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Methodological review

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 the 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.

Contents

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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 (Fig. 1A). The dimensions of the LV wedges typically range from $2\times1.5\times0.9$ to $3\times2\times1.5$ cm and RV wedges range from $2\times1\times0.9$ to $2.5\times1.5\times1.2$ 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 descendent 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 Fig. 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 ± 0.5 °C. A suction line is used to keep the solution level 2–3 mm above the top cut surface of the tissue. The perfusate is delivered at a constant pressure (40–50 mmHg), which is initially set by adjusting the flow rate. The flow rate should be approximately 1–2 ml/min/g of tissue (or 8–14 ml/min), although every preparation differs and the final perfusion rate is to maintain 40–50 mmHg of pressure. The so-called "equilibration time," which is approximately 1–1.5 h, 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 h 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 h 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 250 μm) except at the tip (3–5 mm). The electrodes are placed ~1 cm 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 (~2–3 mm from the Endo surface) using floating glass microelectrodes (Fig. 1A and C-1). The electrodes are made of 125 μm-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 ~3–4 mm) filled with 2.7 M 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.