=== COMPLEX SYSTEMS BIOPHYSICS ===

The Effect of the Extracellular Ca²⁺ Concentration on the Force–Frequency Dependence in the Myocardium of the Guinea Pig: Potentiation by a Pause under Pronounced Hypothermia

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Abstract—The effects of a four-fold decrease in the concentration of Ca²⁺ in a perfusing medium under pronounced hypothermia at 20°C on the contractile responses of the papillary muscles of the right ventricle of a guinea pig, that is, the frequency–force dependence in the frequency range of 0.1–1.0 Hz and the effect of potentiation by a pause at stimulation frequencies of 0.3 and 0.8 Hz, were studied. It was shown that with a decrease in the concentration of extracellular Ca²⁺ there was a significant decrease in the contraction force of papillary muscles from 40 to 70%, reaching a maximum of $72 \pm 3\%$ at a frequency of 0.4 Hz; at the same time, the positive frequency–forcer dependence remained. The absolute values of the effect of potentiation by a pause decreased and the decrease was most pronounced at a lower stimulation frequency ($79 \pm 6\%$ and $40 \pm 19\%$ at 0.3 and 0.8 Hz, respectively). Thus, it was shown that the predominant dependence of contraction on extracellular sources of Ca²⁺ in the myocardium of guinea pigs persisted under pronounced hypothermia; at the same time, an increase in the frequency of stimulation led to an increase in the content of Ca²⁺ in the sarcoplasmic reticulum.

INTRODUCTION

The heart rate is one of the most important indicators of the myocardium. In addition, it has been shown that the heart rate is also an important internal regulator that enables adaptation of the performance of the heart according to the needs of the body. In the myocardium of most mammals, its increase leads to an increase in the contraction force (a positive inotropic effect) [1]. The myocardium of rats and mice is the exception, for which a two-phase type of frequency–force dependence (FFD) was described [2, 3]. The effect of potentiation by a pause is another important indicator of myocardial function, which can serve not only as a qualitative indicator of the content of Ca²⁺ in the sarcoplasmic reticulum (SR) [4], but also as an important diagnostic feature [5, 6].

It is known from the literature that when the concentration of extracellular Ca²⁺ changes, the FFD [7, 8] and the expression of the effect of potentiation by a pause [9] may change.

Abbreviations: FFD, frequency–force dependence; SR, sarcoplasmic reticulum.

Changes in the level of extracellular Ca²⁺ affect a large number of physiological parameters in the heart in addition to the transformation of rhythmoinotropic effects, such as sensitivity to adrenergic stimulation [10], the contractile response of the myocardium [11], and its passive mechanical properties [12]. A decrease in the concentration of Ca²⁺ in the perfusing solution can be used as an additional cardioprotective factor in cold cardioplegia [13–15].

There is no comprehensive description of changes in rhythmoinotropic characteristics in the myocardium of guinea pigs when the concentration of extracellular Ca²⁺ is decreased under pronounced hypothermia. This study was focused on the effect of reducing Ca²⁺ to 0.45 mM in a perfusing medium under pronounced hypothermia (20°C) on the contractile responses of the papillary muscle of the right ventricle of guinea pig, namely, on the frequency–force dependence (the frequency range of stimulation 0.1–1.0 Hz) and the effect of potentiation by a pause at stimulation frequencies of 0.3 and 0.8 Hz.

MATERIALS AND METHODS

The studies were carried out on the papillary muscles of the right ventricle of guinea pigs. In the experiments, guinea pigs of the Agouti breed (males weighing 200−250 g) were used. The animals were kept in the vivarium of the IBC RAS under standard conditions in accordance with the rules for the design, equipment and maintenance of experimental biological clinics (vivariums) in ventilated areas that exclude the occurrence of drafts and sudden temperature changes. The light mode was 12 h of light and 12 h of darkness, the feeding mode was *ad libitum*.The air temperature was maintained in the range of 18−21°C.

All guinea pigs selected for the experiment were delivered to the experimental room from the vivarium at least 1 h before its start. The animals were anesthetized with diethyl ether. The isolated heart was placed in a Tyrode's solution (20°C) of the following composition (in mM): NaCl, 135; KCl, 4; $MgCl_2$, 1; $CaCl_2$, 1.8; $NaHCO_3$, 13.2; NaH_2PO_4, 1.8; and glucose, 11; pH 7.4. The solution was aerated with a gas mixture containing 95% O_2 + 5% CO_2. The isolation of papillary muscles, as well as the stimulation and measurement of the contraction amplitude in the isometric mode were carried out according to the previously described method [16] at a temperature of the perfusing solution of 20 ± 0.1°C. The diameter of the papillary muscles varied from 0.6 to 1.0 mm, while the length varied from 1.0 to 3.0 mm. To study their mechanical activity, an automated apparatus based on a personal computer and ADC−DAC boards (L-Card 154 and L-Card E14-440) was used. The mechanical activity of the muscles was recorded using a 6X-2M mechanotron. At the beginning of each experiment, the preparation was stimulated with rectangular pulses (voltage 5 V; duration 5 ms; a current two times higher than the threshold) with a frequency of 0.3 Hz for 1 h to stabilize the contraction force.

