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Original article

Anatomical and molecular mapping of the left and right ventricular His–Purkinje conduction networks

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ABSTRACT

Functioning of the cardiac conduction system depends critically on its structure and its complement of ion channels. Therefore, the aim of this study was to document both the structure and ion channel expression of the left and right ventricular His–Purkinje networks, as we have previously done for the sinoatrial and atrioventricular nodes. A three-dimensional (3D) anatomical computer model of the His–Purkinje network of the rabbit heart was constructed after staining the network by immunoenzyme labelling of a marker protein, middle neurofilament. The bundle of His is a ribbon-like structure and the architecture of the His–Purkinje network differs between the left and right ventricles. The 3D model is able to explain the breakthrough points of the action potential on the ventricular epicardium during sinus rhythm. Using quantitative PCR, the expression levels of the major ion channels were measured in the free running left and right Purkinje fibres of the rabbit heart. Expression of ion channels differs from that of the working myocardium and can explain the specialised electrical activity of the Purkinje fibres as suggested by computer simulations; the expression profile of the left Purkinje fibres is more specialised than that of the right Purkinje fibres. The structure and ion channel expression of the Purkinje fibres are highly specialised and tailored to the functioning of the system. The His–Purkinje network in the left ventricle is more developed than that in the right ventricle and this may explain its greater clinical importance.

1. Introduction

The Purkinje fibres (PFs) were discovered by Jan Purkinje in 1837 [1] and their anatomy was elegantly studied and illustrated by Sunao Tawara at the beginning of the 20th century [2]. The PFs form the final portion of the cardiac conduction system — they provide a rapid conduction pathway through the ventricles ensuring a coordinated contraction of the ventricles [3]. They are insulated from the ventricular myocardium by a connective tissue sheath, which is lost before the PFs form terminal connections with the ventricular myocardium via specialised junctions in the endocardium [4–7].

The PFs are specialised: most importantly they are fast conducting, in part as a result of a high upstroke velocity during phase 0 of the action potential [8]. They have other distinct action potential characteristics: a prominent early rapid repolarisation (phase 1), a negative plateau potential (phase 2), an increased action potential duration, and spontaneous diastolic depolarisation (phase 4) [8]. Normally the PFs do not exhibit pacemaker activity, because of overdrive suppression by sinus rhythm, but in heart block they act as an escape pacemaker [9]. They also play a role in the generation and maintenance of arrhythmias — they support reentry [10], sustain ventricular fibrillation [7], are susceptible to arrhythmogenic early and delayed after-depolarisations [3,11,12], are linked to torsade de pointes associated with long QT syndrome [13,14] and play a role in arrhythmias after electric shock defibrillation [15].

To understand the physiological and pathophysiological functioning of the PFs, the aim of the current study was to map the anatomy of the His–Purkinje conduction networks in the rabbit heart and the expression of the major cardiac ion channels responsible for the electrical activity of the PFs. In the human, right bundle branch block is relatively common, but may be asymptomatic, whereas left bundle branch block is less common, but more serious, and for this reason both left and right His–Purkinje networks were investigated, by using similar molecular mapping techniques as we used previously for mapping the sinoatrial and atrioventricular nodes in the rabbit heart [16,17].

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2. Materials and methods

Experiments were conducted on 1 to 2 kg male New Zealand white rabbits, which were sacrificed by an overdose of pentobarbital according to the United Kingdom Animals (Scientific Procedures) Act, 1986. Using whole mount immunoenzyme-histochemistry, a marker of the cardiac conduction system, middle neurofilament (NF-M) was labelled to stain His–Purkinje tissue. Similar results were obtained from three hearts and data from two of the hearts are shown here. A 3D computer model of the His–Purkinje networks in one of the hearts was constructed by image analysis and Matlab software and used in numerical simulations to calculate action potential conduction. From a further eight rabbits, samples of free running PFs were carefully micro-dissected under a dissecting microscope using fine forceps without contamination from the left and the right ventricular muscle. Total RNA was extracted from the samples and reverse transcribed and the abundance of mRNA for the major cardiac ion channels responsible for the electrical activity of the PFs was measured using quantitative PCR (qPCR). The abundances of mRNAs were normalised to the abundance of a housekeeper, 28S. For further details of the methods used, see the Data Supplement.

