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COMMENTARY

Why are you talking with snakes? To get new evolutionary insights in cardiac electrophysiology!

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Cardiac electrophysiology: From ionic channels to electrocardiogram

In the heart, cardiac electrical activity is due to the presence of ion channels in the sarcolemma of cardiac myocytes. The most prominent ion channels are voltage gated and selective for Na^+, Ca^{2+}, and K^+. The sarcolemma can form membrane invaginations called t-tubules where the L-type Ca^{2+} channel is highly concentrated. The presence of t-tubules in cardiac myocytes depends on the region and species. The sequential activation and inactivation of ion channels lead to action potentials. In mammalian working heart cells, these are initiated by the opening of Na^+ channels, resulting in the rapid upstroke, followed by a plateau phase due to opening of L-type Ca^{2+} channels. The repolarization arises by opening of various K^+ channels. In ventricular myocytes, the action potential typically lasts hundreds of milliseconds (Fig. 1). Of course, the waveform of cardiac action potential differs between regions (atria versus ventricles) and even across the wall of the ventricle (endocardium versus epicardium) because of differences in the expression and/or the properties of the underlying ion channels. These electrical processes can be detected on the body surface electrocardiogram (ECG) and represent temporal and spatial averages of each phase. The electrical cycle starts with the P wave due to spread of excitation through the atria. The QRS complex is the result of activation of the ventricular walls, whereas the T wave results from repolarization gradients in the ventricular walls.

During this era of translational research, it is refreshing to have a study featured in an earlier issue of *JGP* investigating cardiac electrophysiology in snakes (Boukens et al., 2022). In Antoine de Saint-Exupéry's book *The Little Prince*, first published in New York City during the Second World War, there are two mentions of snakes. At the end of book, the narrator asks the Little Prince: "Why are you talking with snakes?" The reason is that the snake is admirably confident that he has mastered life's mysteries (de Saint-Exupéry, 1943). The study by Boukens et al. (2022) in ball pythons is therefore important in several aspects: first, they provide an advance of scientific knowledge. From an evolutionary point of view, reptiles and mammals share a common ancestor in Amniote, with divergence occurring ∼315 million yr ago (Warren et al., 2008). Reptiles are important for evolutionary and comparative physiological study; in addition, today there is an emphasis for translational research aimed at converting basic research results to human. We, therefore, need to justify the study of nonconventional animal models. For reptiles, there is an obvious translational relevance; they can tolerate long-term hypoxia, whereas mammals do not, especially at the heart level (Alderman et al., 2019). In addition, cardiac cells from reptiles, or at least geckos, routinely proliferate, even in the adult stage. This large-scale regeneration is absent in the heart of mammals. Physicians dream of patients being able to tolerate cardiac hypoxia (and ischemia) and to be able to regenerate damaged myocardium after a myocardial infarction. Finally, reptiles are now pets, and reptile cardiology in clinical veterinary exotic practice is currently underdeveloped; however, smartphones can be used to record ECGs (Cermakova et al., 2021).

From mammals to reptiles

In cardiac electrophysiology, most of the knowledge comes from the class of mammals (see above). There are some striking differences in reptiles. In common with other non-crocodilian reptiles, the snake heart is composed of two atria and a single ventricle (Farrell et al., 1998). There is no atrial–ventricular node or His–Purkinje network that forms the specialized electrical conduction system of higher vertebrates. Despite these structural differences, the reptilian ECG waveform is morphologically similar to the mammalian waveform, with P wave, QRS complex, and T wave representing sequential atrial depolarization, ventricular depolarization, and ventricular repolarization (Figure 1 from Boukens et al., 2022). The convergence of function towards a common characteristic despite large structural differences suggests the importance of the electrocardiographic features for survival.