The Frequency−Force Dependence

The FFD was recorded in the isometric mode in the range of stimulation frequencies from 0.1 to 1.0 Hz. To plot the FFD, the magnitude of the contraction force for each of the stimulation frequencies in the studied range was expressed as a percentage relative to its value at the stimulation frequency of 0.1 Hz, taken as 100% [17, 18].

Recording the Effect of a Pause

A pause was introduced in the stimulation under constant stimulation with a given frequency, at which the contraction force was at a stable level (basic contraction); this led to the potentiation of the first contraction after the pause (a test contraction). The magnitude of the effect was expressed as a percentage relative to the for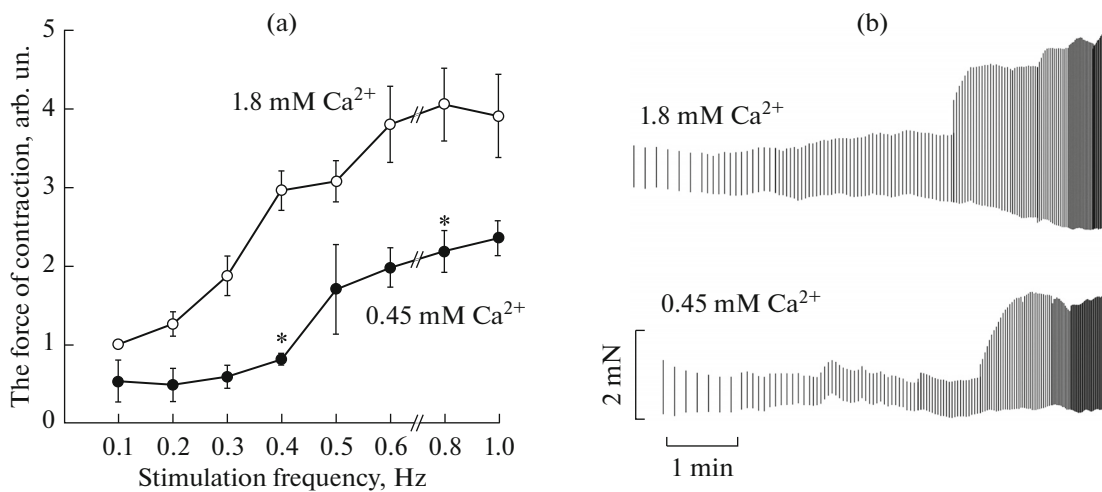ce of contraction at the base frequency of stimulation (0.3 and 0.8 Hz). The data were checked for the normality of the distribution using the Shapiro−Wilk test. The significance of the obtained results was assessed using the Student's paired test and the one-way analysis of variance (one way ANOVA) (according to the significance level $p < 0.05$). The data were presented as averages ± standard error of the mean. Statistical data analysis was carried out using Microsoft Excel 2019 and GraphPad Prism 8 statistical software packages.

RESULTS AND DISCUSSION

The Frequency−Force Dependence

A decrease in the level of Ca^{2+} from 1.8 to 0.45 mM in the range of 0.1−1.0 Hz at 20°C caused a significant (from 40 to 70%) decrease in the contraction force of papillary muscles, reaching a maximum of 72 ± 3% at a frequency of 0.4 Hz (Fig. 1). This fact once again illustrated one of the fundamental features of the myocardium, a significant dependence of the contraction force on the inflow of extracellular Ca^{2+}. At the same time, it should be noted that the decrease in contractility in the myocardium of the guinea pig was significantly more pronounced than that described for the myocardium of the rat [10]. This difference can be explained by the large contribution of SR to the activation of contraction in the rat myocardium under hypothermia [19]. There are data in the literature that indicate that high concentrations of Ca^{2+} can modify the nature of the FFD, masking its individual phases [7, 8]. It should be noted that in our case, a single-phase positive FFD persisted, which is a featurre of the healthy myocardium of most mammals [1] and guinea pigs, in particular [20], including the conditions of hypothermia, as we have shown earlier [21]. At the same time, there are data in the literature according to which an increase in the frequency of stimulation led mainly to a decrease in Ca^{2+} current in guinea pig cardiomyocytes [22, 23]; however, when the contractile response was studied under conditions of blocking of the L-type Ca^{2+} current, the positive FFD occurred [3, 21], which indicated an increase in the role of this mechanism with an increase in the frequency of stimulation. Hypothermia could also reduce the peak values of the Ca^{2+} current; however, its inactivation was slowed and the total amount of Ca^{2+} entering the cell remained at a level close to the initial one [24], which eventually led to an increase in contractility in response to hypothermia [25, 26], in which the L-type Ca^{2+} current played a significant role [19, 27]. An increase in the diastolic calcium level might in itself have a positive inotropic effect [28].

