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Optical and Electrical Recordings from Isolated Coronary-Perfused Ventricular Wedge Preparations

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Abstract

The electrophysiological heterogeneity that exists across the ventricular wall in the mammalian heart has long been recognized, but remains an area that is incompletely understood. Experimental studies of the mechanisms of arrhythmogenesis in the whole heart often examine the epicardial surface in isolation and thereby disregard transmural electrophysiology. Significant heterogeneity exists in the electrophysiological properties of cardiomyocytes isolated from different layers of the ventricular wall, and given that regional heterogeneities of membrane repolarization properties can influence the electrophysiological substrate for re-entry, the diversity of cell types and characteristics spanning the ventricular wall is important in the study of arrhythmogenesis. For these reasons, coronary-perfused left ventricular wedge preparations have been developed to permit study of transmural electrophysiology in the intact ventricle. Since the first report by Yan and Antzelevitch in 1996, electrical recordings from the transmural surface of canine wedge preparations have provided a wealth of data regarding the cellular basis for the electrocardiogram, the role of transmural heterogeneity in arrhythmogenesis, and differences in the response of the different ventricular layers to drugs and neurohormones. Use of the wedge preparation has since been expanded to other species and more recently it has also been widely used in optical mapping studies. The isolated perfused wedge preparation has become an important tool in cardiac electrophysiology. In this review, we detail the methodology involved in recording both electrical and optical signals from the coronary-perfused wedge preparation and review the advances in cardiac electrophysiology achieved through study of the wedge.

Keywords

Electrophysiology; Pharmacology; Ventricle; Arrhythmias; Cellular

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1. Introduction

The electrophysiological heterogeneity that exists across the ventricular wall in the mammalian heart has long been recognized, but remains an area that is incompletely understood. Largely due to practical considerations, experimental studies of the mechanisms of arrhythmogenesis in the whole heart often examine the epicardial surface in isolation and thereby disregard transmural electrophysiology. Significant heterogeneity exists in the electrophysiological properties of cardiomyocytes isolated from different layers of the ventricular wall, and given that regional heterogeneities of membrane repolarization properties can influence the electrophysiological substrate for re-entry, the transmural axis is extremely important in the study of arrhythmogenesis. For these reasons, coronary-perfused left ventricular (LV) wedge preparations have been developed to permit study of transmural electrophysiology in the intact ventricle. Since the first report by Yan and Antzelevitch in 1996 [1], electrical recordings from the transmural surface of canine wedge preparations have provided a wealth of data regarding the cellular basis for the electrocardiogram (ECG) and the role of transmural heterogeneity in arrhythmogenesis. Use of the wedge preparation has since been expanded to other species including rabbits [2–5], pigs [6] and humans [7] using optical mapping studies. The isolated perfused wedge preparation has thereby become an important tool in cardiac electrophysiology. Blinded studies have established the rabbit wedge preparation as a thoroughly validated preclinical model [2–5]. In this review we describe in detail recording of both electrical and optical signals from the coronary-perfused wedge preparation and review the advances in cardiac electrophysiology achieved through study of the wedge.

2. ECG and Action Potential Recordings with Floating Microelectrodes Obtained from isolated Coronary-Perfused Canine Ventricular Wedge Preparations

The canine ventricular wedge preparation, first developed over 15 years ago [1], consists of transmural segments of the right ventricular (RV) or LV free wall. The tissues, excised and perfused through an epicardial coronary artery, are isolated in such a way so that the vessel is situated parallel and nearly equidistant to the top and bottom cut surfaces of the preparations (Figure 1A). The dimensions of the LV wedges typically range from $2 \times 1.5 \times 0.9$ to $3 \times 2 \times 1.5$ cm and RV wedges range from $2 \times 1 \times 0.9$ to $2.5 \times 1.5 \times 1.2$ cm.

In an effort to avoid the antero-lateral papillary muscle, LV wedges are commonly dissected from the antero-apical aspects of the ventricular wall with cuts made parallel to the distal diagonal branch of the left anterior descending (LAD) coronary artery. Wedges suitable for these studies can also be excised from the lateral and posterior walls and cannulated through the left marginal and postero-lateral (PL) branches of the circumflex artery (LCX), as well as from the inferior wall perfused through branches of the posterior descending artery (PDA) commonly branching from the right coronary artery (RCA). For wedges isolated from the RV, the right marginal branch of the RCA is used, which is typically observed in the mid-basal aspect of the free wall; no other segments with appropriate vessels are regularly found in the RV. The conus arteries, which derive from the RCA and/or LCX and supply the right ventricular outflow tract (RVOT), are usually not visible from the epicardial surface of the canine heart. During the cannulation procedure (for step-to-step details see Figure 1B), the preparations are perfused with a cardioplegic solution (consisting of a 4°C-Tyrode's solution [see below] containing 12 mM KCl). Following cannulation, wedges are transported to a pre-filled bath and perfused with a Tyrode's solution of the following composition (in mM): NaCl 129, KCl 4, NaH₂PO₄ 0.9, NaHCO₃ 20, CaCl₂ 1.8, MgSO₄ 0.5, and glucose 5.5. The solution is bubbled with 95% O₂ and 5% CO₂ and the perfusate is maintained at $37 \pm 0.5^\circ\text{C}$.

