

# Electrical and Contractile Properties of the Heart Ventricle in Response to Ambient Temperature Changes in Frog *Rana temporaria*

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## Abstract

The aim of the study was to investigate the dynamics of change in the intraventricular pressure, depolarization and repolarization processes on the ventricular epicardium (VE) in *Rana temporaria* in response to a rise in ambient temperature. By methods of catheterization and electrophysiological mapping the dynamics of the intraventricular pressure change, the processes of depolarization and repolarization on the epicardium of the heart ventricle in adult frogs in a temperature range from 10°C to 20°C were studied. We found that the rise in body temperature by 10°C led to increase of the maximal systolic ventricular pressure (MSVP), maximal value of the MSVP derivative and maximal rate of MSVP decline but to a decrease in dispersion of depolarization time and durations of activation-recovery intervals on the ventral and dorsal sides of VE. The role of the electrical inhomogeneity of the myocardium was shown to be a modulator performing fine adjustment to factors in the external environment of the organism. (**International Journal of Biomedicine. 2017;7(3):180-184.**)

**Key Words:** intraventricular pressure • ventricular epicardium • depolarization • repolarization • temperature • *Rana temporaria*

## Abbreviations

AT, activation time; ARIs, activation-recovery intervals; ECG, electrocardiogram; EDP, end-diastolic pressure; HR, heart rate; MSVP, maximal systolic ventricular pressure; VE, ventricular epicardium.

## Introduction

The ambient temperature can limit or increase absorption of O<sub>2</sub> in animals; thus it has a drastic effect on the cardiovascular system.<sup>(1-4)</sup> Ectothermic animals inhabiting regions with a temperate climate have to compensate for temperature changes in order to cope with the seasonal thermal variations. It is known that ectothermic vertebrate hearts are highly sensitive to temperature effects. Therefore, ectothermic animals can be used to understand mechanisms controlling the electrical and contractile functions of the myocardium in response to changes in environmental temperature.<sup>(5-7)</sup>

At present there are only a few studies of the electrophysiological processes that occur in a frog's heart ventricle under changes in ambient temperature.<sup>(8,9)</sup> It has been shown that when the body temperature in the frogs is

decreased to 10°C, then HR is diminished and the durations of QRS and ST-T complexes of ECG are lengthened. At the same time, the repolarization sequence is changed on the ventricular epicardium (VE). There are no data about cardiohemodynamic in amphibia under temperature effects.

The aim of the study was to investigate the dynamics of change in the intraventricular pressure, depolarization and repolarization processes on the VE in *Rana temporaria* in response to a rise in ambient temperature.

## Materials and methods

### Animals and surgical procedure

The 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).

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Data were obtained from 18 adult frogs *Rana temporaria* of both sexes (age, 2-3 years old). Two groups of animals were investigated: Group I consisted of 8 frogs with body temperature 10°C (body mass, 32±7 g), and Group II consisted of 10 frogs with body temperature 20°C (body mass, 40±5 g). The body temperature was measured rectally by means of a temperature sensor of the hemodynamic apparatus Prucka MacLab 2000 system. Measurements of rectal temperature did not show an essential difference between a frog's body temperature and the chamber of habitat. This is in agreement with the findings of other authors.<sup>(5,10)</sup> As in our previous studies on amphibians,<sup>(11,12)</sup> the animals 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 heart was flushed with Ringer's solution. At the end of the experiment, the animals were euthanized by the intravenous injection of an overdose of alcoholic solution.

