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Effect of an Increase in Heart Rate on the Pumping Function of the Heart Ventricles in Cold-Blooded Animals under Low Ambient Temperature Conditions

Natalya A. Kibler*, PhD; Vladimir P. Nuzhny, PhD, ScD; Dmitry N. Shmakov, PhD, ScD

Institute of Physiology, Komi Science Center, Ural Branch of the RAS
Syktyvkar, Komi Republic, Russia

Abstract

This study investigated the effect of heart rate (HR) on the pumping function of the heart ventricle (HV) in rainbow trout *Oncorhynchus mykiss* acclimated to a temperature of 5–7ºC, and in adult frogs *Rana temporaria* at an ambient temperature of 10–12ºC. The dynamics of intracavitary ventricular pressure was recorded by transmural catheterization. HR was changed by atrial electrocardiostimulation. The increase in HR in rainbow trout resulted in a decrease in the maximal systolic ventricular pressure (MSVP), an increase of end-diastolic ventricular pressure (EDVP), and a reduction in the maximum rates of the rising and falling pressures. In the frog, MSVP and isovolumic parameters (dP/dt_{max} and dP/d_{tmin}) also decreased as HR increased from 24 bpm to 42 bpm. The preload of the frog's HV did not change significantly, compared to the sinoatrial rhythm (SR). The maximum increase in the frequency of atrial stimulation up to 60 bpm in fish and up to 42 bpm in frogs resulted in a significant decrease in the myocardial contractility and deterioration of the pumping function of HV under low ambient temperature conditions. (**International Journal of Biomedicine. 2020;10(3):262-265.**)

Key Words: pumping function • heart rate • heart ventricle • temperature • rainbow trout • frog

Abbreviations

EDVP, end-diastolic ventricular pressure; **HR**, heart rate; **HV**, heart ventricle; **MSVP**, maximal systolic ventricular pressure; **SR**, sinoatrial rhythm.

Introduction

Any stressful changes in the environment trigger a complex of physiological reactions, mainly associated with an increase in cardiac output, which is realized by increasing HR.(1) In cold-blooded vertebrate amphibians, HR variability is considered as a factor that ensures the activity of the heart in extreme conditions that go beyond the usual conditions in the organism's habitat.(2)

In lower ectothermic (cold-blooded) vertebrates in natural conditions, slow sinoatrial heart rhythm (3,4) and the degree of organization of the cardiac conduction system (5,6) do not allow a significant increase in HR. In amphibians, however, an increase in habitat temperature to 20°C results in an increased HR, accompanied by improvement in the contractile function of the ventricle.(7) In fish, especially salmon, cardiac output increases during active movement. During this time, the stroke volume and, to a lesser degree HR, is responsible for the rise of cardiac output during swimming. Increasing the temperature of the surrounding water environment from 3°C to 10°C is accompanied by a significant increase in HR; however, during these conditions, stroke volume and cardiac output vary in different fish species.(8) In a study conducted on strips of ventricular myocardium,(9) the increased HR in rainbow trout (*Oncorhynchus mykiss*) *in vitro* at 4°C, 10°C, and 18ºC, acclimated to 10ºC, revealed no changes in the myocardial contractile function. Currently,

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***Corresponding author:** *Natalya A. Kibler, PhD; Laboratory of Cardiac Physiology, Institute of Physiology, Komi Science Center, Ural Branch of the RAS, Syktyvkar, Komi Republic, Russia. E-mail address: natanadya@mail.ru*

there are insufficient studies of the regularities of the influence of HR on the contractile function of the heart in amphibians and fish at low temperature conditions at which effective cardiac activity is maintained.(10) It is important to conduct a model study of the contractile function of HV in cold-blooded animals (fish and amphibians)at different HRs in low ambient temperature conditions.

The study of the contractile properties of HV with a functionally homogeneous spongy myocardium in lower ectothermic vertebrates (fish and amphibians) under electrocardiostimulatory myocardial stress (an increased HR) will help to explain the mechanisms of the heart function.

The purpose of this work is to study the influence of HR increase on the contractile properties of HV in fish and amphibians, by modified atrial electrocardiostimulation in low temperature conditions.

