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Review

The beat goes on: Cardiac pacemaking in extreme conditions ☆

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ABSTRACT

In order for an animal to survive, the heart beat must go on in all environmental conditions, or at least restart its beat. This review is about maintaining a rhythmic heartbeat under the extreme conditions of anoxia (or very severe hypoxia) and high temperatures. It starts by considering the primitive versions of the protein channels that are responsible for initiating the heartbeat, HCN channels, divulging recent findings from the ancestral craniate, the Pacific hagfish (*Eptatretus stoutii*). It then explores how a heartbeat can maintain a rhythm, albeit slower, for hours without any oxygen, and sometimes without autonomic innervation. It closes with a discussion of recent work on fishes, where the cardiac rhythm can become arrhythmic when a fish experiences extreme heat.

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1. Introduction

Heart rate in craniate animals (chordates with a well-defined head) varies considerably in response to the varying demands placed on the heart to deliver blood to tissues, *e.g.*, during times of exercise, stress, digestion and altered body temperature. In extant animals as diverse as the most primitive craniates (hagfishes), through fishes, amphibians and reptiles, to birds and mammals, cardiac output typically can vary at least 3-fold and such changes are often attributed to an altered heart rate (*e.g.*, Shin et al., 1995; Eliason et al., 2013). Here we consider the control of heart rate under two extreme conditions.

First, we consider environmental anoxia (or very severe hypoxia), which is when the primary role of the circulatory system to transport O_2 and CO_2 between tissues and the respiratory organ is lost, or becomes minimal, due to ambient O_2 being temporarily unavailable. As O_2 levels in arterial blood fall, the tissue's capacity to generate ATP using oxidative phosphorylation becomes progressively limited and

Abbreviations: SL, sarcolemma; I_{Kr}, delayed rectifier K^+ current; HCN, hyperpolarization-activated cyclic nucleotide-gated; I_f, funny current; SR, sarcoplasmic reticulum; RyRs, ryanodine receptors; NCX, sodium/calcium exchanger channel; PO, power output; CTmax, upper thermal tolerance; T_{arr}, arrhythmia triggering temperature.

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even the heart becomes challenged with generating sufficient ATP through glycolytic pathways. Moreover, an anoxic brain may constrain autonomic nervous control (Nilsson, 2001; Nilsson and Lutz, 2004), including that of the cardiac pacemaker. The second environmental condition we consider is supra-optimal temperature in fishes, which is when maximum cardiac performance is in decline due to excessive warming.

Cardiac performance is tightly related to heart rate. In order to understand how cardiac performance is altered under these two extreme environments, extrinsic and intrinsic control of pacemaker activity has been explored to understand the modulation of the rhythm of the heartbeat. We start by considering pacemaker activity in an ancestral craniate heart, that of the hagfish, which has no autonomic innervation. As such, this naturally aneural heart offers a unique approach to the study of the initiation and regulation of the craniate heartbeat.

2. Pacemaker activity in craniates

The agnathan branchial heart shares many functional and anatomical characteristics with vertebrate hearts. Like other craniates, the hagfish heart is multi-chambered and contains cardiac muscle cells (cardiomyocytes) that intrinsically respond to increased stretch (increased cardiac filling) by increasing contractile force (increased cardiac stroke volume), which is termed the Frank–Starling mechanism. Cardiac contractions are also myogenic (initiated within the heart itself). Yet, despite the lack of cardiac innervation (Axelsson et al., 1990; Bloom et al., 1963; Farrell, 2007; Ota and Kuratani, 2007), heart rate in the Pacific hagfish (*Eptatretus stoutii*) can still vary considerably (*e.g.*, fourfold on recovery from anoxia; Cox et al., 2010), albeit much more slowly (it takes approximately 2 h for the changes on recovery from anoxia) than with direct autonomic neural control. Recent discoveries on hagfish heartbeat initiation and cardiac control in the context of prolonged anoxia have provided novel and interesting insights into how the earliest form of the craniate heart is operated. Prior to highlighting these novel and recent discoveries for pacemaking of the hagfish heart, we first provide a brief introduction of the initiation of the heartbeat in vertebrate pacemaker tissues because the initiation of the vertebrate heartbeat is well studied in non-piscine models.

Specialized pacemaker cells (termed the sinoatrial node and located in the sinus venosus, or at the sinoatrial border in more derived vertebrates) initiate the heartbeat. These cells self-generate rhythmic action potentials (Fig. 1) that subsequently trigger action potentials in non-pacing contractile cardiomyocytes in a rhythmic manner. Pacemaker cells have been identified in all major craniate groups from fish to mammals, including hagfish (Jensen, 1965). In fact, pacemaker potentials with shapes similar to those found in mammalian hearts have been recorded in hagfish hearts using sharp-microelectrodes (Jensen, 1965; Arlock, 1975; Vornanen et al., 2002). However, hagfish cardiomyocytes are considerably depolarized at rest (−41 and −48 mV in the atrium and ventricle, respectively) compared to teleost fishes and mammals (−65 mV and −75 mV in the atrium and ventricle, respectively). The action potential of a pacemaker cell differs from that of a contractile cardiomyocyte by having a pacemaker potential, an unstable resting membrane potential that, over time, depolarizes until it reaches the threshold for the action potential upstroke. Historically, two models have been proposed to explain the pacemaker potential in vertebrate pacemaker cells: the “membrane clock hypothesis” and the “calcium clock hypothesis”.