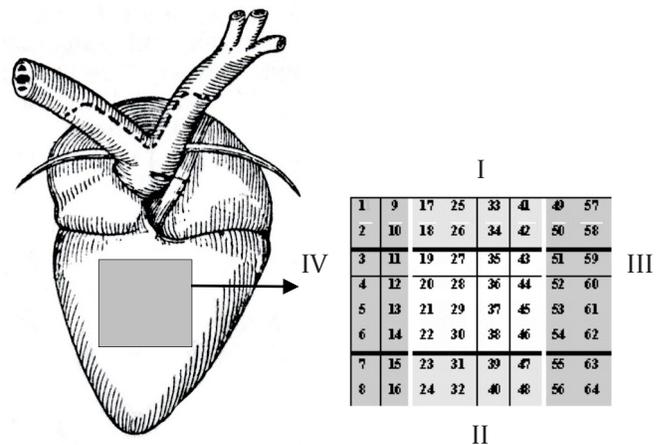
#### Hemodynamic recording

The hemodynamic variables were determined with the Prucka MacLab 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. IP and ECG in standard bipolar limb leads were recorded synchronously. Hemodynamic parameters measured included MSVP (mmHg), EDP (mmHg), maximal value of the MSVP derivative (+dP/dt<sub>max</sub>, mmHg/s), maximal rate of MSVP decline (-dP/dt<sub>min</sub>, mmHg/s). The durations of QRS complex and QT interval were measured also.

#### Electrocardiographic recording and analysis

Unipolar electrograms and ECGs were recorded in reference to Wilson's central terminal. Simultaneous data acquisition was done by means of a custom-designed mapping system (16 bits; bandwidth 0.05 to 1000 Hz; sampling rate 4000 Hz). Standard bipolar limb lead ECGs were recorded with an application of subcutaneous steel needle electrodes. Registration of an epicardial electrogram was performed by using a matrix (5 mm x 5 mm) containing 64 electrodes at the sinoatrial rhythm. The matrix was alternately superimposed on the central portion of the ventral and dorsal sides of VE in such a way that the superior border of the matrix grasped the basal part and the inferior border – part of the ventricular apex (Fig.1).

In every epicardial lead, the activation time (AT), the repolarization time (RT) and the activation-recovery intervals (ARIs) were obtained. The latter was used to assess local repolarization durations. AT, RT and ARIs were determined as dV/dt<sub>min</sub> during QRS complex, dV/dt<sub>max</sub> during ST-T, and the difference between RT and AT, respectively. The values were determined automatically, inspected by the observer and corrected manually if necessary. In each set of simultaneously recorded electrograms, the beginning of the QRS complex in the II limb lead was chosen as a reference time point with respect to which ATs and RTs were measured in a given set of electrograms.



**Fig. 1.** Scheme of the position of the matrix (5 mm × 5 mm) with electrodes on the epicardial surface of the ventral side of the frog ventricle. Numbers from 1 to 64 show the location of the electrodes on the matrix. Black-and-white marking on the matrix shows the boundaries of the epicardial mapping zones.

I - the basal region of the matrix; II - the apical region of the matrix  
III - the right portion of the matrix; IV - the left portion of the matrix.

The dispersion of ATs, ARIs, and RTs of the ventricle were taken as the difference between the maximal and minimal AT, RT and ARIs values in a set of recorded electrograms, respectively.

In order to construct isochronal activation maps, the zero point was assigned to the timing of the epicardial activation breakthrough. Similarly, the zero points in the repolarization maps identify for the earliest repolarization on the epicardium.

Statistical examination was done using statistical package Primer of Biostatistics 4.03 and SPSS 11.5 using Wilcoxon test for paired comparisons and the Friedman test followed by the Wilcoxon test. Data are presented as mean±SD. A probability value of  $P < 0.05$  was considered statistically significant.

## Results

At the body temperature of 10°C, HR in frogs was 24.3±2.3 bpm, and the duration of QRS complex and QT interval was 83.4±4.5 ms and 1375.6±363.2 ms, respectively. When body temperature was increased to 20°C, HR was raised to 38.8±3.1 bpm ( $P < 0.05$ ), but the duration of QRS and QT interval was decreased to 68.7±7.2 ms and 783.3±102.2 ms, respectively.

