Review

Evolution of the Sinus Venosus from Fish to Human

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Abstract: The sinus venosus, the cardiac chamber upstream of the (right) atrium, is a severely underinvestigated structure. Yet, its myocardium harbors the cardiac pacemaker in all vertebrates. In human, ectopic pacemaking and subsequent pathologies may originate from sinus venosus-derived myocardium surrounding the coronary sinus and the superior caval vein. In ectothermic vertebrates, *i.e.*, fishes, amphibians and reptiles, the sinus venosus aids atrial filling by contracting prior to the atrium (atria). This is facilitated by the sinuatrial delay of approximately the same duration as the atrioventricular delay, which facilitates atrial filling of the ventricles. In mammals, the sinuatrial delay is lost, and the sinus venosus-derived myocardium persists as an extensive myocardial sheet surrounding the caval veins, which is activated in synchrony with the myocardium of the atria. The caval vein myocardium is hardly of significance in the healthy formed heart, but we suggest that the sinus venosus functions as a chamber during development when cardiac output, heart rate, blood pressure and architecture is much more like that of ectothermic vertebrates. The remodeling of the sinus venosus in mammals may be an adaptation associated with the high heart rates necessary for postnatal endothermy. If so, the endothermic birds should exhibit a similar remodeling as mammals, which remains to be investigated.

1. Introduction

In human, the embryonic heart chamber the sinus venosus remodels during development to the sinus venarum, and a subpopulation thereof harbors the dominant pacemaker of the heart, which is of paramount importance for initiating the heart beat [1–4]. Sinus venarum myocardium may also give rise to arrhythmias, leading to cardiac morbidity and mortality [5,6]. The chamber sinus venosus can be found in the formed hearts of the oldest extant vertebrates, and while its origin is reviewed extensively [7,8], overviews of the evolutionary changes across vertebrates are rare and focus on morphology [9–11]. Here, we define the sinus venosus myocardium as the myocardium upstream of the (right) atrium, the border of which often is defined by a valve. We review the evolution in form and function and discuss its significance to the heart of man.

2. Molecular Identification and Characterization of the Sinus Venosus

In mammals, chicken and zebrafish, three transcription factors, Shox2, Tbx18 and Bmp4, specifically are expressed in sinus myocardium [12,13]. Shox2 is a transcriptional regulator of Isl1 [14], and transcription factors Isl1 and Tbx3 are only expressed in the part of the sinus myocardium with most of the pacemaker activity [3,15–18]. While the exact role of Isl1 in the sinus myocardium is unknown, Tbx3 imposes a pacemaker-like phenotype upon myocardium when expressed ectopically even in fully differentiated myocardium [19]. Albeit that the molecular phenotype and transcription pathways of the sinus venosus are being unraveled in mammals, chicken and zebrafish, the studies on the other vertebrate classes largely are restricted to anatomical and electrophysiological studies.

3. The Sinus Venosus is Maintained in Evolution

Hagfishes represent the evolutionary oldest class of extant vertebrates, and their systemic heart comprises a sinus venosus, an atrium and a ventricle (Figure 1a,b). The wall of the sinus venosus of hagfishes is composed of connective tissue and a thin layer of cardiomyocytes (less than 100 μm), but the extent of myocardium is not precisely determined [20,21]. The electrocardiograms of hagfishes reveal distinct activations of the sinus venosus that precede atrial activation with a delay of similar duration to that of the atrioventricular delay (Figure 1c). In clades of fishes younger than cyclostomes, three species of cartilaginous fishes and the sturgeon, a basal ray-finned fish, the relative mass of the sinus venosus is around 3% of the total cardiac weight [22]. Flesh-finned fish, encompassing the lungfishes and coelacanths, are phylogenetically close to terrestrial vertebrates and have essentially the same myocardial and electrical patterning as hagfishes (Figure 1d,f) [21,23–25]. The evolutionarily much younger teleost fish, e.g., trout and zebrafish, have very little, if any, myocardium upstream of the sinuatrial junction (Figure 1e) [17,20]. The advantage of this loss remains to be shown. Amphibians, extant representatives of the first terrestrial vertebrates, typically have a sinus venosus consisting of left, right and posterior sinus horns. In contrast, anurans, as typically represented by the *Xenopus* frog, have a voluminous sinus venosus (Figure 1g–i). A configuration similar to that of basal