A suction line is used to keep the solution level 2–3mm above the top cut surface of the tissue. The perfusate is delivered at a constant pressure (40–50mmHg), which is initially set by adjusting the flow rate. The flow rate should be approximately 1–2ml/min per gram of tissue (or 8–14ml/min), although every preparation differs and the final perfusion rate is to maintain 40–50mmHg of pressure. The so-called “equilibration time”, which is approximately 1–1.5 hours, is the time it takes for the ECG parameters to stabilize (i.e. to reach a steady-state) following the stress that the preparation undergoes during the isolation procedure. ST segment depression is common after initial isolation of the wedge preparation. During the equilibration period the ST segment depression normalizes approaching an isoelectric potential. Preparations that continue to manifest an ST segment depression greater than 10 to 15% of the amplitude of the R wave after 1–1.5 hours of equilibration are considered not suitable for further study. The recording of a pseudo-ECG (see below) is therefore a critically important quality control feature.

In some preparations (~20–30%) a continuous rise in pressure occurs (a reflection of an increase in vascular resistance), which is usually not possible to stop by adjusting the perfusion rate. We believe that this phenomenon is the result of vasoconstriction of the coronary microcirculation in wedges that are not well perfused because the chosen artery does not adequately supply the preparation. This rise in coronary resistance may, on occasions, be a consequence of using a relatively high perfusion pressure at the outset, leading to irreversible accumulation of fluids in the extracellular space (edema). Either way, the resultant hypoperfusion leads to a rapid deterioration of the tissue within 2–3 hours associated with swelling, a decrease in the strength of contraction, a decrease in the amplitude of the ECG, widening of the R wave, and progressive development of ST-segment depression and in some cases frequent premature ventricular complexes (PVCs). Under these conditions, action potential (AP) impalement is difficult to achieve.

Despite adequate technique, 20–30% of canine ventricular wedge preparations are not optimal for experimentation. The reason for the high success rate (70–80%) is due to the presence of abundant collateral vessels in the canine heart (as opposed to the pig or the human heart) [8–10]. These anastomotic channels are typically located epicardially.

2.1. Pseudo-ECG and Floating Microelectrode Recordings

A transmural pseudo-ECG is recorded using either two Ag/AgCl half cells or simply two plain Ag/AgCl wires, Teflon-insulated (diameter 250µm) except at the tip (3–5mm). The electrodes are placed ~1cm from the epicardial (Epi) (+) and endocardial (Endo) (–) surfaces of the preparation and along the same axis of the (AP) recordings.

Transmembrane APs can be simultaneously recorded from the Epi (surface or immediate subsurface), Endo and sub-Endo (M cells) regions (~2–3mm from the Endo surface) using floating glass microelectrodes (Figure 1A and 1C-1). The electrodes are made of 125µm-silver wires Teflon® insulated except at the tip. One end of the silver wire is soldered to a jack electrical connector, which in turn is mounted to the microelectrode probe of the intracellular amplifier. The other end is inserted in the glass microelectrode (length ~3–4mm) filled with 2.7M KCl; the glass electrode and the silver wire are brought together with wax melted using a cauterizer. Floating microelectrodes are referenced to a ground wire placed at the bottom of the tissue chamber. Floating microelectrodes are necessary because of the vigorous contraction of the coronary-perfused wedge (see also Addendum to the Wedge Methodology: Pitfalls to Overcome; Online Supplement).

Basic stimulation is applied to the Endo surface in an effort to mimic the direction of physiologic activation of the ventricular wall using a pair of thin silver electrodes insulated except at the tips. Programmed electrical stimulation (PES) is commonly applied to the Epi

surface in an attempt to induce arrhythmias. In some cases, basic stimulation is applied to epicardium as well in order to amplify transmural dispersion of repolarization (TDR). The ECG and the AP signals are amplified, digitized (typically at 2kHz) and analyzed using an analog digital converter acquisition system (Spike 2 for Windows; Cambridge Electronic Design, Cambridge, UK).

2.3. Transmural Distribution of Action Potential Duration

The duration of the action potential duration (APD) measured at 90% repolarization (APD_{90}) is typically shortest in Epi cells and longest in M cells located in the deep subendocardium (Figure 1C-3). APD of Endo is usually shorter than that of the M cell, but longer than Epi. A sharp transition of APD is commonly observed between Epi and deep subEpi (Figure 1C-3 and Online supplement Figure 1S) [11,12]. This transition coincides with a sharp transition of cell orientation in the deep-subepicardium (Figure 1C-2 and 4) [13].

2.4. The ECG of the Wedge

The morphology of the surface (clinical) ECG is an expression of an intricate summation of extracellular voltage gradients during the cardiac cycle, including transmural and tangential gradients, the latter involving apico-basal, interregional, and interventricular gradients. In the wedge preparation, however, the transmural ECG morphology derives largely from the “transmural” extracellular gradients that exist between the Endo and the Epi regions [14].

During Endo pacing, the depolarization wavefront propagating from Endo to Epi creates a positive deflection inscribing the R wave. The onset of the R wave corresponds to earliest activation of Endo, the peak of the R wave coincides with latest activation of Epi (time of maximum transmural voltage gradient), and the end of the R wave is coincident with the end of phase 0 of the AP associated with the latest activation of Epi.

Because the pseudo-ECG is a bipolar recording, the transmural ECG can be approximated by subtracting the AP of the Epi region from that of the M region (i.e. M – Epi) (Online Supplement Figure 2S). In this configuration, it can be clearly visualized that the J wave follows the contour of the epicardial action potential notch. The J wave is inscribed as a result of the transmural gradient present at the time of the development of the Epi notch, and the isoelectric ST-segment is a result of a similar phase 2 voltage across the ventricular wall (see also Online Supplement Figure 7S). The T wave is inscribed largely by transmural differences in the time course of repolarization. The peak of the T wave (T_{peak}) corresponds to full repolarization of Epi, whereas the end of the T wave (T_{end}) is coincident with final repolarization of the M cell AP [1,14] (Online Supplement Figure 2S).