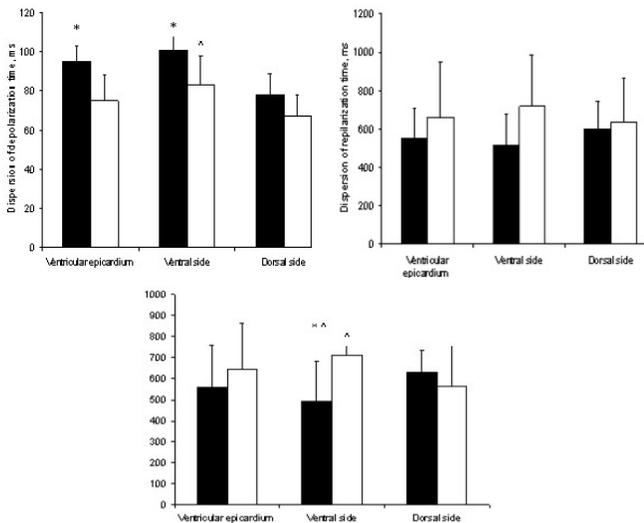
At 10°C, the earliest foci of depolarization were located in the basal part of the epicardial fragment on the dorsal surface ( $P < 0.05$ ), and the latest foci in the left part of the epicardial fragment on the ventral surface. The sequence of activation on the dorsal surface of the epicardium is from the base to the apex ( $P < 0.05$ ) (Table 1). For frogs in Group I, dispersion of AT in both epicardial surfaces was more than for those in Group II ( $P < 0.05$ ) (Fig. 2). The duration of repolarization on the ventral surface of the epicardium was greater than on the dorsal one ( $P < 0.05$ ) (Fig. 2). Dispersion of ARIs time in both epicardial surfaces of the ventricle was 559.0±200.8 ms in total (Fig. 2). At the same time, dispersion of ARIs time was less on the dorsal surface of the ventricle than on the ventral one ( $P < 0.05$ ) (Fig. 2).

**Table 1.**

**Chronotopographic performance of different zones of VE of the frog heart at the body temperature of 10°C (Group I) and 20°C (Group II)**

Epicardial areas of the ventricle		Depolarization time		Repolarization time	
		Group I	Group II	Group I	Group II
VS	I	35.4±12.3	21.8±11.8#	961.5±342.2	855.4±250.7
	II	35.8±10.6	32.5±12.3	950.4±342.5	858.8±249.5
	III	32.7±10.2*	22.5±13.4*	972.2±334.6	828.1±245.6*
	IV	40.2±10.7	38.1±13.1	963.5±346.2	918.2±252.1
DS	I	26.6±9.4†	30.4±9.4†	1243.6±469.8^	813.5±220.1
	II	35.4±10.8	43.2±10.6	1250.0±450.4^	804.7±235.7
	III	29.6±12.7	44.1±14.3	1236.2±478.5^	814.4±221.1
	IV	31.4±14.7	37.2±17.5	1245.7±418.4^	805.7±240.7

\* $P < 0.05$  – in relation to the left part of the epicardial portion of the ventricle; # $P < 0.05$  – in relation to the apical part of the epicardial portion of the LV. ^ $P < 0.05$  – in relation to Group II. I – fragment of the basal region of the matrix; II – fragment of the apical region of the matrix; III – fragment of the right region of the matrix; IV – fragment of the left region of the matrix. VS – ventral side of the VE; DS – dorsal side of VE.



**Fig. 2.** Dispersion of depolarization, repolarization and ARIs times in the frog VE at the body temperature of 10°C and 20°C. \* $P < 0.05$  – in relation to the body temperature of 20°C. ^ $P < 0.05$  – in relation to the dorsal side of the VE. ■ Group I (10°C) □ Group II (20°C)

At 20°C, a wave of depolarization on the ventral and dorsal surfaces of the ventricle extends from the base to the apex ( $P < 0.05$ ). The earliest AT was found in the basal part of the epicardial fragment on the ventral side, and the latest in the right part on the dorsal surface (Table 1). Dispersion of AT on the ventral and dorsal surfaces of the epicardium was 75.4±13.0 ms, and AT on the ventral side of the ventricle was more than that on the dorsal side ( $P < 0.05$ ) (Table 1).