amphibians is found in most amniotes. In reptiles and chicken, the three sinus horns contain myocardium from the sinuatrial border to the pericardial reflection (Figure 1j–n) [26,27]. The sinus venosus is remodeled in the mammalian heart and possibly in the avian heart, as well, and the associated specializations are discussed below.
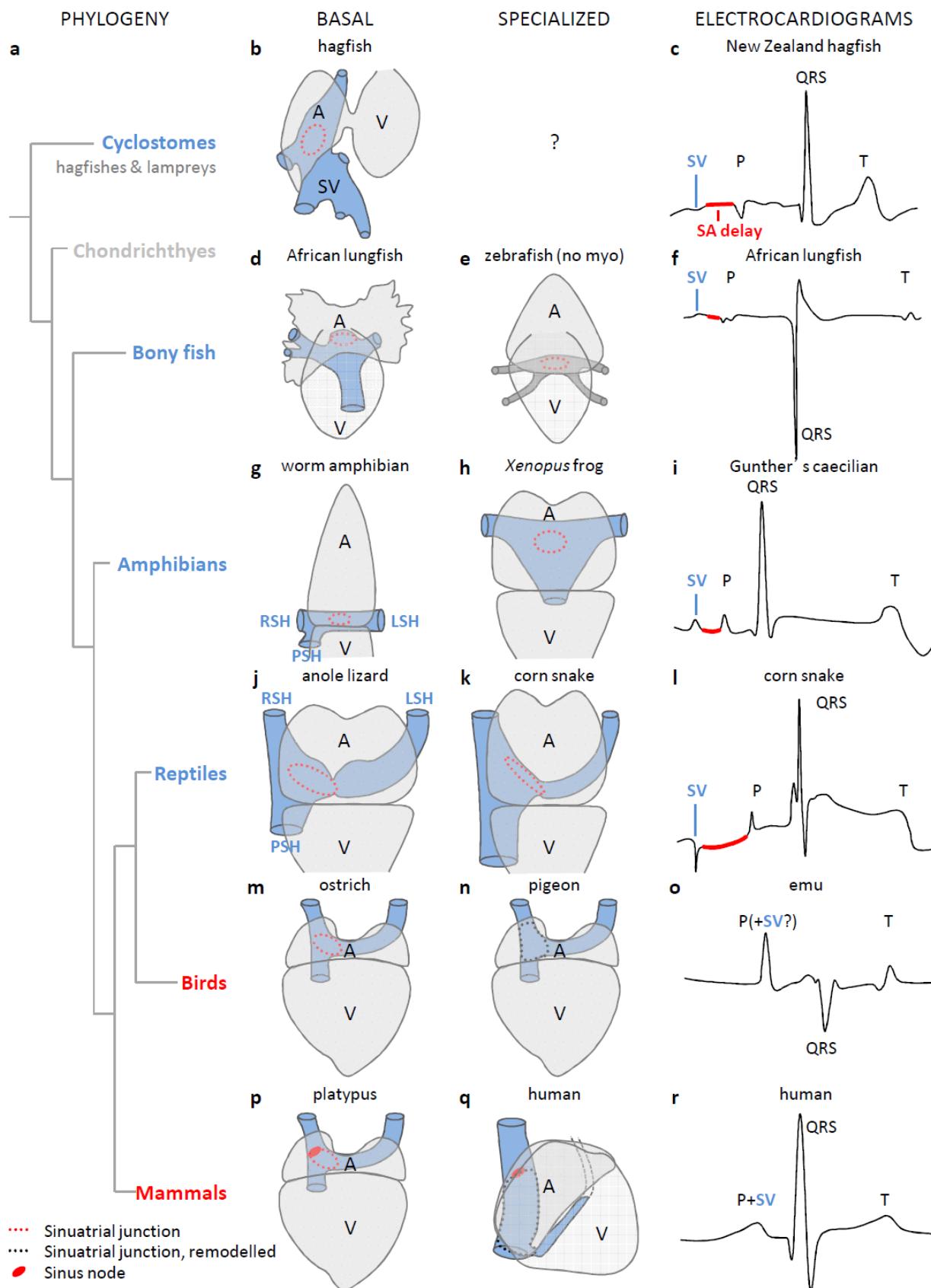
4. The Dominant Pacemaker Generally is Located at the Sinuatrial Border

