(by 18.8 ± 3.6%). Hypercapnia was accompanied by an increase in p_aO_2 (by 7.9 ± 2.4%) and p_aCO_2 (by 6.8 ± 2.1%). Breathing a normoxic gas mixture with helium was accompanied by a small decrease in p_aCO_2, which may have been due to an increase in CO_2 diffusion under the influence of helium [5]. The complex helium-oxygen hypercapnic gas mixture caused an increase in p_aO_2 (by 19.1 ± 3.1%), but did not completely abolish the hypocapnia: p_aCO_2 remained depressed (by 2.7 ± 1.1%). Addition of 3% CO_2 only partially abolished the effect of helium, which induces hypocapnia.

Thus breathing a normoxic helium-oxygen gas mixture does not cause any significant changes in the pulmonary and systemic hemodynamics or in the blood gas composition. Meanwhile the presence of helium in complex gas mixtures may modify the reactivity of the pulmonary and bronchial vessels relative to the action of components introduced into the composition of these mixtures (relative to hypercapnia and hyperoxia in the present investigations).

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CHANGES IN RHYTHM OF THE ISOLATED AND INTACT FROG HEART WITH TIME

T. M. Vinogradova, G. S. Sukhova, A. I. Tumanov, and I. A. Lebedev

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The isolated frog heart still remains one of the most widely used experimental objects [2-9]. However, despite many investigations conducted on this object, there has still been no detailed study of the time course of the rhythm of the isolated frog heart, starting with the time of isolation. It is absolutely essential to know this relationship for the intelligent conduct and evaluation of the results of experiments. We know, for example, that there are differences in the relationship between the frequency of excitation of the heat and temperature for the intact and isolated frog heart [2, 4, 5]. Usually they are linked with the influence of the autonomic nervous system [2, 4]. It is interesting to compare these relationships, allowing for changes in the rhythm of the isolated frog heart with time. The investigation described below was carried out to study these problems (specifically, the time course of the rhythm of the isolated frog heart and the effect of this dynamics on the results of experiments).

Institute for Physico-technical Problems, Moscow. Department of Human and Animal Physiology, Faculty of Biology, M. V. Lomonosov Moscow State University.

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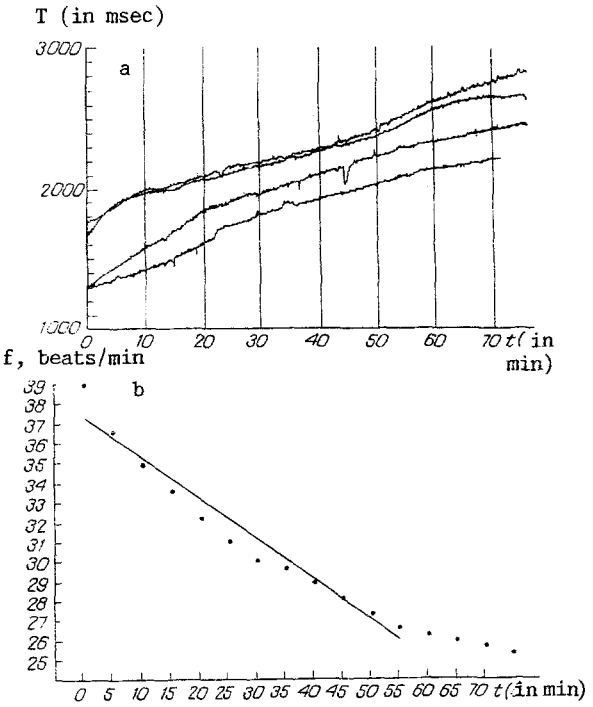

Fig. 1. Time course of rhythm of isolated frog heart between 20 and 95 min after preparation of the specimen. a) Intact intervalograms of periods of excitation of sinus node for four preparations; b) mean changes of frequency of excitation of sinus node with time.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated heart of male frogs (Rana temporaria). The heart was removed by a modified Gramenitskii's method [3]. The stability of the rhythm was studied in two types of preparations and in two time periods: from the time of obtaining the preparation until 1.5 h and from 1.5 h to 2 h 45 min. In the first type of preparation the sinus node and atria were unfolded to make a flat tissue slab, which was placed in oxygenated Ringer's solution. For 20 min after preparation of the specimen, it was kept in that solution at room temperature (18-19°C), after which the rhythm was recorded for 75 min. The time of normal working of the ventricle in a preparation of this type varied from 100 to 120 min. The ventricle then stopped. To study the stability of the rhythm in the 2nd time interval, a 2nd preparation was therefore made. It differed from the first in that the ventricle was removed from it. Recording of the rhythm of the preparation began 1.5 h after it was obtained and also continued for 75 min. Electrical activity of the heart was derived by means of suction cap electrodes and recorded on an N-338-4P automatic writer. The heart rate thus recorded was independent of the site of the cap electrodes (sinus node, atria, ventricle). In most experiments, the sinus node rhythm was judged from the atrial rhythm. The intervals between atrial AP were measured on a ChZ-57 frequency meter with an accuracy of 2 msec, and were led continuously throughout the experiment into a CM-1803 computer. The intervalogram of periods of excitation of the heart was plotted by PC-XT computer. To monitor the time course of changes in the period of excitation of the heart, 20 consecutive periods were averaged every 5 min of the experiment. To study dependence of frequency on temperature of the isolated heart the same preparation was used as in the experiment of series I. The temperature was changed from 5 to 24°C at the rate of about 0.3 deg. C/min. Electrical activity of the heart was recorded by means of suction cap electrodes 20 min after dissection, on an "Élkar" electrocardiograph. The dependence of frequency on temperature of unrestrained frogs was studied in males in the winter period (January to March). To record the ECG, the frog was anesthetized with ether and two electrodes were implanted: one beneath the scapula into the region of the atria, the other beneath the xiphoid process toward the anterior wall of the ventricle. To abolish visual stimulation during the experiment, the frogs were placed in boxes with lightproof walls. The rate of change of temperature was 0.5°C in 10 min. The skin temperature was measured with a mercury

