Journal of Molecular and Cellular Cardiology 86 (2015) 187–198

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Methodological Review

Methods for isolating atrial cells from large mammals and humans

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ARTICLE INFO

Article history:
Received 20 May 2015
Received in revised form 7 July 2015
Accepted 9 July 2015
Available online 15 July 2015

Keywords:
Atrial myocytes
Cell isolation
Human
Large mammal
Dog
Sheep
Pig
Collagenase
Protease
Enzyme

ABSTRACT

The identification of disturbances in the cellular structure, electrophysiology and calcium handling of atrial cardiomyocytes is crucial to the understanding of common pathologies such as atrial fibrillation. Human right atrial specimens can be obtained during routine cardiac surgery and may be used for isolation of atrial myocytes. These samples provide the unique opportunity to directly investigate the effects of human disease on atrial myocytes. However, atrial myocytes vary greatly between patients, there is little if any access to truly healthy controls and the challenges associated with assessing the in vivo effects of drugs or devices in man are considerable. These issues highlight the need for animal models. Large mammalian models are particularly suitable for this purpose as their cardiac structure and electrophysiology are comparable with humans. Here, we review techniques for obtaining atrial cardiomyocytes. We start with background information on solution composition. Agents shown to increase viable cell yield will then be explored followed by a discussion of the use of tissue-dissociating enzymes. Protocols are detailed for the perfusion method of cell isolation in large mammals and the chunk digest methods of cell isolation in humans.

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http://dx.doi.org/10.1016/j.yjmcc.2015.07.006
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1. Introduction

Disease of the cardiac atria is a large and growing global problem. Atrial fibrillation (AF) is the most prevalent arrhythmia in the developed world, affecting 33 million patients globally [1]. Current pharmacological options have imperfect efficacy and substantial adverse side-effects, including drug-induced proarrythmia and both cardiac and non-cardiac toxicity [2–4]. The limited effectiveness of current pharmacology is likely to result from an incomplete understanding of the pathophysiology of this complex heart rhythm disorder. Understanding the processes underlying AF requires investigation at several scales. Whilst studies at a whole-animal, whole-organ and tissue level have provided many insights into the origins of arrhythmia initiation and perpetuation [5], elucidating the pathological changes in ion currents, Ca^{2+} handling, and cellular microstructure requires isolated cardiomyocytes.

Much of our understanding of myocyte physiology has been obtained from rodent models [6–8], from which cell isolation has recently been reviewed [9]. Human electrophysiology, however, differs in several fundamental regards from these species. Examples of this include a five to ten-fold difference in resting heart rate [10], a markedly different complement of ion channels [11] and the presence of some transverse tubules in the atria of large mammals [12–14]. To this end, a potentially more fertile ground for understanding human disease is investigating cardiomyocytes obtained from humans or other large mammals.

This review aims to find the common thread that runs through the atrial isolation protocols that have been evolving for more than 30 years [15]. There is a paucity of papers directly comparing isolation techniques [16] and therefore the method sections from individual citations have been compared. A representative paper describing the protocol used by each research group can be found in the supplemental materials.

The theory underlying atrial myocyte isolation will be reviewed, and factors shown to influence cell yield and quality discussed. Some reagents, whilst improving cell quality for a specific experiment, may be detrimental to the requirements of a different investigation. Knowledge of these principles will therefore allow the isolation protocol to be tailored to the experiment in hand. This will be followed by examples of protocols that have proved successful for obtaining myocytes from humans and large mammals.

1.1. Search strategy

Examples of protocols were found by searching PubMed using the terms “atrial myocytes” OR “atrial cells” OR “heart cells” from 1st October 1980 to 1st April 2015, written in English and excluding reviews. Human studies were found by filtering for “human”, whilst reports on large mammalian species were found using the additional search terms of “dog” OR “canine”, “sheep” OR “ovine”, “goat”, “pig” OR “porcine” OR “swine” NOT “guinea pig”. References from key review articles on atrial electrophysiology were also identified.

2. Saline solutions

The basic composition of all solutions used for cell isolation is usually based on established physiological saline solutions. Historically, these were formulated by empirically adjusting ion concentrations until the solutions were considered acceptable in terms of the behaviour and survival of a specific tissue. Physiological salines which are commonly used in experimental cardiac research are shown in Table 1.

In his famous publications from the early 1880s Sydney Ringer described for the first time the importance of proper extracellular ion concentrations for the physiological function of the heart [17], establishing the importance of Na^+, K^+ and Ca^{2+} ions to maintain continuous beating of an isolated frog heart. To prevent acidification by metabolic products, he included bicarbonate buffers to maintain the cardiac contractions for longer periods. Later Locke [18] adapted Ringer's solution to the mammalian heart, by increasing salt concentrations. Locke also added 5.6 mmol/L glucose (0.1%) which substantially increased the survival time of the heart. Maurice Tyrode's widely used saline was originally developed to maintain contractions of isolated rabbit intestine [19]. He added magnesium and improved buffering by adding phosphate and increasing the concentration of bicarbonate.

Krebs and Henseleit performed experiments on liver sections [20]. They further adapted available physiological salines to resemble the composition of mammalian plasma by including sulphate and increasing the concentration of phosphate. They used higher concentrations of bicarbonate and gassed their solution with carbogen (5% CO_2, 95% O_2) to achieve a pH of 7.4.

Many of the physiological salines used today are based on the formulations described above. Usually more modern buffers, such as HEPES (*4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid*) or MOPS (3-(N-morpholino) propanesulfonic acid) are added to help maintain constant pH as their pK_a values are close to normal blood pH. Furthermore metabolic substrates such as pyruvic acid, β-hydroxybutyrate, glutamic acid, oxalic acid or succinate may be included in addition to glucose. Bovine serum albumin (BSA, 0.1–7%) is commonly used in experimental solutions to maintain colloid osmotic pressure, thereby preventing oedema [21], and to reduce nonspecific binding of proteins and toxins used for experimental research [22]. At higher concentrations, BSA is also used to inhibit enzyme activity [23].

These physiological salines have been optimised for different stages of the isolation process. The following section will describe the typical solutions used for (i) cardioplegia and tissue transport, (ii) Ca^{2+}-free solutions used for tissue dissociation, and (iii) storage solutions that are used once single myocytes have been obtained.

Table 1
Physiological salines used in cardiac research (in mmol/L) [118].

	Ringer [17]	Locke [18]	Tyrode [19]	Krebs and Henseleit [20]
NaCl	116	154	137	117
KCl	1.2	5.6	2.7	4.7
$CaCl_2$	1	2.1	1.8	2.5
$MgSO_4$				1.2
$MgCl_2$			1.1	
$NaHCO_3$	2.7	2.4	11.9	24.8
NaH_2PO_4			0.4	
KH_2PO_4				1.2
Glucose		5.6	5.6	11.1
pH				7.4
CO_2				5%

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