The AP recordings obtained using floating microelectrodes from vigorously beating preparations typically manifest variable amplitudes due to incomplete impalement (Online Supplement Figure 3S). However, the overall morphology of a low amplitude recording, when scaled-up to that of a “full” AP trace, is indistinguishable. It is for this reason that a voltage calibration is generally not provided for action potentials recorded from vigorously beating wedge preparations using floating glass microelectrodes [15–21].

The simultaneous recording of an ECG and action potentials from different sites across the ventricular wall of the wedge has permitted delineation of the cellular basis for the T wave and J wave of the ECG [22] and has identified the T_{peak} - T_{end} interval in the ECG as a marker of TDR, an electrocardiographic marker of arrhythmic risk [23]. Although the intuitive correlation between the AP recordings and the ECG are practically helpful, it needs to be acknowledged that the human heart *in vivo* is considerably more complex than the left or right ventricular wedge preparations and that its ECG is an expression of a much more

complex summation of vectors. This is the basis for a long-standing debate as to what Tpeak – Tend represents [24,25]. While there is general agreement that augmentation of Tpeak-Tend denotes an increase in spatial dispersion of repolarization within the ventricular myocardium, the debate centers principally on whether Tpeak-Tend denotes a measure of TDR. Because precordial leads look transmurally across the ventricular wall, a good correlation between TDR and Tpeak-Tend is expected in these leads of the surface ECG, but not in the bipolar limb leads, including leads I, II, and III, which do not look across the ventricular wall. While Tpeak-Tend intervals measured in these limb leads may provide an index of TDR, they more likely reflect global dispersion, including apico-basal and interventricular dispersion of repolarization [23]. Indeed, recent studies have concluded that augmentation of Tpeak-Tend in limb leads is a marker of increase spatial rather than transmural dispersion of repolarization [26], but when measured in the precordial leads, it is a marker of increased transmural dispersion of repolarization [27].

There are also conditions under which Tpeak-Tend does not provide an accurate measure of TDR in the wedge or in precordial leads, especially when the amplitude of the T wave is reduced due to slowing of phase 3 repolarization. In supplementary Figure 4S, a good correlation of Tpeak-Tend is observed at normal potassium concentrations (4mM); however when the potassium concentration is reduced to 2mM and the amplitude of the T wave is reduced, Tpeak-Tend does not correlate with TDR [28].

A good correlation between TDR and Tpeak-Tend generally occurs in our experimental models of long-QT, short QT, and Brugada syndromes (see sections below) where the transmural dispersion and arrhythmia vulnerability are greatly increased.

Supplementary Figure 5S illustrates that changing the direction of activation from endocardial to epicardial increases Tpeak-Tend as well as TDR. This accentuation of TDR is particularly exaggerated in the presence of I_{K_r} blockers such as cisapride, which augments TDR. As a consequence of the increase in TDR, an extrastimulus delivered to epicardium leads to the development of a polymorphic ventricular tachycardia (VT).

2.5. The Isolated Coronary-Perfused Interventricular Septum

The interventricular (IV) septum is supplied by a single artery originating from either the LAD or LCX, in all but 6% of cases. For this approach, the septum is dissected at the bifurcation of the left coronary into the LAD and LCX arteries, allowing visualization and cannulation of the proximal septal artery (Figure 2). Because of marginal apical perfusion present in some preparations (possibly supplied *in vivo* by the septal perforators branches of the LAD and/or PDA), the inferior-most 1 to 1.5 mm needs to be excised. The coronary-perfused isolated IV septums are placed in a large bath and allowed to recover for 2 hours before the start of recordings. Floating glass microelectrodes are used to obtain AP recordings from the preparation's top surface (a frontal plane located at mid-septum). Like in the wedge preparations, two electrodes positioned at ~1cm from the RV and LV endocardial septal surfaces are used to generate a trans-septal ECG. Point stimulation is applied to either the RV or LV Endo surface.

The distribution of APD₉₀ across the IV septum is illustrated in Figure 2A [29]. As shown in the top panel, APD₉₀ is shortest in RV Endo and increases until reaching a maximum beyond mid-septum, abbreviating thereafter near the LV Endo. The Purkinje fiber AP, recorded from the RV Endo surface displays the longest APD₉₀. The middle panel shows the distribution of APD₉₀ in individual experiments and the lower panel shows composite data from 6 experiments. The spike and dome morphology of the AP was generally most accentuated in the deep subendocardium on both sides of the septum, and least accentuated at the surface and in the mid-septum. Composite data graphed in the lower panel of Figure

2A highlights the biphasic distribution of APD_{90} across the septum. APD_{90} increases progressively as the position of the roving floating microelectrode shifts away from RV Endo, reaches a maximum near mid-septum, and abbreviates again as we approach LV Endo. Of note, spontaneous Torsade de Pointes (TdP) developed in the arterially-perfused septal preparation during LV Endo stimulation following exposure to BayK 8644 (1 μ M) (Figure 2C).