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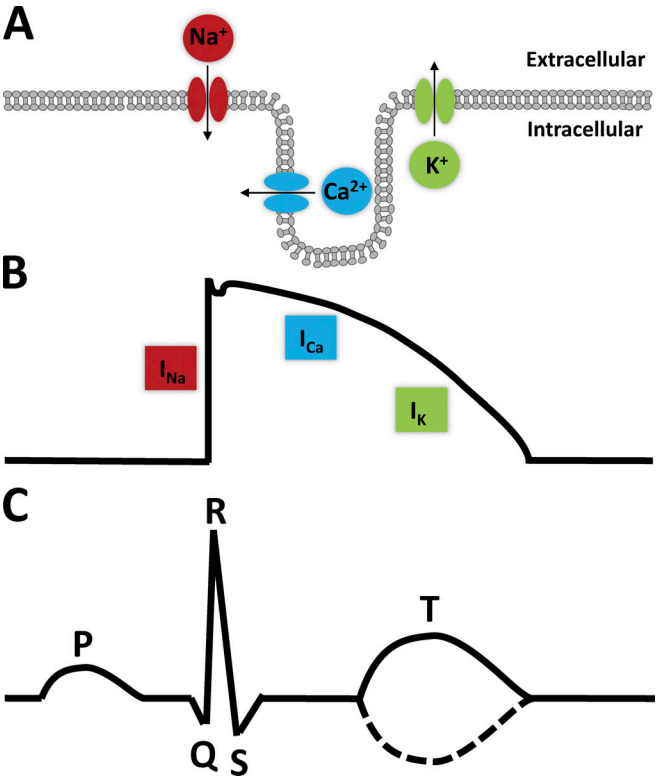

Figure 1. **Cardiac electrical activity. (A)** Several ionic channels are present on the sarcolemma of cardiac myocytes: Na^+, Ca^{2+}, and K^+ channels. Ca^{2+} channels are mainly located in transverses tubules when present. Specific ionic channels composition depends on the species and the region of the heart (e.g., atria versus ventricle). **(B)** Opening and closing of ionic channels are responsible for the action potential. **(C)** At the body level, surface ECG reflects the electrical activity in the entire heart. P wave is the atrial depolarization, QRS is the ventricular depolarization, and T wave is the ventricular repolarization. In some cases, T wave can be negative (dashed line).

Recording a high-quality ECG from a snake is not trivial. The thick, scaly skin of snakes and lizards limits the sensitivity of ECG leads. Furthermore, ECGs in reptiles are quite variable. The T wave is commonly found to be very wide and of variable morphology even in the same species (Lewis et al., 2020), as already described in the 1960s (Valentinuzzi et al., 1969). In addition, reptiles are ectotherms and depend on their environmental temperature to regulate their core temperature. Cardiac function is maximized when a reptile is maintained within its preferred temperature range. Hence, body temperature induces several changes in ECG parameters (Jacob and McDonald, 1975), and this what Boukens et al. (2022) investigated.

The electrical cycle is highly dependent on ventricular structure, which affects the propagation of the activation and repolarization front. Major differences in myocardial structure exist between mammals and reptiles. In mammals, the depolarization wave initiates at the clearly defined sinus node, whereas in snakes there is no sinus node but instead a sinus venosus located caudally to the right atrium, which contains spontaneously depolarizing pacemaker cells (Jensen et al., 2014, 2017). Furthermore, whereas the atrioventricular node in mammals connects to the fast-conducting His-bundle and Purkinje network leading to an endocardial-to-epicardial activation pattern of the two ventricular walls, the atrioventricular junction connects directly to the ventricular myocardium leading to an epicardial activation pattern from the base to the apex of the unique ventricular cavity in snakes (Gregorovicova et al., 2018).

Physiological mechanisms uncovered by Boukens et al.

By using the century-old technique of ECG, custom-made 256-lead electrodes to record local electrograms from the ventral surface, and state-of-the-art technique RNAseq, the authors observed that T-wave polarity could be inverted by increasing temperature from 25 to 35°C in an anesthetized python. They revealed that this effect is not due directly to temperature itself but results from catecholaminergic stimulation, the sympathetic system being dependent on temperature. Deciphering the analysis of RNAseq, they suggested that heterogeneity of catecholamine-associated genes could explain this inversion of polarity during sympathetic activation by accentuating ventricular repolarization heterogeneity. In conclusion, a change in the ventricular temperature itself is not involved in the modification of the T-wave polarity.