Regardless of the configuration of the sinus venosus, it always harbors the dominant pacemaker. In the mammalian heart, dominant pacemaker activity is located in an anatomically distinct sinus node at the border of the right atrium and the right sinus horn or superior caval vein [2,28–30]. The avian sinus node is positioned similarly, but typically is less distinct if at all anatomically identifiable [2,31,32]. The transcription factors, Isl1 and Tbx3, are markers of the sinus node and are expressed in cells with high levels of automaticity in mouse, human and chicken [3,14,33,34]. Even in zebrafish, where the sinus node has not been identified anatomically, Isl1 marks pacemaker cells at the sinuatrial junction [17]. Cells of the sinuatrial junction of zebrafish and trout may have the so-called spindle, elongated spindle and spider morphologies, like the cells of the mammalian sinus node [17,35]. In ectotherms and endotherms alike, the sinuatrial junction or sinus node is richly innervated by sympathetic and parasympathetic branches of the autonomous nervous system [36–38].

Figure 1. Anatomical and electrical characteristics of the sinus myocardium in evolution. (**a**) Phylogeny of vertebrates with ectotherms in blue and endotherms in red. (**b**) Hagfishes represents the oldest class of vertebrates, and their hearts have a muscular (blue) sinus venosus (SV) (ventral view; the red dotted line indicates the sinuatrial orifices). (**c**) The hagfish ECG reveals a distinct wave for the activation of the sinus venosus followed by a delay (red) before atrial activation (P). (**d**) Lungfishes, the sister group to terrestrial vertebrates, have a myocardial sinus venosus. (**e**) In teleost fish, the sinus venosus has very little myocardium or no myocardium, except on the sinuatrial border, like in the zebrafish. (**f**) The ECG of the African lungfish reveals sinus venosus activity. (**g**–**i**) Worm amphibians and salamanders are considered basal amphibians, and in frogs (e.g., *Xenopus*) and toads, the sinus venosus is a large cavity rather than vessels with myocardial sleeves, as in the worm amphibians. (**j**–**k**) In reptiles, the sinus venosus is much like in basal amphibians and consists (generally) of the posterior, left and right sinus horns, and a specialization may be the regressed left sinus horn (**k**). (**l**) Recordings from the posterior sinus horn. (**m**) In basal birds, like ostriches and emus (Palaeognathae), but also chicken (Neognathae), the sinus venosus is much like that in reptiles. (**n**) In many birds, the sinus venosus is partially atrialized, because the sinuatrial junction is remodeled. (**o**) The ECG of birds does not show an SV wave, possibly because the sinus venosus has been synchronized with the atria (P(+**SV?**)). (**p**) Basal mammals, like the monotreme platypus, but also placental mammals, like the armadillo, maintain a reptile-like sinus venosus. (**q**) In man, much of the sinus venosus is atrialized, and the left sinus horn regresses to the coronary sinus, but sinus venosus-derived myocardium is still found upstream of the remodeled sinuatrial junction. (**r**) In man, there is no sinus wave on the ECG, probably because

the sinus venosus activation is synchronized with the atria (P + **SV**). Based on [4,10,20,27,28,30,39–50] (**a,e,j–k,m–r**), modified from [9,21,51–54] (**b–d,f,i,l**) and unpublished observation (**g**; de Bakker, Wilkinson and Jensen). A, atria; LSH, left sinus horn; PSH, posterior sinus horn; V, ventricle.