Materials and Methods

Animals and surgical procedure

This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The Animal Care and Use Committee of Institute of Physiology of the Komi Science Center of the Russian Academy of Sciences approved the experimental protocol (approved number: 29).

Fish. The experiments were carried out on 14 rainbow trout (*Oncorhynchus mykiss*) of both sexes, weighing 1.1±0.2 kg, adapted for several weeks to the natural water temperature of 5-7°C in pond-farm cages. Each fish was immobilized and fixed in a water-filled tray with its body positioned on the back. A rubber hose was inserted into the oral cavity, through which, to provide artificial respiration, river water at a temperature of 5–7°C was continuously passed through the gills under low pressure. The body temperature of the experimental animals during the study was maintained within 5–7°C. A longitudinal incision was used to open the chest cavity and pericardium, exposing the heart and the myocardium of the ventricle.

Frog. Experiments were performed on 14 adults (2–3 years old) frogs (*Rana temporaria*) of both sexes, weighing 34 g – 47 g. All animals demonstrated normal sinus rhythm on an ECG. The frogs were anaesthetized by placing them for 3 minutes in a jar containing 40% ethanol. After that, the ventral thoracic wall was removed and the pericardium was cut open. During the experiment, the body temperature of each animal was in the range of 10-12ºC and the heart was flushed with warm Ringer's solution (10–12ºC). In the laboratory, the animals' body temperature equilibrated rapidly with an ambient temperature that corresponded to the data of other researchers. At the end of the experiment, the animals were euthanized by an intravenous injection of an overdose of alcoholic solution.

Hemodynamic recording

In both fish and frogs, the hemodynamic variables were determined with the Prucka Mac Lab 2000 system (GE Medical System, GmbH). The pressure in the ventricle was measured with a catheter (internal diameter, 1 mm) filled with the heparinized 0.9 % saline inserted via the free wall into the ventricular cavity. Invasive monitoring of the pressure was carried out using transducers, transforming blood pressure inside of the vessels as the transducer registered mechanical changes. Intraventricular pressure and ECG in standard bipolar limb leads were recorded synchronously. Hemodynamic parameters measured included: MSVP (mmHg), EDVP (mmHg), maximal value of the MSVP derivative (dP/dt_{max}, mmHg/s), maximal rate of MSVP decline (dP/dt_{min}, mmHg/s). The durations of QRS complex and QT interval were measured also.

Pacing

The HR in fish was changed by pacing with a CEECX–3 pacemaker. The atrium was stimulated, with the stimuli lasting 2ms and amplitude 2–4V in the range from sinus rhythm to the maximum HR, at which disorders occurred in heart activity. In our study, HR of 60bpm is the maximum frequency in trout under the given temperature conditions, above which rhythm disturbance occurred.

In frogs, HR was changed by pacing the right atrium from 0.4 to 0.7Hz with step 0.1Hz (24-42 bpm). The right atrium was stimulated with cathodic impulses (duration 3.5ms; twice-diastolic threshold). The amplitude of impulses was 3–5V. The duration of pacing period was 1 min.

The results were statistically analyzed using BIOSTAT 4.03 program with use of Wilcoxon and Mann-Whitney criterion. Data are presented as mean±standard deviation (M±SD). A probability value of $P<0.05$ was considered statistically significant.

Results

Fish. The initial HR at SR ranged from 18 bpm to 27 bpm at t=5–7°C. The duration of the QRS complex in the ECG increased at HR, increasing to 60 bpm (148.3±21.4 ms; $P<0.05$), relative to SR (102.7±12.5 ms). The QT interval shortened with the increase in HR up to 60 bpm (506.6±93.0 ms; $P<0.05$), relative to SR (1208.2±249.4 ms).

MSVP was significantly reduced ($P<0.02$) at imposed rhythms from 30 bpm to 60 bpm, relative to SR (Fig.1).

