

mechanical dispersion, however, longer QTc was also related to better longitudinal strain values (155). These findings point to the significance, and complexity, of proper electro-mechanical coupling.

The papers in this thesis have not primarily addressed the issue of electro-mechanic dispersion; however, in Papers I and III, a complex bi- or tri-phasic adaptation pattern is seen following HR increase in study subjects with healthy ventricles. We believe that this is a global reflection of the regional VR dispersion seen in previous animal and invasive human experiments on a smaller scale (89, 90, 97). The possible significance of a relationship between our findings and dispersion in electro-mechanics is perhaps best understood from a quote from Markhasin et al.: "Our studies support the notion that 'well-organized' inhomogeneity is a pre-requisite for normal cardiac function, and suggest that electrical and mechanical heterogeneity are normally 'synergistic'."(100) In other words, heterogeneity from the level of ion channel density and intrinsic APD differences can be transferred to optimised adaptation of contractility and relaxation in the whole heart in response to HR change.

We therefore suggest that a computerised 'whole heart' model of dispersion could reproduce our findings in global VR dispersion by taking into account regional dispersion in repolarization time in response to HR changes. In a clinical context, investigating patterns of adaptation in VR dispersion in patients with structural heart disease and ICD could reveal adaptation patterns associated with increased arrhythmia risk.

5.5 ULTRA-RAPID CARDIAC MEMORY IN RESTITUTION AND ADAPTATION

Most cardiologists probably associate the term 'cardiac memory' (CM) with the T wave vector adaptation to the preceding activation wave front vector, i.e. negative T waves in leads II, aVF and III after a prolonged period of apical ventricular pacing. This is a function of activation-induced CM (which is further divided into long-term and short-term, see the introduction). Previously, 20 minutes was the shortest observed period to evoke activation-induced (short-term) CM (156), but in Paper II, we found that a VP period as short as 8 minutes was sufficient to induce transient activation-induced memory; hence, this is a novel finding. However, this was a post hoc analysis, and the focus of this thesis is on the importance of ultra-rapid CM in VR adaptation to changes in HR.

In contrast to activation induced-memory, ultra-rapid CM is an interval-dependent memory function (see the introduction). The molecular mechanisms behind ultra-rapid CM are not fully disclosed, and there are important differences between the mechanisms controlling restitution (APD/QT reaction to alterations in previous RR intervals) and adaptation to sustained rate changes. In a comprehensive review, Eisner et al. provide an overview of the ultra-rapid memory effects as a function of incomplete recovery of voltage-gated ion channels and ion concentrations (48). For restitution, incomplete recovery of the L-type calcium current (I_{CaL}) and incomplete deactivation of potassium currents (I_{Kr}, I_{Ks}) together with decreasing current in the sodium-calcium exchanger (NCX) were suggested as important contributors to immediate APD/QT shortening (48, 157). Regarding the slow changes corresponding to QT adaptation in our studies, this is probably an effect of ionic accumulation more than the kinetics of membrane currents, since the latter are too fast to explain hysteresis over 2–3 minutes (48). Based on data from experiments on ventricular tissue from humans and dogs together with computer simulations, Pueyo et al. identified the effects on I_{CaL} and I_{Ks} as the most important contributors to the fast phase of adaptation. During the slow phase of APD/QT adaptation, their results also point to the intracellular sodium concentration, sodium-potassium pump and NCX as important for APD/QT shortening (58). However, there are conflicting data on the importance of the sodium-potassium pump for APD/QT adaptation (48).

As discussed in the previous section, the heart muscle shows significant dispersion in electro-mechanical properties (101, 158). However, we convey the theory that the tri-phasic adaptation pattern in Papers I and III is a consequence of different adaptation times in different regions of the heart. Possibly, this reaction pattern can also be influenced (attenuated or reinforced) by myocardial dispersion in ultra-rapid memory. That is, based on regional differences in voltage-gated ion channels and active pumps, the incomplete recovery of currents and decreased ion transport that constitute the ultra-rapid CM would presumably also show regional variation. What benefits would the influence of ultra-rapid CM on regional adaptation time provide? Returning to the Duplex experiments conducted by the Markhasin group and the paradigm-shifting paper by Katz and Katz (discussed in the section above), in the pumping function of the heart, heterogeneity in mechanical properties is necessary to achieve optimal function (98, 99). However, as pointed out by Solovyova et al., myocardial heterogeneity is not a ‘static’ property of the ventricular wall (99). Instead, slow adaptive changes in electro-mechanic coupling and calcium handling tune electro-mechanical heterogeneity to optimise overall heart function (99, 102). Hysteresis (delay) is an essential function in such physiological electro-mechanical adaptation of the heart (see