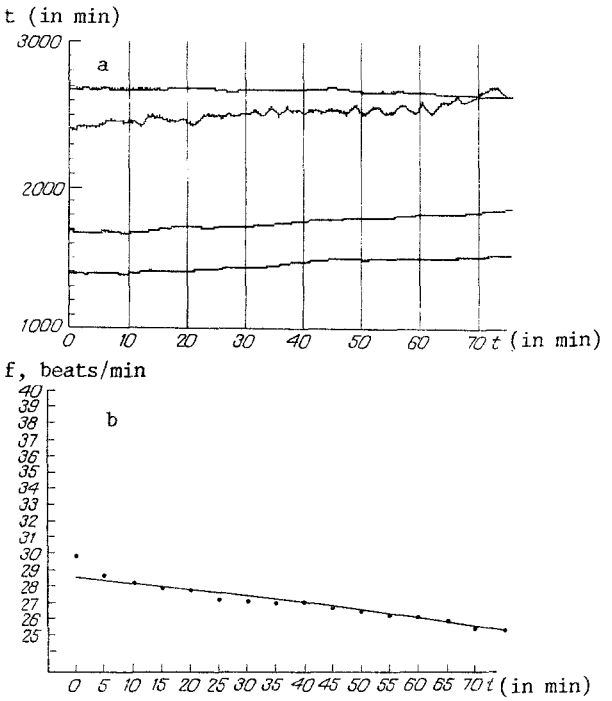

Fig. 2. Time course of rhythm of isolated heart from 100 to 175 min after preparation of the specimen. a and b) As to Fig. 1.

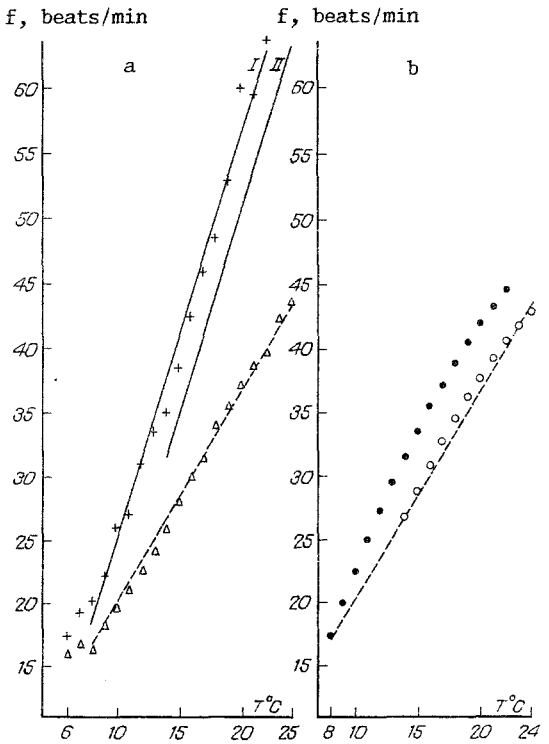

Fig. 3. Effect of dynamics of rhythm of isolated frog heart on results of an experiment. a) Experimental frequency versus temperature graphs for intact and isolated heart; regression lines for intact heart drawn as continuous line (I – our own data, II – data from [5]), for isolated heart by broken line. b) Comparison of experimental frequency versus temperature graph of isolated heart with calculated graph; experimental dependence shown by broken line, version calculated by equation (3) as circles: filled circles for calculations using our own data for the intact heart, empty circles for data for the intact heart taken from [5].

thermometer located between the hind limb and the lateral surface of the frog. The ECG was recorded during daylight in frogs at rest.

EXPERIMENTAL RESULTS

In the experiments of series I, changes in the period of excitation of the isolated frog heart with time were studied on 10 preparations. The results of these experiments are given in Fig. 1. Despite individual differences in the dynamics of rhythmic activity of the different preparations, the mean heart rate during 55 min of the experiment fell as an almost linear function from 38.9/min initially to 26.6/min after 55 min. With a longer time interval the change of frequency took place more slowly, to reach 25.4/min at the 75th minute of the experiment. The fall of frequency of excitation of the heart with time during the 55-min period can be described by the regression equation: $f_t = 37.16 - 0.21t$ (1), where t is the time elapsing after the beginning of the experiment. It is more convenient to use the normalized graph of this relationship. For this purpose we divide equation (1) by the initial frequency, which was 37.16, so that:

$$f_t/f_0 = (1 - 0.0057 \cdot t), \quad (2)$$

where f_0 is the initial frequency and t the time elapsing after the beginning of the experiment. By means of this equation, knowing the initial frequency and the time elapsing after the beginning of the experiment, it is possible to calculate the momentary value of the frequency (f_t).