3. Experimental Models derived from the Ventricular Wedge Preparations using the floating microelectrode technique

3.1. The Long QT syndrome

The inherited long-QT (LQT) syndrome is attributable to a number of genetic mutations that cause prolonged ventricular repolarization and an increased risk of TdP and sudden cardiac death. The most common mutations are associated with: 1) loss of function of the slowly activating delayed rectifier potassium current (I_{Ks}) due to mutations in the *KCNQ1* encoding the α -subunit of $KvLQT1$ or $Kv7.1$ (LQT1); 2) loss of function of the rapidly activating delayed rectifier potassium current (I_{Kr}) due to mutations in the gene *KCNH2* encoding the α -subunit of hERG or $Kv11.1$ (LQT2); and 3) late I_{Na} gain of function due to mutations in the gene *SCN5A* encoding the α -subunit of $Na_v1.5$ (LQT3).

Experimental models of LQT1 [30,31], LQT2 [31,32] and LQT3 [31–33] have been developed in the canine LV wedge preparation using chromanol 293B (an I_{Ks} blocker), d-sotalol (an I_{Kr} blocker) and ATX-II (a sea anemone toxin capable of causing a large increase in late I_{Na}), respectively (Figure 3). Figure 3, panels A and D illustrate a model of LQT1 [30]. Exposure of the preparation to 30 μ M chromanol 293B dramatically increased repolarization time (Online Supplement Figure 6S). Addition of 100nM isoproterenol to the perfusate, used as a surrogate of enhanced sympathetic drive, led to a dramatic increase in TDR and Tpeak-Tend making the tissue more vulnerable to arrhythmogenesis (Figure 3A and Online Supplement Figure 6S). This model of prolonged QT interval and broad-based T wave in response to inhibition of I_{Ks} , in combination with an exquisite sensitivity to sympathetic stimulation, remarkably recapitulates the clinical features of LQT1. Similar clinically relevant LV wedge models of LQT2 (Figure 3B and D) and LQT3 (Figure 3C and F) have also been developed using d-sotalol [31,32] and ATX-II [31–33], respectively. These pharmacologic models of LQT2 and LQT3 also closely recapitulate the clinical phenotype.

Experimental models of rare forms of LQT (LQT7 or Andersen-Tawil syndrome and LQT8 or Timothy syndrome) have also been shown to reproduce the ECG and arrhythmic manifestations of these clinical syndromes (Online Supplement Figure 4S) [18,28,29].

Similarly to the “congenital” models of LQT, acquired models of LQT, mainly related to drug-induced I_{Kr} block have been developed [16] reproducing the ECG manifestations of acquired LQT, including prolonged QT intervals and the development of TdP arrhythmias (Online Supplement Figure 5S).

3.2. The Short QT syndrome

The Short QT (SQT) syndrome is an inherited syndrome characterized by a short QT (QTc < 360ms) interval in the ECG and a high incidence of ventricular tachycardia and ventricular fibrillation (VT/VF) in infants, children, and young adults. SQT1, involves nucleotide variations in *KCNH2* resulting in a gain of function in I_{Kr} [34]. In SQT2 a missense mutation in *KCNQ1* ($KvLQT1$) causes a gain of function in I_{Ks} [35]. SQT3 involves a gain of function mutations in *KCNJ2*, the gene that encodes for the inward

rectifier channel I_{K1} . SQT4 is caused by mutations in the α_1 -subunit of cardiac L-type calcium channel, whereas SQT5 is caused by mutations in the β_2 -subunit of the cardiac LTCC, SQT6 is associated with loss of function mutations in *CACNA2D1*, the gene encoding the $\alpha_2\delta$ -subunit of LTCC [36]. Mutations in the calcium channel often lead to ST-segment elevation as well as SQT, thus creating a combined Brugada/Short QT syndrome [37]. In SQT1, SQT2, and SQT3, the ECG commonly displays tall peaked T waves due to acceleration of phase 3 repolarization and an augmented Tpeak-Tend interval suggesting that TDR is significantly increased. Left ventricular wedge models of the SQT1 demonstrate that an increase in I_{Kr} preferentially abbreviates the Epi AP, thus increasing TDR and creating the substrate for re-entry [38]. The I_{Kr} agonist PD118057 was also shown to cause a preferential abbreviation of the pectinate muscle AP, thus creating a large dispersion of repolarization and refractoriness in the right atrium, which creates the substrate for the development of AF [39].

3.3. The Brugada syndrome

The Brugada syndrome (BrS) [40] is characterized by right precordial ST-segment elevation and closely coupled PVCs associated with high risk for VF. BrS has been associated with mutations in eleven different genes. Over 300 mutations in *SCN5A* ($Na_v1.5$, BrS1) have been reported in 11–28% of BrS probands [41–43]. Mutations in *CACNA1C* ($Ca_v1.2$, BrS3), *CACNB2b* ($Ca_v\beta 2b$, BrS4) and *CACNA2D1* ($Ca_v\alpha 2\delta$, BrS9) are found in approximately 13% of probands [44,45]. Mutations in glycerol-3-phosphate dehydrogenase 1-like enzyme gene (*GPD1L*, BrS2), *SCN1B* (β_1 -subunit of Na channel, BrS5), *KCNE3* (MiRP2; BrS6), *SCN3B* (β_3 -subunit of Na channel, BrS7), *KCNJ8* (BrS8), *KCND3* (BrS10) and *MOG1* (BrS11) are more rare [46–52]. These genetic defects cause a loss of function of I_{Na} or I_{Ca} , or a gain of function of I_{to} . These and other genetic variants, may give rise to subclinical forms or acquired forms of BrS.

