Journal of Molecular and Cellular Cardiology 50 (2011) 642–651

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original article

Molecular architecture of the human specialised atrioventricular conduction axis

I.D. Greener [a,1], O. Monfredi [a,1], S. Inada [a], N.J. Chandler [a], J.O. Tellez [a], A. Atkinson [a], M.-A. Taube [a], R. Billeter [b], R.H. Anderson [a], I.R. Efimov [c], P. Molenaar [d], D.C. Sigg [e], V. Sharma [e], M.R. Boyett [a,2], H. Dobrzynski [a,*,2]

[a] University of Manchester, UK
[b] University of Nottingham, UK
[c] Washington University, St Louis, USA
[d] Queensland University of Technology & University of Queensland, Australia
[e] Medtronic Inc., USA

ARTICLE INFO

Article history:
Received 28 August 2010
Received in revised form 16 December 2010
Accepted 17 December 2010
Available online 21 January 2011

Keywords:
Atrioventricular node
Ion channels
Gap junctions
Arrhythmias

ABSTRACT

The atrioventricular conduction axis, located in the septal component of the atrioventricular junctions, is arguably the most complex structure in the heart. It fulfils a multitude of functions, including the introduction of a delay between atrial and ventricular systole and backup pacemaking. Like any other multifunctional tissue, complexity is a key feature of this specialised tissue in the heart, and this complexity is both anatomical and electrophysiological, with the two being inextricably linked. We used quantitative PCR, histology and immunohistochemistry to analyse the axis from six human subjects. mRNAs for ~50 ion and gap junction channels, Ca^{2+}-handling proteins and markers were measured in the atrial muscle (AM), a transitional area (TA), inferior nodal extension (INE), compact node (CN), penetrating bundle (PB) and ventricular muscle (VM). When compared to the AM, we found a lower expression of $Na_v1.5$, $K_{ir}2.1$, Cx43 and ANP mRNAs in the CN for example, but a higher expression of HCN1, HCN4, $Ca_v1.3$, $Ca_v3.1$, $K_{ir}3.4$, Cx40 and Tbx3 mRNAs. Expression of some related proteins was in agreement with the expression of the corresponding mRNAs. There is a complex and heterogeneous pattern of expression of ion and gap junction channels and Ca^{2+}-handling proteins in the human atrioventricular conduction axis that explains the function of this crucial pathway.

1. Introduction

The atrioventricular conduction axis (or atrioventricular node) is located in the septal component of the atrioventricular junctions, and is arguably the most complex structure in the heart. It has a multitude of functions, such as providing a critical delay between atrial and ventricular systole, protecting against life-threatening rapid supraventricular rates, and providing contingent pacemaking in the event of failure of the sinus node [1]. It is also the site of one of the most common cardiac arrhythmias, namely atrioventricular nodal reentrant tachycardia. Like any other multifunctional tissue, complexity is one of its key features. This complexity is both structural and functional. Histological analysis of the specialised muscular axis responsible for conduction of the cardiac impulse across the plane of atrioventricular insulation reveals various components common to all mammals, including the human [2]. Inferiorly, between the coronary sinus and tricuspid valve annulus, is found the inferior nodal extension (INE). The INE connects to the compact node (CN). The CN occupies the apex of the triangle of Koch, becoming the penetrating bundle (PB), or bundle of His, when it penetrates into the central fibrous body. Also connecting to the CN is a transitional area (TA) made up of transitional atrial myocytes. Electrophysiological data relating to the function of the axis has largely been derived from rabbit preparations. Amongst the most important findings in terms of function is the presence of dual inputs to the axis [3,4]. The fast pathway is located antero-superiorly, whereas the slow pathway is located between the orifice of the coronary sinus and the attachment of the septal leaflet of the tricuspid valve. The fast pathway is thought to correspond to the TA and the slow pathway to the INE [5,6].

In the rabbit, three cell types are widely accepted as making up the atrioventricular conduction axis. As compared to atrial myocytes with a fast Na^+-dependent action potential upstroke and negative resting potential, N (typical nodal) cells have a slow Ca^{2+}-dependent upstroke and a more positive diastolic potential, whereas the transitional AN and NH cells have an intermediate upstroke and diastolic potential [7]. N cells are thought to make up the INE (slow pathway) and CN, the transitional AN cells are thought to make up the TA (fast pathway), and the transitional NH cells are thought to be present in the PB [7,8].

* Corresponding author at: Cardiovascular Medicine, Faculty of Medical and Human Sciences, University of Manchester, Core Technology Facility, 46 Grafton Street, Manchester M13 9NT, UK. Tel.: +44 161 275 1182.
E-mail address: halina.dobrzynski@manchester.ac.uk (H. Dobrzynski).
[1] Joint first authors.
[2] Joint senior authors.

0022-2828/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.yjmcc.2010.12.017

Electrophysiological data regarding the human atrioventricular conduction axis have largely been restricted to electrode recordings during physiological testing and modification of arrhythmic substrates. Clearly, fast and slow pathways exist in humans [9]. More recently, Efimov and colleagues have provided further insights into the electrophysiology of the axis in the human with optical action potential recordings [10]. Due to limited access to live human tissue, there is no information regarding specific ionic currents or cell–cell coupling properties in the human. To remedy this deficiency, we have undertaken a molecular analysis of transcripts and proteins governing electrical excitability, hoping to provide further insight into the behaviour of the human atrioventricular conduction axis.