Papers I and II) to accommodate slow adaptation of the electro-mechanical function by gradual shortening of APD, and hysteresis is a function of ultra-rapid CM (41, 48, 58). Thus, ultra-rapid memory functions could presumably be of importance in the heterogeneous electro-mechanical adaptation of different myocardial regions. We therefore propose that the tri-phasic pattern of VR adaptation seen in Papers I and III is a reflection of regional dispersion (heterogeneity) in repolarization and a heterogeneous adaptive process, and that ultra-rapid memory functions are important modulators of such 'adaptive heterogeneity'.

5.6 QT ADAPTATION IN A REPOLARIZATION DISORDER (LQTS)

In LQTS, patients with intermediate or high arrhythmia risk are usually easy to identify; for example, QTc > 500 ms points to a high risk regardless of genotype and the occurrence of syncope during beta blocker treatment entails a high risk of future events (120, 121, 159, 160). On the other hand, approximately 25% of all patients with LQTS have normal QTc and a low risk for life-threatening cardiac events. In a study including 469 LQTS patients with normal QTc (QTc ≤ 440 ms) the event rate for aborted cardiac arrest and SCD was 0.13% per year (from birth through age 40 years) (125).

Although the risk in LQTS individuals with normal QTc is low, they are at a significantly increased risk of malignant arrhythmias compared with their healthy relatives (125). Risk assessment and therapy decisions are especially challenging in this group of patients. Development of additional phenotypic characterisation beyond QTc at rest is therefore of need.

Even though QTc in the fourth minute of stress test recovery is used as a diagnostic criterion, routine risk assessment does not include evaluation of the QT response to increased HR (118, 120). Therefore, an important question is, 'Could the QT response to HR increase help in risk assessment and prognostic evaluation, especially in LQT1, where the arrhythmia risk is linked to exercise or stress?' In a Holter recording study by Halamek et al., the QT interval adapted faster to RR changes in LQT1 patients than in healthy individuals (161). In contrast, following exercise testing, QT hysteresis increased in LQTS vs. controls, as reviewed by Gravel et al. in (69). However, data on LQTS-mutation type were available in only two of the six studies on QT hysteresis following exercise (162-167). In these two studies, recovery was prolonged in LQT2 vs. LQT1 and controls (162, 163). Interestingly, the beta blocker

treatment seemed to normalise hysteresis in two studies (163, 167), which was also seen in a post hoc observation in Paper IV.

In Paper IV, we found significantly shorter QT adaptation to HR increase by atropine in patients with LQT1. This is in line with the results of Halamek et al. (161). In LQT1, the pathogenic mutation causes a reduction in the current of the slow inward rectifying potassium channel (I_{Ks}). At resting HR, the contribution from I_{Ks} to repolarization is small in humans (168). However, at increasing HR, and especially in situations with sympathetic stimulation, I_{Ks} plays an important role in APD/QT shortening (76, 168). This is why symptoms in LQT1 generally occur in these situations (126). Further studies are needed to provide a mechanistic explanation for the shorter QT adaptation time in LQT1 human subjects. However, from the results in Paper IV, we can conclude that the hysteresis function in QT adaptation is strongly associated with the I_{Ks} function, and that QT adaptation therefore possibly constitutes a method for individual phenotypic characterisation in LQT1.

Atropine testing has been proven safe, at least for asymptomatic LQT1 individuals. The QT adaptation time can hopefully be a valuable tool to identify the true low-risk population of asymptomatic individuals with LQT1 and normal or near-normal QTc.

5.7 METHODOLOGICAL ASPECTS AND LIMITATIONS

There are several important limitations to the studies in this thesis, and for a thorough review, the reader is referred to the individual papers. However, there are methodological considerations that were of significance throughout the thesis studies.