**Table 2.**

**ARIs duration different areas on the ventral and dorsal sides of the VE of the frog at the body temperature of 10°C (Group I) and 20°C (Group II)**

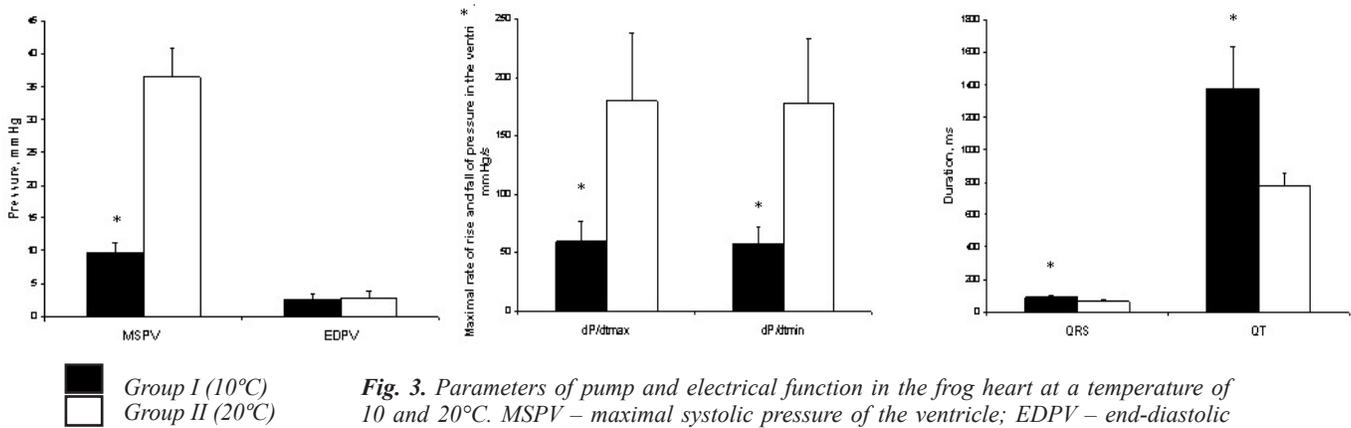
Epicardial areas of the ventricle		Group I	Group II
Total duration of ARIs both sides of the epicardium of the ventricle of the frog		1067.0±153.4*	800.8±33.5
VS	Duration of ARIs of VS	918.7±203.6##*	828.4±96.3#
	I	926.6±150.4*	837.1±252.7
	II	915.5±149.4*	816.3±270.2
	III	939.4±141.3*	806.6±207.2^
	IV	919.7±152.4	869.1±203.9
DS	Duration of ARIs of DS	1215.4±325.9*	772.1±101.0
	I	1220.5±360.2*	782.4±128.6
	II	1214.1±343.4*	762.4±137.3
	III	1235.0±294.3*	764.4±200.5
	IV	1217.7±318.2*	769.5±147.2

\* $P < 0.05$  – in relation to Group II; ^ $P < 0.05$  – in relation to the left side epicardial fragment of VS of the ventricle; # $P < 0.05$  – in relation to the duration of ARIs on DS of the epicardial portion of LV. I – fragment of the basal region of the matrix; II – fragment of the apical region of the matrix; III – fragment of the right pane of the matrix; IV – fragment of the left region of the matrix; VS – ventral side of the VE; DS – dorsal side of VE.

It should be noted that at this temperature the duration of ARIs on the ventral surface of the VE was increased as compared with the dorsal surface ( $P < 0.05$ ) (Table 2). On the ventral side of the epicardium the largest increase in repolarization duration was observed in the left part as compared with the right part ( $P < 0.05$ ) (Table 2). In Group II, the repolarization durations both on ventral and dorsal surfaces of the ventricle, as well as the total durations of ARIs, were less than in Group I ( $P < 0.05$ ) (Table 2). Dispersion of ARIs time in the ventral and dorsal surfaces of the ventricle was 645.4±219.8 ms; on the ventral side it was more than on the dorsal side ( $P < 0.05$ ) (Fig. 2). There was an increased dispersion of ARIs time on the ventral surface of the epicardium as compared with Group I ( $P < 0.05$ ).