PHYLOGENY | BASAL | SPECIALIZED | ELECTROCARDIOGRAMS

a | **b** hagfish | | **c** New Zealand hagfish

Cyclostomes — hagfishes & lampreys
Chondrichthyes
Bony fish
Amphibians
Reptiles
Birds
Mammals

d African lungfish | **e** zebrafish (no myo) | **f** African lungfish

g worm amphibian | **h** *Xenopus* frog | **i** Gunther's caecilian

j anole lizard | **k** corn snake | **l** corn snake

m ostrich | **n** pigeon | **o** emu

p platypus | **q** human | **r** human

RSH, LSH, PSH, A, V, SV, QRS, P, T, SA delay, P(+SV?), P+SV

···· Sinuatrial junction
···· Sinuatrial junction, remodelled
●   Sinus node

Most studies in ectothermic vertebrates show that the dominant pacemaker activity is located at the sinuatrial junction, albeit that an anatomical sinus node is absent [20,21,35,37,55,56]. In addition, some studies report pacemaking upstream of the sinuatrial junction at variable positions [53,57], and the dominant pacemaker activity of the heart of man may shift within the sinus node region [29,58,59]. This resembles the embryonic stages of mouse and chicken, where dominant pacemaker activity is not yet confined to the sinus node, as underlined by the broad expression of the sodium channels related to spontaneous depolarization, like Hcn4 [60–65]. Further, myocardium of the right sinus horn of the formed mammalian heart may retain high levels of automaticity [4,66].

5. In Ectotherms, the Sinus Venosus Promotes Cardiac Output through Atrial Filling

In fishes, and probably many amphibians and reptiles, the contraction of the sinus venosus contributes significantly to atrial filling [21,24,37], which, in turn, augments ventricular filling, which is important for the maintenance of cardiac output [67]. In ectothermic vertebrates, passive filling of the ventricle is less pronounced than in mammals, which makes atrial contraction a major contributor to ventricular filling [37,68,69]. However, the absence of myocardium in the sinus venosus of most teleost fish is difficult to reconcile with a significant role of the sinus venosus for atrial filling in fishes (Figure 1e). Pressure build-up by contraction of the sinus venosus would require valves between the veins and the sinus venous, which, however, to the best of our knowledge, have not been reported for ectothermic vertebrates [37,69,70]. In contrast, a sinuatrial valve composed of two myocardial leaflets is present at the sinuatrial junction of most vertebrates, diminishing regurgitation from the (right) atrium [3,9,20,21,24,37,71–73].

6. The Sinus Venosus is Atrialized in Endotherms

Both the sinuatrial valve and the sinus venosus are formed during mammalian embryonic development. Later, from around Carnegie stage 16 of human development onward, the left sinus horn (coronary sinus) partly fuses with the left atrium, and the sinuatrial valve regresses, particularly the left leaflet. Of the right leaflet of the sinuatrial valve, its superior portion becomes the Eustachian valve and its inferior portion the Thebesian valve guarding the orifice of the coronary sinus [74,75]. A sinus septum, which is also present in reptiles [20,21,35,37,55,56], partly separates the sinus horns and contributes to the tendon of Todaro of the formed human heart and is then a useful landmark for the identification of the atrioventricular node [74,75]. The now atrialized sinus venosus is called the sinus venarum (Figure 1q) [74,75]. Concomitantly, genes expressed in the atria and the ventricles (Nkx2.5, Cx40 and Anf) progressively become expressed in distal parts of the sinus myocardium in man, mouse and basal mammals, like shrews [3,62,76–79]. This occurs after Carnegie stage 14, corresponding to approximately 33 days of development in man and 14.5 days in mouse. In the adult mammalian heart, much of this myocardium remains upstream of the right atrium. Furthermore, the sinuatrial valve is retained in species from all three mammalian subclasses, e.g., platypus (monotreme), American opossum (marsupial) and woolly monkey, and in many birds, e.g., ostrich and chicken [10,40,48,71,77,78,80,81]. This suggests that the sinus venosus has not, or only partially, morphologically atrialized in these cases (Figure 1m,p). Nevertheless, the ECG of mammals and birds does not show a "SV" (sinus venosus) wave for sinus myocardial activation (Figure 1o,r). This is probably because the sinus horns (caval