2. Materials and methods

We obtained specimens including the area of the triangle of Koch and the adjacent membranous part of the septum from six diseased human hearts acquired from the Prince Charles Hospital District, Chermside, Australia (ethics approval, EC2565; work also ethically approved by University of Manchester). A clinical profile of the patients is shown in the Data Supplement. We used quantitative PCR (qPCR), histology and immunohistochemistry to investigate the molecular make-up of the components of the atrioventricular conduction axis. For qPCR, we sampled the components of the axis, along with the atrial muscle (AM) and ventricular muscle (VM), as outlined in Fig. 1A, from 30 to 50 (60 μm thick) frozen sections, which underwent a novel and rapid haematoxylin and eosin (H&E) staining protocol [11]. In brief, from multiple quick H&E stained sections, tissue from each region (TA, INE, CN, PB) were micro-dissected by hand under a dissecting microscope with a fine surgical needle. Each region was identified by its histological appearance. The identification was guided by Masson's trichrome stained sections as well as sections immunolabelled for Cx43 such as those in Fig. 1. Total RNA was extracted from a given region from all relevant tissue sections. This was repeated for all six hearts.

Following extraction of total RNA from the samples and reverse transcription to produce cDNA, the amounts of ~50 cDNAs for selected cell type-markers, connexins, ion channels, Ca^{2+}-handling proteins and receptors were measured using qPCR. The data were analysed using the 'ΔCT technique': to correct for variations in the amount of total RNA in the different samples, the amounts of the different cDNAs measured were normalised to the amount of cDNA for a housekeeper (ribosomal protein, 28S). The amount of 28S cDNA was similar in each tissue investigated (data not shown). For a given ion channel etc., the amount of mRNA is proportional to the amount of cDNA and, therefore, this method allows the abundance of the *same* mRNA in different tissues to be compared. Tentative conclusions can also be drawn about the relative amounts of *different* mRNAs from the relative amounts of the corresponding cDNAs. However, the conclusions must be *tentative*, because the efficiency of the reverse transcription step (to produce cDNA) varies for different mRNAs. If the difference between two cDNAs was greater than 10-fold, the difference is likely to reflect to a difference in the abundance of the two corresponding mRNAs (rather than difference in efficiency of reverse transcription). However, if the difference between two cDNAs was less than 10-fold, the result must be interpreted cautiously. Data for *individual* transcripts are plotted here, whereas the data for *groups* of related transcripts are plotted in the Data Supplement (Figs. S1–S7) to facilitate comparison. All transcripts in each sample were measured in triplicate. In general, the three measurements were close. The average of these three measurements was then taken and the mean ± SEM of the average values for each heart was calculated – the n number for the calculation is, therefore, the number of hearts. In figures, the mean ± SEM is shown; in addition, the individual averages are shown as open circles. The SEM (as well as the open circles) shows the variability between hearts. Significant

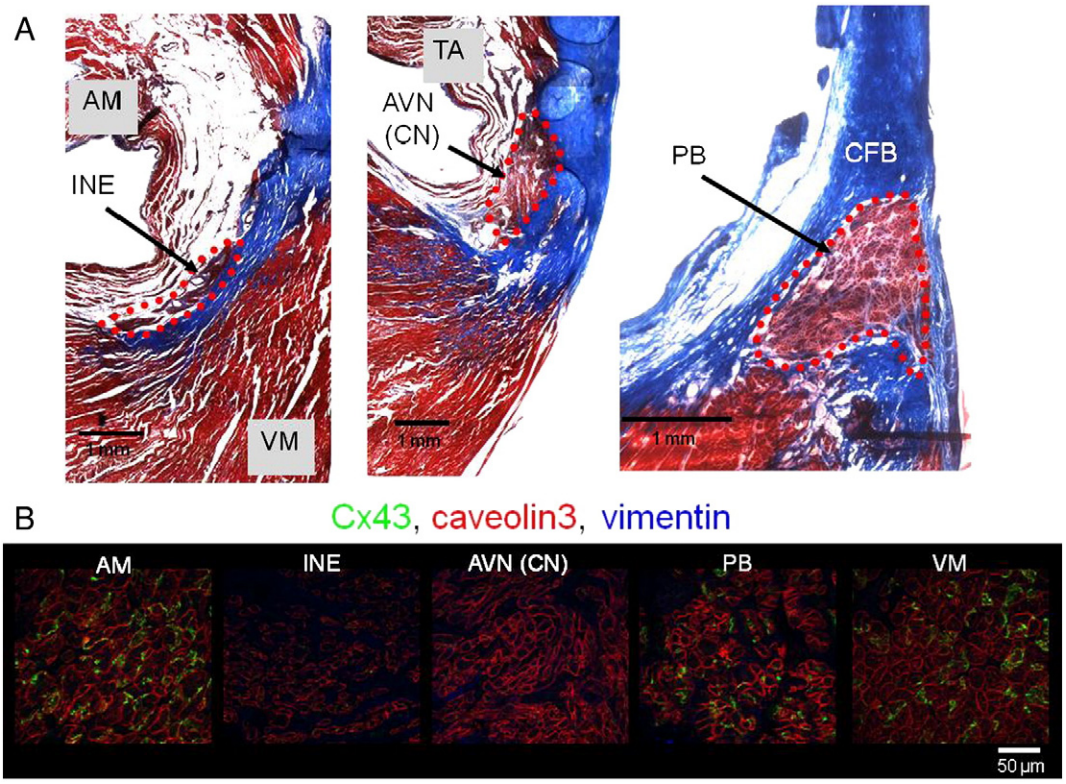

Fig. 1. Histological and immunohistochemical characteristics of human atrioventricular conduction axis. A, Masson's trichrome stained sections through INE (left), CN (middle) and PB (right). Myocytes stained purple, connective tissue blue. Dotted lines highlight areas (INE, CN and PB) sampled for qPCR. B, high magnification images of triple immunolabelling of Cx43 (green signal; at gap junctions), caveolin3 (red signal; within cell membrane) and vimentin (blue signal; within fibroblasts) in AM (left), INE, CN and PB. CFB, central fibrous body.