Variability of the Rhythm of Isolated Rat Heart

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Variability of the rhythm of Langendorff-perfused isolated rat heart was compared to that of human and rat hearts *in vivo*. The rate and variability of the rhythm of isolated rat heart differed from those *in vivo*. It was shown that intracardiac mechanisms regulating heart activity affect the main parameters of heart rate variability.

Key Words: *heart rate variability; isolated heart*

Methods for mathematical analysis of heart rate variability (HRV) and the concept of a double-circuit regulation of heart rate (HR) proposed by R. M. Baevskii offer possibilities to study peculiarities of the regulatory systems. It is believed that HRV reflects the state of extracardial regulation: the sympathetic and parasympathetic nervous systems and humoral and metabolic mechanisms [2]. HRV changes in patients with cardiac insufficiency are of considerable prognostic significance [6,7]. This problem holds much promise for studies of the autonomic regulation of isolated organs during transplantation.

The isolated heart is not under control of exogenous neurohumoral factors. However, the heart can respond (at the level of the intracardiac nervous system) to environmental changes. It is known that cells of the sinoatrial node respond to mechanical stretching by accelerated pacemaker activity [5]. Furthermore, generation of the cardiac rhythm depends on ionic balance in pacemaker cells [1]. It was proposed that cardiac rhythm fluctuations can be due to blood pressure changes in the sinoatrial node artery [5]. These data suggest that intracardiac regulatory systems are involved in the realization of slow-wave and/or intermittent changes in HR [4].

The model of Langendorff-isolated heart simulates its functioning under physiological conditions: ionic composition of perfusion Krebs—Henseleit solution is similar to that of the blood, and pH, temperature, and pressure approximate physiological levels. At the same time, these experimental conditions affect metabolism of the isolated heart and HR parameters.

Previous experiments revealed variability of the rhythm of the isolated rabbit heart [9]; the spectrum of some fluctuations differed from that observed *in vivo*. On the other hand, it was reported that HRV disappears after denervation of the heart [8]. Here we studied HRV in Langendorff-perfused isolated rat heart using an improved method of cardiointervalogram (CIG).

MATERIALS AND METHODS

Experiments were performed on 20 outbred albino male rats weighing 150-200 g and narcotized with 35 mg/kg Nembutal. Before isolation of the heart, the animals were intraperitoneally injected with 500 IU/kg heparin. Langendorff-isolated heart was perfused with oxygenated Krebs—Henseleit solution (95% O_2 and 5% CO_2). Electrocardiogram (ECG) of the isolated heart was recorded using original clip electrodes. In intact animals, ECG was recorded using subcutaneous needle electrodes (standard bipolar lead II). Epinephrine hydrochloride was added (10 μg intracoronary) to study HRV changes in the isolated heart induced by humoral factors.

Beat-to-beat fluctuations in the cardiac rhythm were analyzed by *R-R* interval recording. *R-R* interval histogram was presented by vertical bars with heights equal to the duration of the cardiac cycle (the interval

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TABLE 1. Heart Rate and Its Variability in Human and Rat Hearts *in Vivo* and Isolated Rat Heart (*M*±*m*)

Parameter	Human	Rat	Isolated rat heart
HR, bpm	67.4±2.6*	294.2±8.6	176.1±9.8*
R-R_m, msec	889.1±37.2*	208.3±6.5	359.3±22.7*
Mode, msec	907.1±36.9*	208.1±6.5	357.1±21.6*
ΔX, msec	269.3±5.5*	12.9±1.7	18.9±4.5
Standard deviation, msec	52.7±2.0*	2.9±0.3	4.3±0.9*
Mode amplitude, %	37.0±1.6*	72.0±3.1	58.1±5.8*

Note. *$p<0.05$ compared to rat heart.

between consecutive R waves). R-R interval histogram was used for visual analysis of HR and for processing and presentation of experimental data reflecting HRV and characteristic changes in the cardiac rhythm under unsteady conditions.

The distribution of R-R intervals (as random values) was analyzed by constructing histograms of R-R intervals or variational curves, whose points corresponded to bases or centers of the corresponding vertical bars on the histogram. The following parameters were analyzed: mode, mode amplitude, and variational range (ΔX).

We calculated standard deviation and mean R-R interval (R-R_m), which reflects the cardiac rhythm and inversely depends on HR. Mode, mode amplitude, and ΔX were also analyzed.

CIG was constructed by continuous monitoring on an MAVRS-01 device [3]. This device allowed us to visualize ECG or CIG, to construct sliding histogram from 1-37 current R-R intervals, and to calculate R-R_{min}, R-R_{max}, mode, and HR. R-R interval histogram was constructed with discretization steps of 5, 10, 15, 20, and 25 msec and an accuracy equal to $^1/_2$ of that estimated by the original method for R waves in ECG (not more than 1 msec). The necessary condition for this discretization step is the correspondence to the R wave with an accuracy of 1 msec.

CIG was recorded after a 15-min adaptation of the heart to isolation-perfusion conditions and analyzed by Pulsar-95 software. Segments consisting of 50-100 R-R intervals without artifacts and electrical instability of the heart were analyzed. HRV parameters were calculated and data were processed using Statistica 5.0 software (Student's t test).

RESULTS

Variability of the cardiac rhythm of Langendorff-perfused isolated rat heart was revealed at a discretization step of 5 msec. Parameters of HRV in Langendorff-isolated heart (Table 1) significantly differed from those typical of rat and human hearts *in vivo*.