veins) are activated simultaneously with the atria [28,30,82–85], thereby masking the sinus activation by the larger P wave (Figure 2). Interestingly, in rabbit, the sinus horn myocardium activates and contracts in synchrony with the atria and has contractility under the regulation of sympathetic and parasympathetic agonists, like atrial and ventricular myocardium (Figure 2d) [86,87]. In mammals, therefore, the sinus myocardium seems to be atrialized in terms of electrical activity and expression of genes, but not in strictly morphological terms. Late in chicken development, the sinus myocardium is activated prior to the atria [65], similar to the situation in ectotherms, but it is not known whether the sinus venosus becomes synchronized with the atria around or after hatching.

Figure 2. The sinus venosus-derived myocardium is electrically synchronized with the atria in mammals. (**a**) In dog, the extensive sinus myocardium of the right sinus horn (RSH) and the azygos vein (az) is activated in synchrony with the right atrium (RA) (notice that the atrial activation was not mapped in its entirety). (**b**) Sinus myocardium is less extensive in man than in dog, but is activated in synchrony with the right atrium. (**c**) In the case of persistent left sinus horn (congenital malformation), this part of the sinus is also synchronized to the atria. While malformed, the presence of two sinus horns resembles that of basal mammals and the rabbit. (**d**) In rabbit, activation of the sinus horns is synchronized with the atria. Activation spreads far cranially, which indicates extensive myocardial investment in the vessels. Modified from [83] (**a–c**) and [84] (**d**). az, azygos vein; A, atria; LA, left atrium; LSH, left sinus horn; PSH, posterior sinus horn; RSH, right sinus horn; RA, right atrium; V, ventricle.
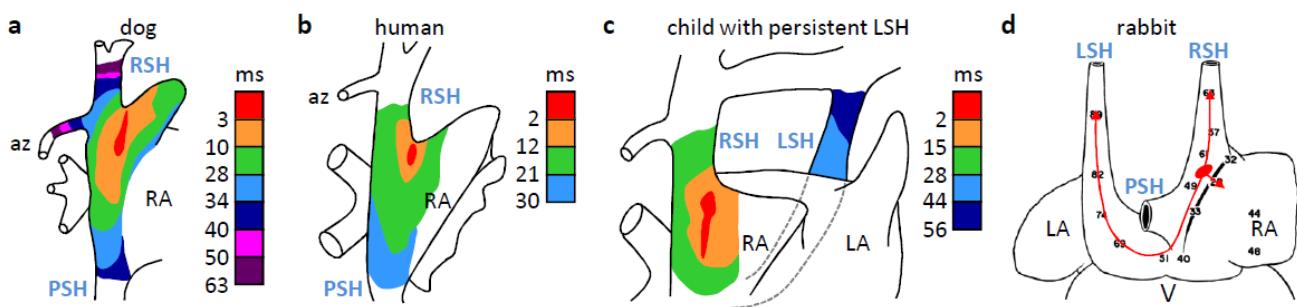
7. Why is the Sinus Venosus Atrialized in Mammals?