In series II (10 experiments) the spontaneous rhythm was recorded in preparations of the sinus node together with the atria 90 min after dissection and for 75 min. The results of these experiments are shown in Fig. 2. Changes in the excitation frequency of the heart during this time interval were significantly smaller than previously. During 75 min of observation the mean excitation frequency of the heart fell as a linear function from 29.7/min initially to 25.3/min after recording for 75 min. The fall of the heart rate is described by the regression equation $f_t = 28.5 - 0.045t$, where f_t is the momentary frequency and t the time elapsing after the beginning of the experiment. The average angle of slope, which is 0.045 ± 0.00004, is significantly less ($p < 0.05$) than in the experiments of series I (0.21 ± 0.0002). Comparison of the graphs (Fig. 1b and 2b) and of the angles of slope leads to the conclusion that the preparation of the sinus node with atria, kept in Ringer's solution for 1.5 h, has a more stable rhythm. It is more appropriate to conduct pharmacological tests of small doses of substances on this preparation.

In the experiments of series III on 23 preparations the effect of temperature on the excitation frequency of the isolated heart was studied. Within the range from 8 to 24°C, heart rate was a linear function of temperature, with the regression equation $f = 1.65T_h + 3.71$, where T_h is the temperature of the isolated heart, equal to the temperature of the surrounding medium (see the broken line in Fig. 3a, b).

In series IV, consisting of 33 experiments on 12 intact resting animals, the heart rate was measured and its dependence on temperature studied from 6 to 24°C. With a rise of body temperature a regular increase was observed in the excitation frequency of the heart. A graph giving the general results of all the experiments is shown in Fig. 3a. It will be clear from this graph that dependence of the frequency of the intact heart on temperature between 8 and 22°C is close to linear. It is described by the regression equation $f_{int} = 3.17T_b - 7.06$, where T_b denotes body temperature (Fig. 3a, I). The good agreement between our own data and those in [5] will be noted. The frequency versus temperature graph of the intact heart in [5] was recorded over the temperature range from 15 to 26°C and was described by the regression equation $f_{int} = 3.18T_a - 16.04$, where T_a is the ambient temperature, which was 1°C higher than the body temperature. The regression line with correction for body temperature is shown in Fig. 3a, II. The difference between the mean angles of slope of the frequency versus temperature graphs of the intact (3.17 ± 0.10) and isolated hearts (1.65 ± 0.04) is significant ($p < 0.05$). Thus the response of the heart to a rise of temperature in vitro is not an exact reproduction of the response in vivo. Let us examine how a steady fall of frequency of the isolated heart with time can affect experimental results. It will be evident that in the isolated heart we see the result of two processes: an increase of frequency due to a rise of temperature, and a decrease of frequency with time. Consequently, the frequency of the isolated heart must be equal to the frequency of the intact heart less a correction for a decrease of frequency. This can be described in the form of the modified equation (2):

$$f_{isol} = (1 - 0.0057 \cdot t) \cdot f_{int}, \quad (3)$$

where f_{int} is the frequency of the intact heart at a given temperature, f_{isol} the frequency of the isolated heart at the same temperature, and t the time elapsing after the beginning of the experiment.

Let us compare the result calculated by equation (3) with the experimental frequency versus temperature graph of the isolated heart. In the experiments the temperature rose from 5°C at the rate of about 1/3°C/min. On the basis of this time course of the experiment and knowing the frequency versus temperature relationship of the intact heart, we can calculate the frequency versus temperature dependence of the isolated heart. In Fig. 3, in one case (filled circles) we took as the basis our data on the frequency versus temperature relationship of the intact frog heart, and in the other case (empty circles) the frequency versus temperature dependence of the intact heart from [5]. Clearly, good agreement is observed between the experimental points and points calculated by equation (3). Moreover, if data in [5] are used as the basis, agreement is virtually complete. Consequently, it can be concluded that the difference in the course of the frequency versus temperature relationship of the intact and isolated frog heart is largely due to a steady fall of the excitation frequency of the isolated heart with time. This example demonstrated how important it is to take account of the intrinsic dynamics of the cardiac rhythm. This aspect has long been understood, widely studied, and used in the analysis of rhythmic activity of the human heart [1]. No such data have hitherto existed for the isolated frog heart. In our experiments we did not analyze the mechanisms involved in the response of the intact frog heart to temperature, and we cannot therefore draw any conclusions regarding the role of the autonomic nervous system in this response. We have simply shown that if the real dynamics of the cardiac rhythm is disregarded, this may lead to false conclusions and hypotheses regarding the nature of the phenomena observed.

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