HR in the isolated heart decreased by 40% (176 *vs.* 300-400 bpm in Nembutal-anesthetized rats) probably due to the maintenance of metabolism under these conditions. R-R_m and mode exceeded the corresponding *in vivo* parameters by 72%. ΔX of the isolated heart was 47% higher than that *in vivo*. Standard deviation reflecting dispersion of R-R intervals and ΔX increased to 152% of that *in vivo*. Mode amplitude of the isolated heart was only 19% below *in vivo* level, which indicated that the increase in HRV during perfusion of the isolated heart was less pronounced than the reduction in HR (Fig. 1, *a*, *b*).

TABLE 2. Changes in the Rate and Variability of the Rhythm of Isolated Rat Heart after Single Injection of Epinephrine (*M*±*m*)

Parameter	Initial level	Time after epinephrine administration, min		
		1	3	15
HR	147.6±15.7	228.8±10.8*	179.8±21.0	158.8±26.9
R-R_m	446.7±54.2	264.8±13.2*	354.2±43.8	409.3±66.4
Mode, msec	446.9±54.7	264.2±12.9*	360.8±36.0	432.5±70.0
ΔX, msec	28.1±6.2	9.2±1.5*	22.0±6.1	13.8±3.8*
Standard deviation, msec	6.9±1.2	2.6±0.4*	5.4±1.0	4.1±0.8*
Mode amplitude, %	42.3±6.9	64.7±7.9*	69.2±8.1*	46.0±7.9

Note. *$p<0.05$ compared to the initial level.

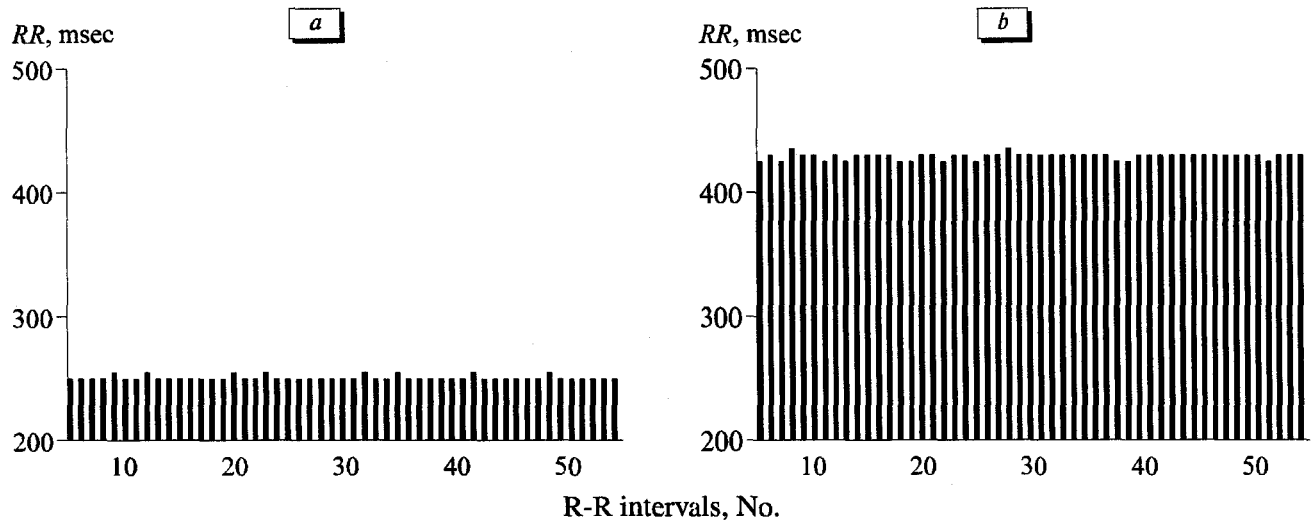

Fig. 1. Cardiointervalogram of the heart *in vivo* (*a*) and isolated rat heart (*b*).

Under physiological conditions, HRV in humans was much greater than in rats and surpassed this parameter during perfusion of Langendorff-isolated rat heart. This was manifested by considerable ΔX and standard deviation (Table 1).

Various factors, including neuromediators and hormones, affect initial variability of the rhythm of isolated rat heart. Intracoronary injection of 10 μg epinephrine not only produced positive chronotropic effect, but also 2-fold decreased HRV of the isolated heart (Table 2).

One minute after administration of epinephrine, HR increased by 55%, R-R_m and mode decreased by 41%, ΔX and standard deviation were reduced by 68 and 62%, respectively, and mode amplitude increased by 52% compared to the initial levels.

By the 3rd min after administration of epinephrine, HRV tended to normal. ΔX and standard deviation decreased, while mode amplitude did not significantly change compared to that recorded 1 min post-injection. HR gradually decreased, and R-R_m and mode increased. HR remained high 15 min after administration of epinephrine. Mode amplitude returned to the initial level, while ΔX and standard deviation remained below the control (by 28 and 65%, respectively).

These data indicate that in the absence of extracardial regulation during perfusion of isolated rat heart, the cardiac rhythm was lower and HRV was higher than *in vivo*. Hence, HRV depends not only on extracardiac, but also on intracardiac regulation. Some humoral factors, including epinephrine, affect variability of the rhythm of the isolated heart under near-physiological conditions.

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