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Review article

The avian embryo to study development of the cardiac conduction system

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ABSTRACT

The avian embryo has long been a popular model system in developmental biology. The easy accessibility of the embryo makes it particularly suitable for *in ovo* microsurgery and manipulation. Re-incubation of the embryo allows long-term follow-up of these procedures. The current review focuses on the variety of techniques available to study development of the cardiac conduction system in avian embryos. Based on the large amount of relevant data arising from experiments in avian embryos, we conclude that the avian embryo has and will continue to be a powerful model system to study development in general and the developing cardiac conduction system in particular.

Contents

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1. Introduction

The avian embryo has long been a popular model system in developmental biology. More than 300 years B.C., even Aristotle appreciated the value of the chick to study embryonic development (Stern, 2005). Since then, numerous elegant experimental techniques have been developed, utilizing the specific advantages of avian embryos. The technical commentary here provides an overview of different methods used in avian embryos, specifically to elucidate the development of the cardiac conduction system (CCS). We first provide an overview of cardiac and CCS development in general and then discuss the particular advantages and disadvantages of the avian embryo to study CCS development. Finally, the techniques most effective for exposing the embryo in the egg and studying the electrophysiology and molecular differentiation of the developing CCS are described, with a short discussion of results obtained from these experiments.

2. General cardiac and CCS development and functioning

During gastrulation, mesodermal cells arise from the primitive streak and subsequently migrate cranially and laterally to form the cardiogenic plates. The first sign of cardiomyocyte differentiation is evident in this region at approximately Hamburger and Hamilton (HH) (Hamburger and Hamilton, 1951) stage 8–9 in chick, when cardiac troponin-I (cTnI) and sarcomeric myosin (MF20) are first detectable. Fusion of bilateral plates of splanchnic mesoderm establishes the primary heart tube (PHT) (Buckingham et al., 2005; Abu-Issa and Kirby, 2007). Subsequently, cells are added to the PHT from undifferentiated mesoderm, which is situated at the arterial and venous poles of the heart. Complex migration, proliferation and differentiation of different cardiac cell types (cardiomyocytes, endothelial cells, epicardial cells, (myo)fibroblasts and smooth muscle cells) finally establish the mature 4-chambered heart, with its own (coronary) vasculature, specialized conduction system and valvular apparatus (Jongbloed et al., 2012). In addition, neural crest cells migrate into the heart from the dorsal region of the rhombencephalon and take part in processes that include induction of aortopulmonary septum formation (Poelmann and Gittenberger-de Groot, 1999).

The CCS initiates and coordinates electrical activation of the myocardium, which is essential for normal functioning of the heart. Rhythmic contraction of the PHT starts around HH 10, and differences in conduction velocity are already evident by HH13 (de Jong et al., 1992). The cells contributing to the CCS are derived from precursors within the myocardial cell lineage (Gourdie et al., 1995). The adult CCS has several components (Fig. 1): the sinoatrial node (SAN) consists of pacemaker cells responsible for initiating atrial electrical activation. Myocardial cells contributing to the SAN originate from the sinus venosus myocardium, an initially U-shaped region of myocardium with pacemaking capacity (Vicente-Steijn et al., 2010) encompassing the bilateral cardinal veins (putative caval veins, that will remodel in favor of the right side during subsequent development). The sinus node will eventually become the primary pacemaker of the heart. After depolarization of the atria, the electrical current is delayed in the atrioventricular node (AVN), thereby ensuring adequate filling of the ventricles. A significant part of the AV node most likely originates from the AV canal, a component of the primary heart tube, although recent data indicate an additional sinus venosus contribution to the superior part of the node (Aanhaanen et al., 2010; Kelder et al., 2015). After a short delay, the AV bundle, the bundle branches and Purkinje fibers propagate the electrical current at high velocity to the ventricular myocardium.

An important structure for proper CCS function is the annulus fibrosus, a layer of dense fibrous cells, which electrically isolates the atria and ventricles, with the exception of the region where the AV bundle (also referred to as His or common bundle) penetrates this layer. This electrical isolation is essential for correct timing of atrial and ventricular activation. The fibrous cells contributing to the annulus fibrosus originate from the monolayer of epithelial cells lining the heart, the epicardium. The epicardial layer in turn is derived from the proepicardial organ (PEO), a cauliflower-like structure, which protrudes from the sinus venosus surface at the venous pole of the heart into the pericardial cavity. Cells derived from the PEO attach to the myocardium of the heart and then migrate over its entire surface. Through epithelial-to-

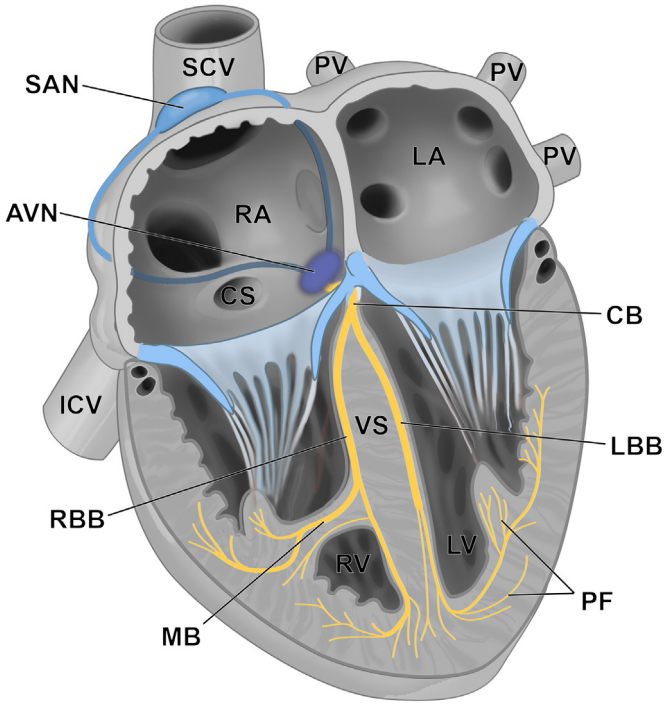

Fig. 1. *The components of the cardiac conduction system.* Schematic drawing of the components of the adult CCS. AVN: atrioventricular node, CB: common bundle, CS: coronary sinus, ICV: inferior caval vein, LA: left atrium, LBB: left bundle branch, LV: left ventricle, MB: moderator band, PF: Purkinje fiber network, PV: pulmonary vein, RA: right atrium, RBB: right bundle branch, RV: right ventricle, SAN: sinoatrial node, SCV: superior caval vein, VS: ventricular septum. Modified after: Jongbloed et al. Differentiation. 2012 Jul;84(1):131-148.