Mammals, like birds, have much higher heart rates than ectothermic vertebrates [67,88], suggesting that at higher heart rates, the sinus venosus becomes functionally redundant: the atria contributes much less to ventricular filling in mammals than in ectotherms [37,68,69], and the sinus venosus may no longer promote cardiac output through enhanced filling of the right atrium. If so, the sinus venosus of birds should be synchronized with the atria, which remains to be shown. Following this reasoning, teleost fish without sinus myocardium should have higher heart rates than basal bony fishes and cartilaginous fishes, but this is not apparent [89]. The atrialization of the sinus venosus may serve to enlarge the right atrium, but the ratio of atrial to ventricular mass is roughly the same in ectothermic and endothermic vertebrates [27].

8. Arrhythmias Originate from Sinus Venosus-Derived Myocardium

Atrial fibrillation is the most common cardiac arrhythmia in man and responsible for an increased risk of stroke. While the majority of the cases of fibrillation arise from myocardium of the pulmonary veins [90], approximately 10% of the arrhythmias originate from the superior caval vein and the coronary sinus and its orifice, which are derived from the sinus horn myocardium [5,91,92]. One factor that is important for the atrialization of sinus horn myocardium is Pitx2 [62], and gene variants close to the PITX2 locus are associated with atrial fibrillation [93]. Thus, anomalous patterning of the sinus horn myocardium may lead to arrhythmogenesis, but the mechanism is not completely understood [94] (intriguingly, pulmonary venous myocardium does not occur in ectothermic vertebrates [26,27]).

Unlike mammals, in ectothermic vertebrates, a delay exists between the activation of the sinus horns and atria, referred to as the sinuatrial delay. Thus, ectothermic vertebrates may be protected from arrhythmias driven by sinus horn myocardium, like the ventricles of the mammal heart are protected from atrial arrhythmias by the delay of the atrioventricular node [4]. Resembling the sinuatrial delay, recent studies on mammal hearts show that sinus node activation may initiate well before atrial activation [59].

9. Why is the Sinus Venosus-Derived Myocardium Retained in the Mammalian Heart?

It is tempting to view the sinus venosus-derived myocardium of the formed heart of man as an evolutionarily inherited structure without function where disease may originate, like the intestinal appendix. We suggest, however, that during the developmental period, when cardiac performance is low, contractions of the sinus venosus contribute to cardiac output, as they do in adult ectotherms. During the stages around septation, hearts of mammals, birds and reptiles are morphologically very alike [27], and the expression of genes is mostly conserved [88,95]. Further, fetal heart rates in mammals, birds and reptiles, when extrapolated to 37 °C for reptiles, are approximately 100–200 beats per minute, and systemic blood pressures are very low, less than 10 mmHg and much below mature values of approximately 100 mmHg [96–102]. If the mammalian sinus venosus is functional during development, a substantial sinuatrial delay should be present, as it is in chicken [65]. We are looking forward to any study showing this in mammals.

10. Conclusions

(1) In most ectothermic vertebrates, the sinus venosus is a cardiac chamber, because it has myocardium, has a distinct electrical activation pattern and contributes to atrial filling by contracting.

(2) In contrast to other vertebrates, most teleost fish have no myocardium in the wall of the sinus venosus, except at the sinuatrial junction that harbors the pacemaker. The mechanism and advantage of this absence is unexplained.

(3) In mammals, the embryonic sinus venosus may remodel to the sinus venarum of the right atrium, as, for instance, in man. The sinus venosus-derived myocardium becomes electrically synchronized with the atria, and the sinuatrial delay seen in ectothermic vertebrates is lost. Birds probably resemble mammals rather than ectotherms.

(4) It is not known why the sinus venosus atrializes in mammals. Possibly, it is a mechanical disadvantage to have three sequential chambers, the sinus venosus, the atria and the ventricles, at the high heart rates necessary for endothermy.

(5) The cardiac pacemaker is generally on the sinuatrial border, but it remains unexplained why only mammals develop a morphologically clearly distinct sinus node.

(6) The functional significance of the sinus venosus-derived myocardium of the formed mammalian heart is not clear. Possibly, it is an inescapable remnant of development, where contraction of the sinus venosus may contribute significantly to cardiac output by aiding atrial filling.

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Author Contributions

All authors contributed to writing and reading of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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