There has long been interest in the basis of cardiac repolarization and T-wave alternans in relation to arrhythmogenesis. In mammals, temperature also plays a role in Ca alternans, which can lead to T-wave alternans (Millet et al, 2021). Classically, it is recognized that the T wave reflects the time of repolarization which differs in different parts of the ventricles: apex versus base, left versus right ventricle, or epicardium versus endocardium (Meijborg et al., 2014). However, despite decades of research, the genesis of the electrocardiographic T wave is still incompletely understood and subject to controversy (Opthof et al., 2009; Patel et al., 2009). In humans, the current explanation is that the total dispersion of repolarization along all anatomic axes determines the T wave (Opthof et al., 2016). In the snake, the lack of conducting tissue makes it more likely that the T wave is due to the difference in repolarization between the apex and the base of the ventricle (Boukens et al., 2016).

The way forward

The study by Boukens et al. (2022) prompts further investigations.

Snakes actively adjust thermoregulatory behavior to raise their body temperature during digestion, exhibiting a postprandial thermophilic response that accelerates digestion at the expense of higher metabolic rates (Tattersall et al., 2004). Indeed, at rest, important vagal "cholinergic" tone controls heart rate (as in humans; Wang et al., 2001b), and Boukens et al. (2022) suggest that adrenergic tone in snakes is also similar to mammals as indicated by the effect of propranolol on basal heart rate. Vagal stimulation is also important in regulating cardiac function in snakes by affecting QT duration and QT–RR relationship (Valentinuzzi et al., 1970). During exercise, increased

adrenergic tone is observed accounting for increased heart rate. By contrast, an increase in heart rate during postprandial thermophilic state occurs under low adrenergic tone (Wang et al., 2001b), suggesting that a non-adrenergic, non-cholinergic factor could act directly on the heart (Wang et al., 2001a). The delicate balance between sympathetic and parasympathetic tone should be investigated.

The RNA sequencing data from Boukens et al. (2022) are intriguing. The β-adrenergic receptor type 1 appears to be the dominant type in snake, with no mention of type 2 receptor, which is the dominant β-adrenergic receptor in lower vertebrates, e.g., frogs (Skeberdis et al., 1997). The expression of CACNA1G, which codes for the T-type Ca^{2+} channel, is unusual in ventricular myocytes of mammalians. A cellular study to confirm the functional presence of β receptors and to characterize ion channels is essential. However, this may be complicated by the fact that the structure of the reptilian heart consists of a thick inner spongy myocardium that derives its oxygen and nutrient supply directly from the blood within the ventricular cavity, surrounded by a thin outer compact layer supplied by coronary arteries (Farrell et al., 1998). Patch clamp and calcium cycling studies have already been done in some reptiles, turtles, and lizards (Galli et al., 2006, 2009); these should also be done in ball pythons. Some cellular electrophysiological data are available from snakes (Abramochkin et al., 2020). The L-type Ca^{2+} channel is also highly regulated by temperature and the adrenergic signaling pathway, whereas the T-type Ca^{2+} channel is not. Of course, excitation–contraction coupling will be slightly different from mammals because of the lack of t-tubules in reptiles (Perni et al., 2012). Detailed electrophysiological and calcium cycling will increase our understanding of evolutionary steps.

To return to *The Little Prince*, the book describes that the narrator, before drawing a sheep, first draws a boa constrictor having eaten an elephant. This description is highly relevant to cardiac physiology (Wang and Rindom, 2021). Grown-ups perceive the elephant inside the snake as a hat. Of course, the Little Prince notices the drawing. "You are not fair, little prince," the narrator says, whereupon the Little Prince responds "I don't know how to draw anything except boa constrictors from the outside and boa constrictors from the inside" (de Saint-Exupéry, 1943).

Indeed, grown-ups have lost their playfulness and are unable to perceive "important things."

The work by Boukens et al. (2022), in addition to providing important new insights in evolutionary cardiac electrophysiology, emphasizes the importance of studying lower vertebrates and invites us to re-read *The Little Prince*, not only for mention of snakes.

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