Atrioventricular conduction disturbances. Especially in Paper I, episodes of Wenckebach block precluded the completion of the study protocol. The maximum steady-state HR was set close to the initial Wenckebach point, which unfortunately did not provide a large enough margin to allow for 1:1 conduction throughout the protocol in many study subjects. In Papers II and III, AV conduction problems were a minor issue, probably because of the lower maximum HR, and in Paper IV, there were no episodes with AV block due to the pharmacological modulation of the autonomic nervous system that enhanced AV conduction.

T end detection difficulties. The customised software used in the annotation of PQRST automatically excludes pacing spikes, creating an artefact, which (when it appears at the end of the T wave) can interfere with the T end annotation. In addition, at high HRs, the merging of the P wave with the T wave from the preceding beat was also a source of potential error in the detection of T end (although the tangent method was used). Manual annotation was necessary in several recordings. In Paper II, the T wave after VP was flattened, which precluded both automated and manual T end annotation in several registrations.

Curve fitting procedures. In all papers, curves were fitted to the recorded data, and registrations were not used if the curve approximation was unable to adequately fit the set of data points. The evaluation of the accuracy of the fitted curves was done prior to the calculation of quantitative measures and statistical analysis.

5.8 ETHICAL CONSIDERATIONS

All four papers were based on results from human experiments conducted in three different projects. The research projects were performed in accordance with the principles of the Declaration of Helsinki. The research projects are all approved by the regional ethics committee: Paper I (Dnr: 095-14), Papers II and III (Dnr: 1010-16) and Paper IV (Dnr: 1021-15). Study subjects participated voluntarily, and all of them provided written informed consent.

In Paper I, the invasive ablation procedure was prolonged by approximately 20 minutes. The pacing protocol was tolerated well by the study subjects, however the high rate of failure is worth considering from an ethical viewpoint. Could the number of successful protocols have been increased if the protocol was modified? If so, could the results have been more robust? It is an obligation for scientists to ensure that the results from human and animal experiments give a yield as high as possible. Perhaps, lowering the highest pacing CL would have allowed for more protocols to pass without difficulties in AV conduction. However, a change in protocol during the study would also have entailed a risk of decreased significance of the results.

In Papers II and III, the procedure was completely non-invasive, and the highest pacing rate was 120 bpm. No study protocol was aborted due to study subject discomfort.

Paper IV used atropine through intravenous access to increase HR. Side effects included accommodation problems, tiredness and dry eyes and mouth. These

effects are common and expected, and occurred in both healthy control subjects and in study subjects with LQT1. However, participants with LQT1 carried an additional risk of ventricular arrhythmias following atropine injection. Since the participants were asymptomatic mutation carriers, the risk of arrhythmias was considered low. The study setting was prepared for the treatment of ventricular arrhythmias, and the experiments were supervised by at least one experienced cardiologist. In line with our assumptions regarding arrhythmia risk, all study procedures were uneventful.

6 CONCLUSIONS

The adaptation of VR to changes in HR is characterised by an exponential accommodation of the QT interval; however, during the time of this adaptation to a new steady state, a more complex reaction is seen in measures of global dispersion. This observation can perhaps, in part, explain the established correlation between changes in HR and arrhythmias. In subjects with LQT1, QT adaptation during HR increase was more rapid compared with healthy individuals. This difference could constitute the rationale for an 'atropine stress test' in LQT1 to further differentiate arrhythmia risk on the phenotype level, primarily to identify true low-risk individuals.

FUTURE PERSPECTIVES

This thesis has dealt with the adaptation of cardiac repolarization to changes in HR in humans. The initial complex response in VR dispersion adaptation is possibly a reflection of a vulnerable window to ventricular arrhythmias. A future research perspective is therefore to investigate whether there are certain patterns in the adaptation of VR dispersion that entail increased arrhythmia risk.

Another prospect is computer simulations of 'whole heart' models under the hypothesis that programmed regional differences in APD adaptation to HR change could reproduce a global tri-phasic VR adaptation pattern. That is, could our observations be explained in terms of ion channel dynamics and interactions using computer simulations? Possibly, the differences in VR dispersion adaptation between healthy and disease conditions (e.g. LQTS) could be reproduced in silico, with improved understanding of arrhythmia induction and maintenance as objectives.

Improvement of prognostic evaluation in LQTS patients is a future aim. We believe that the assessment of the QT adaptation time (QT hysteresis) can help identify individuals with the lowest risk of arrhythmias with potential implications for therapy decisions.

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