Experimental models recapitulating the BrS phenotype, including J point elevation, ST-segment elevation and arrhythmogenesis, have been developed using coronary-perfused RV wedge preparations. Exposure to sodium channel blockers (flecainide) and/or calcium channel blockers (verapamil), combined sodium and calcium channel blockers (terfenadine), potassium channel openers (pinacidil) and I_{to} agonists (NS5806) [12,15,20,53] has been shown to recapitulate the electrocardiographic and arrhythmic manifestations of several of the BrS genotypes. In all of these models, depression or loss of the dome of the RV Epi AP underlies the ST-segment elevation, and the heterogeneities within the RV Epi and between Epi and Endo are responsible for creating the substrate for the development of phase 2-reentry and polymorphic VT (Online Supplement Figure 7S).

4. Optical Mapping of Transmural Electrical Activity in Isolated Coronary-Perfused Ventricular Wedge Preparations

Optical mapping describes the recording of electrical activity using voltage-sensitive dyes and allows simultaneous sampling from hundreds of sites simultaneously. It is therefore ideally suited to the study of the temporal-spatial basis of arrhythmias in multicellular preparations such the wedge. Moreover, optical mapping of voltage (V_m) can be combined with simultaneous recording of intracellular calcium, thus yielding further insights into the cellular basis of electrophysiology and arrhythmias. Such optical recordings are sensitive to motion artifact and so preparations must be immobilized, either mechanically or with uncoupling agents, some of which may have effects on cellular electrophysiology [54], [55]. The spatial resolution, depending on the exact parameters used in each system, is such that each optical AP, derived from a single pixel of the detector, represents the summation of the electrical activity from a number of cells. This means that an optical AP has some important

differences from an electrical AP [56]. The typical rise time of an optical AP (~10ms) is a function of the rise time of the cellular AP (~1–2ms) and blurring due to non-synchronous activation across the pixel area and within the depth of the sensitive volume [57]. For similar reasons, optical APs tend not to exhibit a spike and dome morphology. A comprehensive review of optical mapping is beyond the scope of the current paper but several excellent reviews are available [58], [59].

Rabbit wedge preparations for optical mapping are made in a very similar way as those used for AP recordings with floating microelectrodes (see Figure 4). Hearts are excised and immersed in ice cold Tyrode's solution. As shown in Figure 4, the aorta is opened, and the left main coronary arterial ostium identified. A modified cannula is passed into the artery with perfusate (Tyrode's solution at 37°C) running to avoid air embolism. As soon as the cannula is in position the flow rate is immediately increased to 30ml/min. The correct cannula position is verified by visual inspection, by identifying washout of blood from the perfused area of myocardium, and by observing an appropriate pressure rise in the perfusion system (~50–60mmHg). The time from excision to cannulation and perfusion is < 4 minutes. The RVOT is then removed to allow identification of the cannula marker for suturing, and the cannula is secured by over sewing. The left and right atria and the RV free wall are then removed. In most preparations this could be done without compromising perfusion; in some wedges suturing of atrial and RV vessels is required to maintain perfusion pressure. At this stage, the preparation is loaded with voltage-sensitive dye by a slow bolus injection through an injection port in line with the cannula. An incision is then made through the IV septum at the base of the aorta and any unperfused tissue at the base of the aorta is then removed. Any cut vessels causing a reduction in perfusion pressure are sutured closed. Once the perfusion is stabilized, the septum is removed with a microtome blade, leaving a cut surface of perfused LV free wall for optical imaging. Care must be taken to produce a uniform transmural surface to allow optimal focusing of the optics, and this can usually be achieved using a microtome blade. The wedge preparation is then mounted in a custom-built bath which allows the transmural surface to be orientated for optical imaging and incorporates pseudo-ECG electrodes and stimulating electrodes similar to those described above (see Figure 5A). Prior to optical imaging the preparation must be immobilized. Due to the orientation of the cut surface with respect to the major axes of contraction, mechanical restraint, which has been used for immobilizing Langendorff perfused hearts during Epi imaging, is usually insufficient for the wedge and pharmacological uncouplers are required. Most optical mapping studies now employ blebbistatin, which has little effect on ventricular electrophysiology [55]. Pseudo-ECG parameters and/or floating microelectrode recordings can be obtained to ensure that the uncoupler does not affect the electrophysiology of the wedge.

4.1. Optical Mapping of Transmural Activation: Insights into electrotonic coupling and modulation of APD

Optical mapping based on fluorescent signals from voltage-sensitive dyes allows an image of the electrophysiology across the cut-surface of the wedge. As shown in Figure 5C, maps of activation time and APD₉₀ can be generated indicating values of conduction velocity and action potential duration that are within the physiological range. One of the many measurements made to test the viability of the rabbit wedge preparation is to examine the values of the optical APs recorded at the Epi edge on the cut surface and compare these with optical APs recorded on the Epi surface at various distances from the cut edge. As shown in Figure 5D, APD₉₀ values on the cut surface were comparable to those recorded across the Epi surface suggesting that APs recorded from the cut surface were representative of APs recorded from a similar area of the intact myocardium.