Thus, a decrease in the level of Ca^{2+} to 0.45 mM under hypothermia did not cause noticeable changes in the FFD and extracellular sources of Ca^{2+} played a leading role in activating the contraction, which was indirectly confirmed by the positive FFD.

Fig. 1. The effect of Ca^{2+} concentration in perfusing solution on the frequency–force dependence in the papillary muscles of the right ventricle of guinea pig ($n = 5$). (a) The dependence of the contraction force on the frequency of stimulation. The abscissa axis is the frequency of stimulation, Hz; the ordinate axis is the force of isometric contraction relative to the force at frequency of stimulation of 0.1 Hz, taken as one. The data are presented as average values ± error of the mean (* marks a significant difference from the initial frequency, $p < 0.05$). (b) Original recordings of papillary muscle contractions at Ca^{2+} concentrations of 1.8 and 0.45 mM.
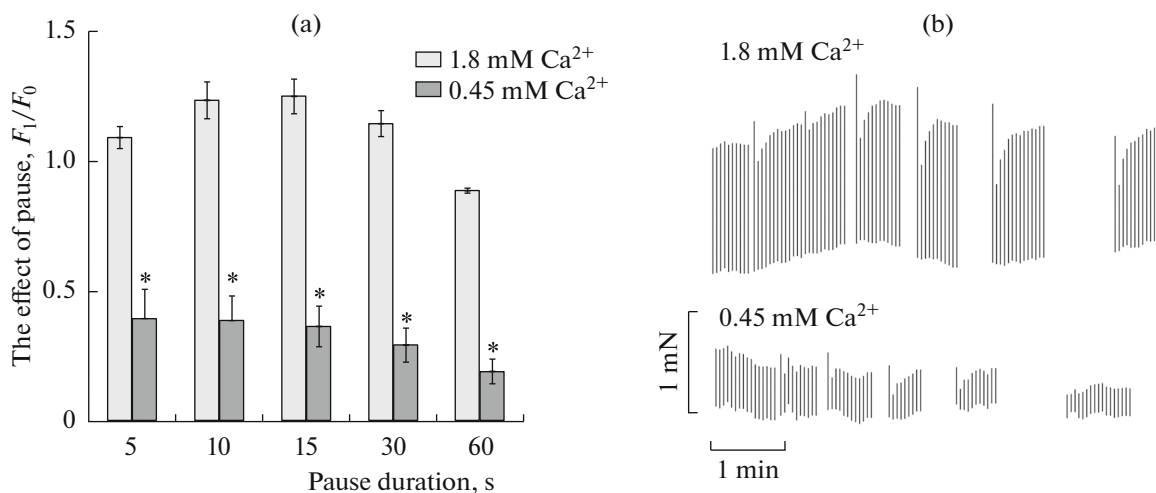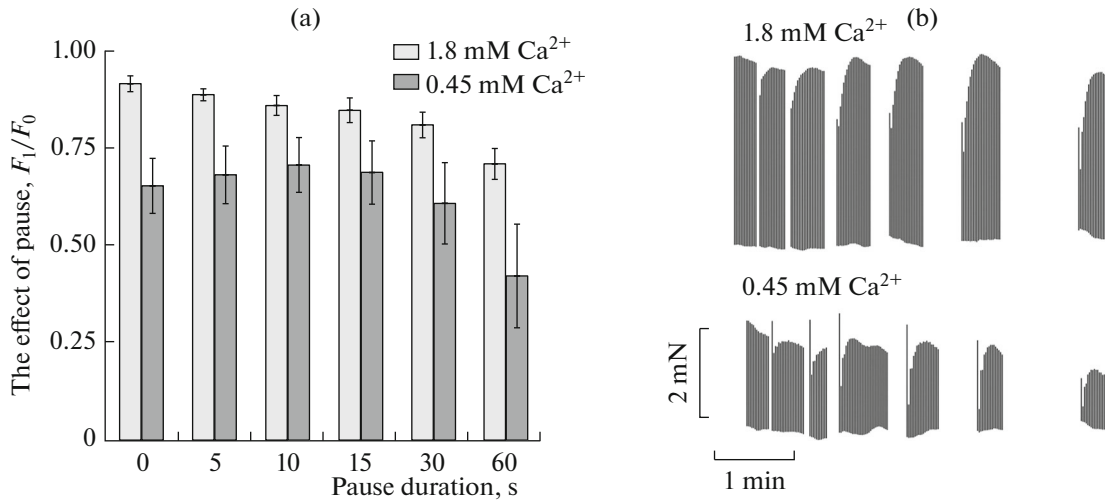