The repolarization sequence was directed from right to left on both surfaces of the VE (Table 1). We did not find apicobasal differences in termination time of repolarization (Table 1). Dispersion of termination time of repolarization on the ventral and dorsal surfaces of the epicardium was 662.9±285.0 ms. There were no differences in dispersion of termination time of repolarization between the ventral and dorsal sides of the epicardium in both groups of animals (Fig. 2).

Thus when body temperature in frogs was increased to 20°C, then the dispersion of AT and ARIs on the ventral and dorsal sides of the VE were diminished.



**Fig. 3.** Parameters of pump and electrical function in the frog heart at a temperature of 10 and 20°C. MSPV – maximal systolic pressure of the ventricle; EDPV – end-diastolic pressure of ventricle; dP/dt<sub>max</sub> – maximal value of the MSVP derivative; dP/dt<sub>min</sub> – maximal rate of MSVP decline; QRS – duration of QRS complex in ECG; QT – duration of QT interval in ECG. \*P<0.05 – in relation to body temperature of 20°C.

### Dynamics of change of intraventricular pressure

When the body temperature in frogs was increased by 10°C, the indexes of the pump function of the ventricle were increased roughly by a factor of three: MSVP (9.0±3.1 vs. 36.2±6.8 mmHg;  $P<0.05$ ), dP/dt<sub>max</sub> (55.1±7.3 vs 182.8±62.3 mmHg/s;  $P<0.05$ ) and dP/dt<sub>min</sub> (49.5±6.7 vs 175.0±60.8 mmHg/s;  $P<0.05$ ). At the same time, EDP was not changed (Fig. 3).

## Discussion

The present investigation showed that in frogs, in response to the change in body temperature from 10°C to 20°C, the studied indexes of the pump function of the ventricle were increased, but dispersion of depolarization time and duration of ARIs on the ventral and dorsal sides of the VE were decreased.

The rise of body temperature in studied animals causes a decrease in the duration of ARIs on the both surfaces of the VE (the tissue level of the heart organization). And at the cellular level, the duration of the action potential of ventricular cardiomyocytes is shortened in ectothermic animals with an increase in ambient temperature,<sup>(13-15)</sup> that is, the same trend is observed.

Our study showed that in frogs, with the rise of ambient temperature there was an increase of HR and shortening of the duration of the ventricular complex of the ECG. These findings were in consistent agreement with previously obtained data.<sup>(8,9)</sup>

The regional (ventral-dorsal) changes in the repolarization process on the epicardium of frog ventricles at body temperature of 20°C are presented here for the first time. We assume that it is probably one of the mechanisms that ensure an adequate contractile act in response to the temperature effects.

We have found that the rise in body temperature in frogs from 10°C to 20°C causes an increase in heterogeneity of repolarization in the VE. The lack of significant changes in the excitation sequence of the VE, in dispersion of ARIs times and the termination of repolarization, possibly is due to heterogeneous change in the action potential duration of cardiomyocytes in different epicardial areas. According to the

contemporary paradigm,<sup>(16-18)</sup> the electrical and mechanical inhomogeneity of the myocardium is a modulator performing fine adjustments to the factors of the internal and external environments of the organism; that is, it performs an adaptive function.

At the same time, it is possible that there is a limiting effect of electrical heterogeneity of the myocardium to the optimal HR for a given species.<sup>(12)</sup> The obtained results point to the existence of adaptive potential or functional reserve in the heart of the studied animals that occurs in response to the change in the ambient temperature.

### Limitations and perspectives of the study

In our study, the frogs were anaesthetized by 40% ethanol. In experimental work with amphibians, authors have used different drugs for anaesthesia: methanesulfonate (MS-222),<sup>(19-20)</sup> ether<sup>(5,8)</sup> and urethane.<sup>(21)</sup> Therefore, further experiments are necessary to compare effects of these drugs on the amphibian cardiovascular system. The present experiments are limited in that they provide an assessment of the processes of depolarization and repolarization only on the fragments of VE. It would be interesting to broaden these studies using ultrasound methods and an increased temperature range to examine in detail the adaptive features of cardiohemodynamics in amphibia.

## Competing interests

The authors declare that they have no competing interests.

## Sources of Funding

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