Optical mapping of the canine wedge revealed that transmural conduction velocity and wavefront configuration depends on the activation sequence, with Endo-to-Epi propagation occurring rapidly along a planar wavefront and Endo-to-Epi propagation displaying slower conduction and a curved wavefront [60]. This was linked to reduced expression of connexin 43 (Cx43) in the subepicardial layers [61] and the increased source-sink mismatch experienced by a non-planar wave, although the contribution of the Purkinje fiber network could not be assessed. Similar transmural activation wavefronts and conduction velocities were observed in the rabbit wedge (Figure 5C) and were then recapitulated in simulations which did not include a Purkinje fiber network [62], suggesting that transmural heterogeneity in ventricular fiber orientation and electrotonic coupling play an important role in producing the activation sequence-dependence of transmural impulse propagation.

Significant transmural heterogeneity in APD between discrete cell layers across the ventricular wall has been identified using electrical techniques, as described in the first part of this review. Optical approaches have expanded our knowledge of transmural heterogeneity by allowing multisite mapping of the entire surface. For example, in canine wedges, functional M cells, which displayed APD prolongation at long cycle lengths, were shown to be variably distributed in small clusters throughout the ventricle [63]. Optical mapping of transmural APD in a small number of wedges from human hearts has also revealed islands of midmyocardial APD prolongation during Endo stimulation at very slow pacing rates [7]. However, at physiological stimulation rates a progressive reduction in APD from endocardium to epicardium during Endo stimulation was observed [64]. In the rabbit wedge, transmural gradients of APD were dependent on activation sequence (see Figure 5C) [62], supporting a dominant role for electrotonic influences in transmural modulation of APD and suggesting that intrinsic cellular differences may not be so important in determining the transmural repolarization sequence in rabbit ventricular myocardium at physiological stimulation rates as they appear in canine wedges. This behavior may be characteristic to the hearts of smaller animals in which the ventricular wall is much thinner [62]. It is noteworthy however that rabbit wedge preparations studied using regular microelectrode techniques and at relatively slow pacing rates show behavior similar to that of the canine wedge [65].

4.3. Transmural Optical Mapping to Identify Arrhythmia Mechanisms

As described earlier, electrical measurements made in the wedge preparation has been instrumental in delineating the possible cellular mechanisms of arrhythmogenesis in LQT. An increased propensity to formation of early afterdepolarizations (EADs) as well as significant TDR have been identified, raising the possibility that TdP could arise either from triggered activity or by re-entrant mechanisms. However, extrapolation of these cellular phenotypes to the mechanism of arrhythmia induction at the tissue level was not possible with discrete electrical recordings. Using transmural optical mapping in a canine model of LQT2, Akar *et al* were able to demonstrate the re-entrant nature of TdP and to identify the co-location of the border of the M cell region and the functional line of block preceding re-entry [63], thereby establishing the link between TDR and arrhythmogenesis in LQTS. In experimental models of LQT3 transmural optical mapping was also used to probe the relative contribution of enhanced EAD formation and increased TDR to arrhythmogenesis and identified that in LQT3, EAD-induced triggered activity interacted with extreme repolarization gradients to produce arrhythmias which were induced by a focal mechanism but maintained by re-entry [66]. The capability of optical mapping studies to differentiate between different arrhythmia induction mechanisms in different contexts is a major advantage of the technique.

4.4. Transmural Abnormalities of Calcium Handling, Delayed After-depolarizations and Triggered Arrhythmias

Elevated diastolic calcium is a feature of a number of proarrhythmic disease states. At the cellular level, elevated diastolic Ca^{2+} leads to overload of the sarcoplasmic reticulum (SR) and thereby predisposes to spontaneous SR Ca^{2+} release, delayed after-depolarizations (DADs) and DAD-triggered arrhythmias. This is another situation in which the transmural axis is key; as SR Ca^{2+} overload and spontaneous Ca^{2+} release (SCR) is more likely to arise in endocardial cells under conditions of enhanced Ca^{2+} entry (typically I_{Kr} blockade and β -AR stimulation) [67]. Using dual optical mapping of voltage and Ca^{2+} across the transmural surface of canine wedges, it could be shown for the first time that under conditions of normal coupling, endocardial SCR could provoke both DADs and focal ectopic beats [68]. This mechanism has also been identified in wedges from failing hearts following chronic rapid pacing [69]. In keeping with these findings subsequent optical mapping studies comparing the Endo and Epi surfaces have identified stronger Ca^{2+} - V_m coupling at Endo sites [70]. Under different experimental conditions, designed to model ryanodine receptor (RyR) dysfunction in canine (rapamycin and β -AR stimulation), SCR, DADs and focal arrhythmias were again observed, but in this case arose preferentially from the epicardium [71]. In contrast to enhanced Ca^{2+} entry, during which endocardial arrhythmias could be linked to slower Ca^{2+} reuptake to the SR and higher diastolic Ca^{2+} [68], in RyR dysfunction the Epi arrhythmias appeared to be dependent on faster SR reuptake kinetics [71].