Fig. 2. (a) The effect of Ca^{2+} concentration in perfusing solution on the effect of pause at stimulation frequency of 0.3 Hz in papillary muscles of the right ventricle of guinea pig at 20°C ($n = 5$). The abscissa axis is the duration of the pause; the ordinate axis is the force of the first contraction after the pause relative to the force of contractions at the base frequency of stimulation (0.3 Hz), taken as one. The data were presented as average values ± error of the mean (* marks a significant difference from the initial frequency, $p < 0.05$). (b) The original recordings of papillary muscle contractions.

The Potentiation by a Pause

In the control, the effect of potentiation by a pause at a stimulation frequency of 0.3 Hz reached a maximum at a pause for 15 s and was 25 ± 7%; it gradually decreased with a further increase in the duration of the pause and declined at a pause for 60 s (Fig. 2). With a decrease in the concentration of Ca^{2+} in the perfusing solution, there was a significant decrease in both the force of the base contraction at 0.3 Hz and the contraction after a pause, which, as in the control condi-

tions, progressively decreased with increasing pause duration, reaching a minimum at 60 s, while the drop level was 64 ± 11 and 79 ± 6% at pauses of 5 and 60 s, respectively. The expression of the pause effect at the same time practically did not change.

A slightly different picture was observed at a stimulation frequency of 0.8 Hz; initially, the introduction of a pause led to a decrease in the test contraction relative to the baseline. At the same time, a decrease in the Ca^{2+} concentration in the solution to 0.45 mM led

Fig. 3. (a) The effect of Ca^{2+} concentration in perfusing solution on the effect of pause at stimulation frequency of 0.8 Hz in papillary muscles of the right ventricle of guinea pig at 20°C ($n = 5$). The abscissa axis is the duration of the pause; the ordinate axis is the strength of the first contraction after the pause in relation to the strength of contractions at the base frequency of stimulation (0.8 Hz), taken as one. The data were presented as averages ± error of the mean. (b) The original recordings of papillary muscle contractions.

to the potentiation of the test contraction instead of a decline. The absolute value of the test contraction decreased similarly to the decrease at the stimulation frequency of 0.3 Hz; however, this decline was much less pronounced and ranged from a minimum of 18 ± 9% at a pause for 10 s to 40 ± 19 at a pause for 60 s (Fig. 3); this decrease was not significant for any of the studied pause durations. Our results were in good agreement with the literature data, according to which the content of Ca^{2+} in guinea pig SR progressively decreased with increasing pause duration [29, 30]. It was unexpected that the decrease in the potentiation effect was less pronounced at a higher frequency of stimulation (Figs. 2 and 3); while it is known from the literature that contractility at higher frequencies is mainly associated with extracellular sources of Ca^{2+} [3]. This might be explained as follows: at a low frequency of contractions under hypothermia, the SR contained a relatively small amount of Ca^{2+} and the effect of potentiation by a pause would strongly depend on the inflow of extracellular Ca^{2+} [31]. At the same time, an increase in the frequency of stimulation would contribute to the filling of SR [30] and reduce the dependence of the effect of potentiation by a pause on extracellular Ca^{2+}; while the dependence on extracellular Ca^{2+} was more pronounced at longer pause durations, which was similar to the literature data [32]. Thus, a decrease in the extracellular concentration of Ca^{2+} under pronounced hypothermia contributed to a more expressed manifestation of the effect of potentiation by pause. In addition, a smaller effect on the absolute values of the first contraction after a pause at a stimulation frequency of 0.8 Hz might indicate an

increase in the content of Ca^{2+} in SR at an increased stimulation frequency.

CONCLUSIONS

It was shown for the first time that the following patterns were characteristic of the papillary muscles of the right ventricle of guinea pig with a decrease in the concentration of Ca^{2+} in the perfusing solution from 1.8 to 0.45 mM under pronounced hypothermia (20°C): a positive frequency–force dependence persisted, which might indicate the predominance of extracellular sources of Ca^{2+} in the activation of contraction; the absolute value of the effect of potentiation by a pause decreased and the expression of this reduction decreased with an increase in the frequency of stimulation, which might indicate an increase in the content of Ca^{2+} in SR with an increase in the frequency of stimulation.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. The experiments were carried out in compliance with the rules of the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes (European

Communities Council Directive (86/609)EEC) and in accordance with the requirements of the IBC RAS Ethics Commission.

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