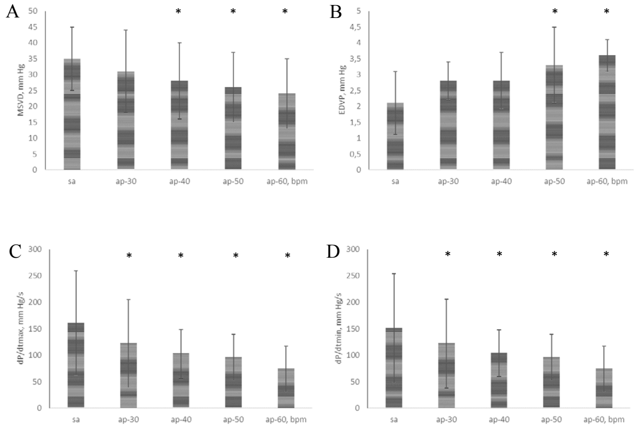

Fig 1. *Hemodynamic parameters of the heart of rainbow trout with an increase in HR: A- MSVP; B - EDVP; C - the maximum rate of the ventricular pressure rise (dP/dt_{max}); D - the maximum rate of the pressure fall ($dP/dt_{min.}$ sa – sinus rhythm; ap – atrium pacing rhythm. 30, …, 60 - HR in beats/min, respectively. * $P<0.05$ - in relation to the sinus-atrial rhythm.*

During this time, EDVP increased significantly with the increase in HR, compared to SR. Increased HR led to a synchronous change in the isovolumic indices (dP/dt$_{max}$ and dP/dt$_{min}$). Starting with the frequency of 30bpm and up to a maximum of 60bpm, these indicators were reduced ($P<0.02$), relative to SR.

Frog. At t=10–12°C, HR in frogs ranged from 23bpm to 29bpm. The duration of the QRS complex did not significantly change with the increase in HR (from 27 to 44 bpm), compared to SR (76.2±20.8 ms), which distinguishes the change in this indicator obtained in fish. The duration of the QT interval decreased ($P<0.05$) with the increase in HR, compared to SR. MSVP and isovolumic indices (dP/dt$_{max}$ and dP/dt$_{min}$) decreased ($P<0.05$) with the increase in the imposed HR from 32 bpm to 44 bpm. However, the preload of HV in frogs with the increased HR did not significantly change, compared to SR (Fig.2), in contrast to the resulting EDP in fish.

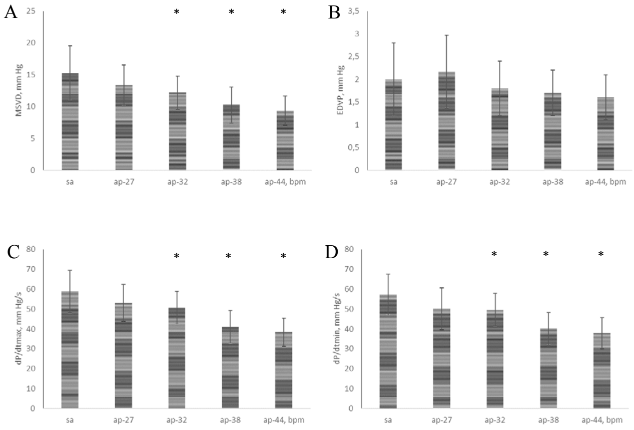

Fig. 2. *Hemodynamic parameters of the heart of frog with an increase in HR: A- MSVP; B - EDVP; C - the maximum rate of the ventricular pressure rise (dP/dt$_{max}$); D - the maximum rate of the pressure fall (dP/dt$_{min}$ sa – sinus rhythm; ap – atrium pacing rhythm. 27, ..., 44 - HR in bpm, respectively. *P<0.05 - in relation to the sinus rhythm.*

Discussion

Fish. In relation to the initial rhythm in trout, MSVP, dP/dt$_{max}$ and dP/dt$_{min}$ decreased while EDVP increased with the increase of HR. In rainbow trout adapted to a temperature of 5–7°C at SR in the frequency range of 21.6±4.9 bpm, the pumping function of the heart was probably within the upper limit of the physiological norm and the further increase in HR caused its deterioration. Previously, research on myocardial strips by Coyne et al. showed that the pumping function of the HV muscle in trout depends on the concentration of intracellular and extracellular calcium, and that changes in HR do not significantly affect the contractile function.