5. Heart Failure and Repolarization Alternans

Understanding the mechanisms of ventricular arrhythmias in heart failure (HF) remains a major challenge after decades of electrophysiology research. The recognition of the association between microvolt T-wave alternans and arrhythmias in the 1990s led to interest in whether beat-to-beat fluctuations in T-wave morphology could be associated with a pro-arrhythmic state. Using optical mapping in guinea-pig epicardium Pastore and Rosenbaum were able to identify that during rapid pacing different areas of myocardium displayed APD alternans with opposite phase, or discordant APD alternans [72]. Such discordant alternans was associated with increased spatial dispersion of repolarization, thus predisposing to unidirectional conduction block and re-entry. Underlying gradients of APD were thought to influence the development of discordant alternans, and so studies of transmural APD alternans in normal and failing hearts attempted to determine whether this was a pathophysiologically relevant mechanism of arrhythmia. Optical mapping studies in both post-infarct HF in the rabbit [73] and following pacing induced heart failure in the canine [74] wedge determined that heart failure did indeed predispose to transmural APD alternans and to ventricular arrhythmia (see Figure 6). In the canine rapid pacing model, which displays significant increases in TDR in failing hearts there was an associated increase in discordant alternans. But in the rabbit post-infarct model there was no increase in TDR and no increase in discordant alternans to account for the increased incidence of arrhythmias. In keeping with the data from the rabbit wedge, transmural optical mapping studies in wedges from failing human hearts did not show any increase in TDR or discordant APD alternans [7], perhaps reflecting the fact that the majority of human heart failure is ischemic in origin. Alternans in the amplitude of the optical AP was identified in both rabbits and humans and was associated with the development of conduction block and ventricular arrhythmia in the former (see Figure 6) [73]. The cellular basis for alternans in the amplitude of the optical AP is as yet, unclear, but may represent a failure of 1:1 conduction throughout the sensitive volume. Indeed in transmural optical mapping studies of failing human wedges, alternating conduction velocity restitution was responsible for conduction failure [7]. Given the possible mechanistic link to life-threatening arrhythmias in patients with heart failure, the role of conduction in amplitude alternans is an important area for future study.

6. Summary

The isolated coronary-perfused ventricular wedge preparation, from which a pseudo-ECG and transmembrane action potentials can be simultaneously recorded, has given us extraordinary insights into the cellular basis for the ECG and the cellular basis for the electrocardiographic changes encountered under a wide variety of clinical conditions associated with the development of life-threatening ventricular arrhythmias. Isolated wedge preparations provide an important bridge between cellular studies and the function of the heart as an intact organ.

Optical mapping has enabled recording of signals from many hundreds of sites, providing additional important insights into the normal electrophysiology of the heart and pathophysiological mechanisms. Dynamic spatial changes in electrophysiology at the tissue level can be appreciated for the first time, providing an important bridge between cellular studies, microelectrode recordings and the function of the heart as an intact organ. In the future, technical improvements and novel optical probes are likely to increase the impact of this powerful technique.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The isolated coronary perfused ventricular wedge preparation was developed in 1996.
- Since then it became an important tool in cardiac electrophysiology research.
- It provided a wealth of data regarding the cellular basis for various ECG waves.
- It demonstrated the role of transmural heterogeneity in arrhythmogenesis.
- We provide the detailed method to work with this powerful experimental model.

Figure 1.

A. Schematic of the ventricular wedge preparation. Shown are an isolated left ventricular (LV) wedge, floating microelectrodes in the M region and on the epicardial (Epi) surface, a pacing electrode in contact with the endocardial (Endo) surface, two Ag/AgCl half cells placed at approximately 1 cm from Endo [electrocardiogram (ECG) (-)] and Epi [ECG (+)] and a polyethylene cannula inserted (and fixed) into a diagonal branch of the left anterior descending coronary artery. **B.** Step-by-step method used to attach a cannula inserted into the coronary artery of a wedge preparation. Shown is a schematic of the ventricular wedge preparation with its coronary vessel. A 6-0 coated braided silk suture is shown as a thread consisting of a free-ending and a curvilinear trace at the opposite end representing the needle. The tip of the cannula is shaped by exposing a polyethylene tubing (P160) to a heat source (a candle, for instance) while slowly rotating and gently pulling the tubing. The shaped tip is then connected to slightly larger diameter tubing, which in turn is hooked to the perfusion system. **C.** Transmural distribution of action potential duration (APD) and tissue resistivity (R_t) across the ventricular wall. **C-1:** schematic diagram of coronary-perfused canine LV wedge preparation. Three floating microelectrodes were used to record transmembrane action potentials simultaneously from Epi, M, and Endo sites. A transmural ECG is recorded along the same transmural axis across the bath, registering the entire field of the wedge. **C-2:** Histology of a transmural slice of the LV wall near the epicardial border. Region of sharp transition of cell orientation coincides with region of high R_t in C-4 as well as the region of sharp transition of APD in C-2. **C-3:** Distribution of conduction time (CT), APD₉₀, and repolarization time (RT = APD₉₀ + CT) in a canine LV wall wedge preparation

paced at basic cycle length of 2000ms. A sharp transition of APD_{90} is present between epicardium and subepicardium. **C-4:** Distribution of total R_t across the canine LV wall. Transmural distances at 0% and 100% represent epicardium and endocardium, respectively. $*P < 0.01$ vs. R_t at midwall. R_t increases most dramatically between deep subepicardium and epicardium. Error bars represent SE ($n = 5$). [Panel C: Modified from Antzelevitch [13], with permission].

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Figure 2.

Electrophysiology of the isolated coronary-perfused canine ventricular septum. **A.** Distribution of action potential (AP) duration across the interventricular septum. Top panel: Distribution of AP morphologies and AP duration at 90% repolarization (APD_{90}) as a function of the trans-septal distance, expressed as percent from right ventricular (RV) endocardium (Endo) to left ventricular (LV) Endo; Middle panel: Graph of APD_{90} as a function of the trans-septal distance in 6 distinct septal preparations; Bottom panel: Graph of composite data from the 6 septal preparations shown in middle panel. The shortest APs are found in RV endocardial sites in 5 of 6 preparations and the longest APs are found in the mid-septum (6 of 6 preparations). Each point represents mean + SD. Basic cycle length = 2000 ms, RV Endo stimulation. * $p < 0.05$ 60% vs. 0%, 20%, 40%, 100%. # $p < 0.05$ 80% vs 0%, 100%. **B:** Photograph showing the location of the left anterior descending artery (LAD) and the septal artery used to cannulate the canine septal preparation. **C:** BayK 8644 ($1 \mu\text{M}$)-induced Torsade de Pointes (TdP) arrhythmia in a coronary-perfused septal preparation. Spontaneous TdP episode developed following a 2-second pause. LV Endo stimulation. The arrhythmia self-terminated after 10 seconds. Electrocardiogram=(ECG). Modified from [29], with permission.