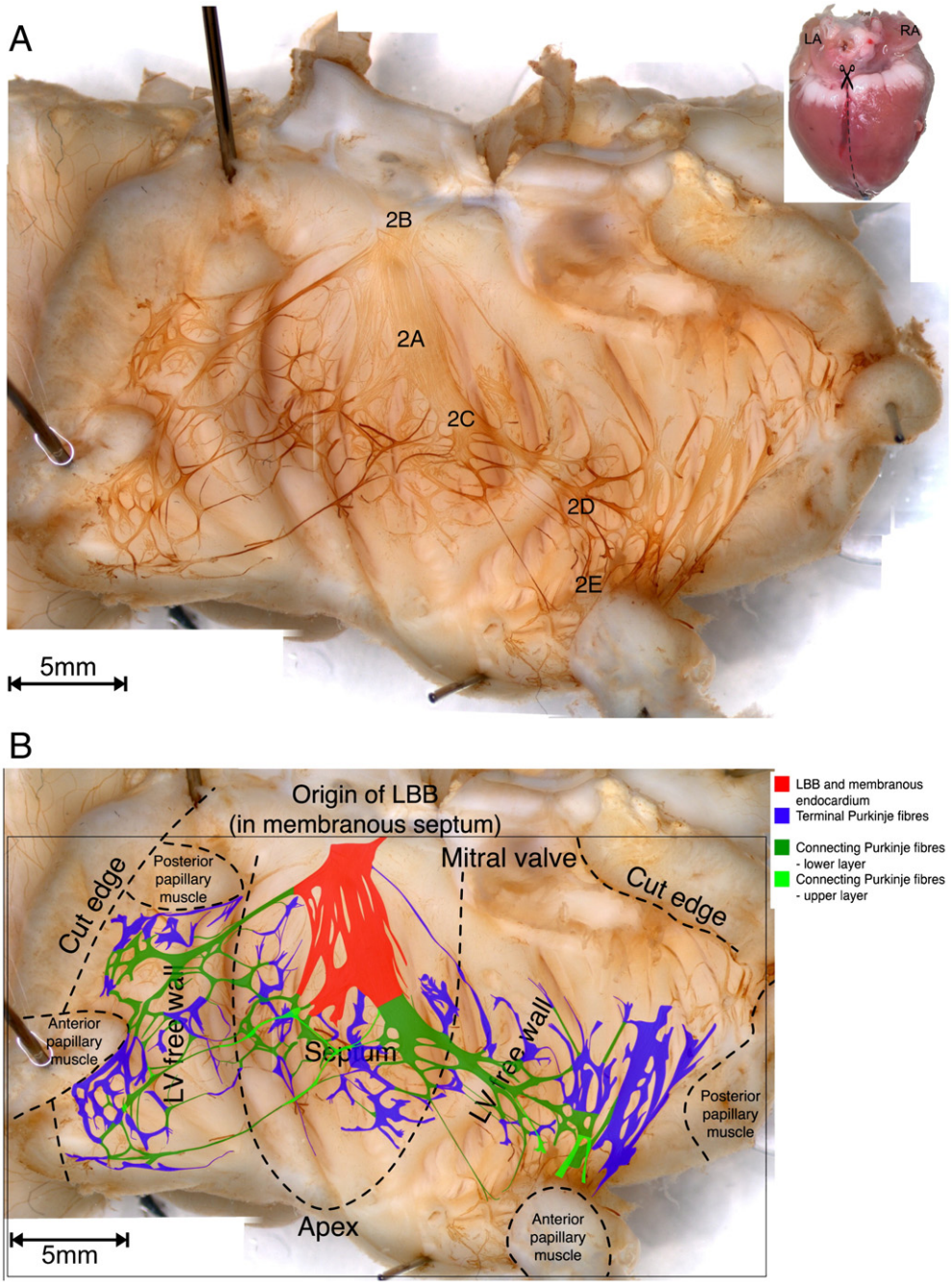

Fig. 1. His–Purkinje network in the left ventricle of the rabbit. A, macroscopic image of immunoenzyme-labelling (dark brown signal) of NF-M on the endocardial surface of the left ventricle. Labels indicate the location of high magnification images in Fig. 2. B, outline of the His–Purkinje network (structures displaying positive immunolabelling). The network has been segmented into different parts — shown in different colours. The dashed lines mark the cut edge of the ventricular free wall, the papillary muscles, and the oval border between the interventricular septum (centre) and free wall (left and right of centre).