The ion channels of the fish heart, being plastic objects, actively react to the electrical parameters of the heart, depending on the ambient conditions and the way of life of fish. The density and kinetics of the sodium current (INa) are essential for generating the action potentials in fish myocardium. For example, at low temperatures, in burbot and rainbow trout the high INa density supports a relatively high HR.(11) Because the main role in activating contraction in the fish myocardium is given to the flow of extracellular Ca^{2+} through the sarcolemma, the electromechanical synchronization and direction of myocardial contraction are limited not only by the size, but also by the duration and direction of the action potential.(12)

It has been established(13) that an increase in HR by more than 2 times (in our study, increasing HR in fish up to 60 bpm) reduces the functional heterogeneity of the myocardium, which is manifested in almost simultaneous depolarization of the layers in the ventral and dorsal parts of the ventricle, which indicates how intensely those parts are involved in the contractile process. The functional homogeneity of the depolarization process of the myocardium is probably related to the almost simultaneous opening of Na^{+}- and Ca^{2+}-dependent channels, which increases the contribution of the calcium component of the depolarizing current to enhancing the operation of the Na\Ca pump under Ca^{2+} cardiomyocyte overload.(14) In our experiment, in the electrocardiogram in fish during atrial stimulation with the frequency exceeding the physiological HR, the changes in the QT interval that were revealed may be a result of the formation of dynamic functional heterogeneity of the myocardium. As a result, HR exceeds the ability of cardiomyocytes to provide intracellular Ca^{2+} homeostasis with the formation of the conduction block, which is manifested in the ECG by the broadening of the QRS complex.(14)

At the maximum imposed HR of 60bpm, we found a short period in which hemodynamic parameters were stabilized, the so-called limit of reducing the contractile properties of HV. The increase in the ventricular preload in trout with the increase in HR is due to the increased contribution of α-adrenoreceptors, which compensates for the reduction in the time it takes for the ventricle to fill. It is possible that, under the conditions of our experiment, with increased preload and reduced contractility of HV, the mechanism of homeostatic physiological functions of the myocardium is activated, at which the maximum HR of 60 bpm is the maximum functionally effective frequency. Above that frequency, there develop structural disorders in HV and fatal rhythm disturbance. Thus, an increase in HR at the artificial atrial rhythm at a constant water temperature (5–7°C) does not improve the contractile properties of HV in fish. In contrast, the results obtained earlier(13) suggest that with the increase of HR the mechanical load on the ventricular base area is distributed and the electrical and mechanical properties of the ventricular myocardium are desynchronized.

Frog. Previously, we showed that in amphibians one of the mechanisms for maintaining the functional activity of the heart in conditions of high ambient temperature with an increase in HR is an increase in electrical heterogeneity on the ventral surface of the ventricle in the area of the arterial cone.(2) The discovered electrical heterogeneity of the myocardium indicates that the myocardial tissue of the amphibian heart is able to change its functional properties depending on external conditions.(7) Regionally determined changes in the electrophysiological properties of the myocardium are based on the changes in metabolism throughout the body.(15) Electrical stimulation of the atrium at the increase in HR in amphibians does not cause an increase in EDVP, unlike in fish. Rather, it reduces the systolic blood pressure and isovolumic parameters of HV. It is known that the modulating effect on the strength and HR is realized through changing the action potential in order to discharge cardiomyocytes when performing excessive load.(16) During an increase of HR beyond the physiological norm for this species, this mechanism apparently stops working, which leads to the phenomenon of calcium overload of cardiomyocytes. The natural variability of HR as a factor of increasing the contractile function is realized through the increase in the electrical heterogeneity of the myocardium, while high HR reduces the electrical heterogeneity of the myocardium, and is the cause of changes in the contractile properties of the heart. Thus, we have shown that the increase in HR above the typical indicators for amphibians at an ambient temperature of 18–20°C is accompanied by a decrease in the contractile function of the myocardium.(2)

Thus, the increased HR in lower cold-blooded animals under low habitat temperature leads to a significant decrease in the myocardial contractility, as well as deterioration of the pumping function of HV, and it cannot be considered as an adaptive factor that ensures the activity of the heart in extreme conditions that go beyond the usual habitat of the animal.

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Competing Interests

The authors declare that they have no competing interests.

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