Figure 3.

LQT1, LQT2, and LQT3 models of long QT syndrome. **A–C.** Action potentials (APs) simultaneously recorded from endocardial (Endo), M, and epicardial (Epi) sites of coronary-perfused canine left ventricular wedge preparations together with a transmural echocardiogram (ECG). Basic cycle length = 2,000 ms. Transmural dispersion of repolarization (TDR) across the ventricular wall, defined as the difference in repolarization time between M and Epi cells, is denoted below ECG traces. LQT1 model was pharmacologically mimicked using isoproterenol (Iso) + chromanol 293B (an I_{Ks} blocker). LQT2 was created using the I_{Kr} blocker d-sotalol + low extracellular K^+ concentration ($[K^+]_o$). LQT3 was pharmacologically mimicked using the sea anemone toxin ATX-II to augment late I_{Na} . **D–F.** effect of isoproterenol (Iso) on LQT1, LQT2, and LQT3 models. In LQT1, isoproterenol produces a persistent prolongation of action potential measured at 90% repolarization (APD₉₀) of the M cell and the QT interval (at 2 and 10min), whereas APD₉₀ of the epicardial cell is always abbreviated, resulting in a persistent increase in TDR (D). In LQT2, isoproterenol initially prolongs (2 min) and then abbreviates the QT interval and APD₉₀ of the M cell to the control level (10min), whereas APD₉₀ of the Epi cell is always abbreviated, resulting in a transient increase in TDR (E). In LQT3, Iso produced a persistent abbreviation of the QT interval and APD₉₀ of M and Epi cells (at 2 and 10min), resulting in a persistent decrease in TDR (F). * $P < 0.0005$ vs. control. † $P < 0.0005$; ‡ $P < 0.005$; ††† $P < 0.05$ vs. 293B, d-sotalol (d-Sot), or ATX-II. [Modified from Antzelevitch [13], with permission].

Figure 4.

The method for producing the left ventricular (LV) wedge preparation in the rabbit. **A.** Illustration of the isolated whole heart, along with the modified cannula, which includes a marker to guide suturing, a bleb which sits distal to the suture and a bevelled tip to facilitate cannulation with minimal risk of arterial trauma. **B.** Illustration of cannulation employed for the perfused LV wedge preparation. The ascending aorta is opened with a longitudinal incision (1) and the left main coronary ostium is identified (2) and cannulated (3). From the other side of the heart, the right ventricular outward tract (RVOT) is then removed (4), the cannula position is ascertained using the suture maker and the bleb and a suture is placed between the two (5) and the cannula is then secured (6). Next the atria and right ventricular (RV) are removed (7), followed by the interventricular septum (IVS) (8), before the transmural imaging surface is cut with a dissecting blade (9). LA: left atrium, RA: right atrium

Figure 5.

A. Left ventricular (LV) wedge preparation mounted for transmural optical imaging. The imaging window is indicated by the black square. The extracellular disc electrodes for recording the pseudo-electrocardiogram can be seen above and below the preparation, as can pairs of epicardial and endocardial stimulating electrodes. **B.** CCD-based optical mapping system. **C.** Isochronal maps of transmurular activation time during endocardial and epicardial stimulation with corresponding contour maps of transmurular action potential measured at 90% repolarization APD₉₀. **D.** Mean epicardial APD₉₀ for each row of optical pixels back from the cut surface (open squares), expressed as a percentage of mean APD₉₀ across the whole epicardial imaging window (grey square). The mean subepicardial value from transmural imaging is shown for comparison (black square). [Modified from [73] with permission]

Figure 6.

A. Transmural action potential duration measured at 90% repolarization (APD_{90}) alternans magnitude as a function of pacing cycle length in normal and failing hearts. A leftward shift in the threshold for alternans is observed in heart failure. Significant transmural heterogeneity is also seen, with greater magnitudes of alternans detected in the subepicardium (Subepi) compared with the subendocardium (Subendo) and midmyocardium (Mid) in both normal and failing hearts. HF: heart failure. **B.** Optical action potential (AP) alternans with rapid pacing in a normal heart, showing the transmural imaging surface with the endocardial (Endo) border uppermost and the epicardial (Epi) border lowermost. The pseudo-electrocardiogram (pseudoECG) and optical AP traces recorded from the pixels

indicated, show alternating morphology. **C.** Corresponding transmural contour maps for APD_{90} and AP amplitude for beats 1 and 2 showing alternans of both parameters in the Subepi region. **D.** AP duration (APD) and AP amplitude (APA) alternans for ventricular fibrillation (VF)-prone and VF-resistant hearts (including normal and failing). There is no difference in the magnitude of APD alternans between VF-prone and VF-resistant hearts, but the magnitude of APA alternans is significantly greater in VF-prone hearts, compared with the VF-resistant hearts. [Modified from [73] with permission]

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