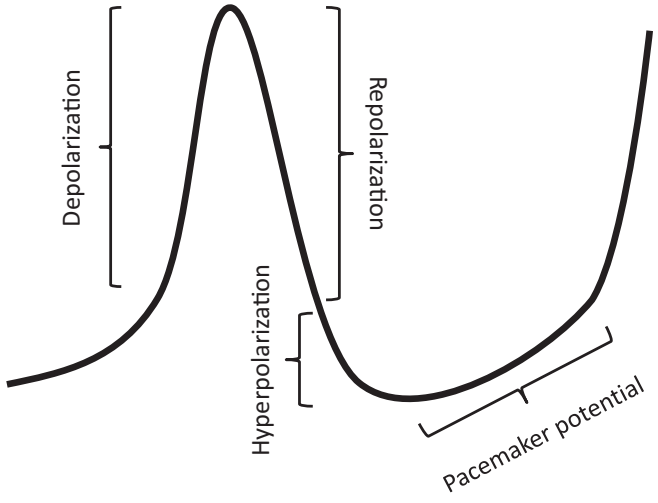

Fig. 1. The major electrical stages of a cardiac pacemaker cell action potential. Upon the reaching of T-type Ca^{2+} threshold potential, an influx of Ca^{2+} depolarizes the cell. Efflux of potassium repolarizes the cell, and even hyperpolarizes it, activating the pacemaker potential. This steady influx of Na^{+} and K^{+} gently depolarizes the cell back to the threshold for T-type Ca^{2+}. See text for more details.

The “membrane clock hypothesis” proposes that all ion currents affecting pacemaker rhythmicity are located in the sarcolemma (SL) of the pacemaker cell. Following the spike of the action potential, an outward delayed rectifier K^{+} current (I_{Kr}) causes the SL membrane to become highly hyperpolarized (negatively charged compared to the threshold of the Ca^{2+} channels), which activates the hyperpolarization-activated cyclic-nucleotide gated (HCN) channels located in the SL membrane. This then allows a slow influx of Na^{+} and K^{+} (termed the funny current, I_f), which slowly depolarizes the cell membrane (Brown et al., 1979a; Brown and DiFrancesco, 1980; DiFrancesco and Ojeda, 1980; Yanagihara and Irisawa, 1980; Doerr et al., 1989; Baker et al., 1997; DiFrancesco, 2010). This gradual depolarization (the pacemaker potential) occurs until the threshold potential to activate T-type Ca^{2+} channels is reached. Rapid entry of Ca^{2+} into the pacemaker cells *via* these T-type Ca^{2+} channels creates the upstroke of the action potential and the depolarization again activates I_{Kr}, restarting the cycle (Hagiwara et al., 1988; Doerr et al., 1989; Zhou and Lipsius, 1994).

The “calcium clock hypothesis” proposes that the ion currents that affect pacemaker rhythmicity are located primarily in the sarcoplasmic reticulum (SR) membrane as well as in the SL (Vinogradova et al., 2004). With this model, depolarization of the cell membrane during the pacemaker potential is initiated by spontaneous release of Ca^{2+} from the SR stores into the cytoplasm (termed Ca^{2+} sparks that can be visualized using calcium imaging techniques) through ryanodine receptors (RyRs) in the SR membrane (Rubenstein and Lipsius, 1989; Huser et al., 2010; Lyashkov et al., 2007; Maltsev and Lakatta, 2007). As Ca^{2+} builds up in the cytoplasm from these Ca^{2+} sparks, the sodium/calcium exchanger channel (NCX) removes one Ca^{2+} ion in exchange for 3 Na^{+} ions, with the result of each exchange being the net movement of 1 positive ion into the cell (Shigekawa and Iwamoto, 2001) and a slow depolarization of the membrane potential. The coupling of SR Ca^{2+} release with translocation of Ca^{2+} out of the pacemaker cell *via* NCX is aided by the co-localization of NCX channels in the SL and RYRs in the SR. Recently, Monfredi et al. (2013) bought the membrane and calcium clock models for vertebrate pacemaker cells together by proposing that the depolarizations caused by both I_f and SR-Ca^{2+} release-stimulated NCX reinforce each other to bring about the pacemaker potential.

A simple pharmacological approach has been used to test these membrane and calcium clock models in hagfish hearts by applying selective pharmacological channel blockers (Wilson and Farrell, 2013). When isolated hagfish hearts are placed into physiological saline held at 10 °C, they continue to beat rhythmically at about 21 bpm for over 24 h. When the ventricle is separated from the atrium, each chamber continues to beat rhythmically, the atrium at the same rate, but the ventricle at 41% (8 bpm) of the atrial rate (Wilson and Farrell, 2013). HCN channels in the SL can be blocked by zatebradine and ZD7222 and such application would provide support for the membrane clock hypothesis if it reduced heart rate. When 0.05 mM of zatebradine, the HCN blocker, is applied to the whole heart, heart rate is reduced by 61% (8.5 bpm; Wilson and Farrell, 2013). With 5 mM zatebradine, spontaneous atrial contractions ceased completely, while ventricular