CHRONOINOTROPIC EFFECTS IN LANGENDORFF PERFUSED RAT HEART

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If healthy heart responds by an increase of contractility to the acceleration of rhythm then negative force-frequency relationship (FFR) is developed in failed heart. In experimental conditions in perfused rat heart it is possible to obtain the positive as well as negative FFR. Transient force response has been poorly described in details, but FFR have been considered mostly as steady state phenomenon.

The aim of the investigation was to study FFR in whole rat heart under different experimental conditions using analysis of force transient response; to study the effect of different extracellular calcium concentrations on force transition dynamics, and hence the resulting sign of chrono-inotropic relation.

Materials and Methods. On total of 15 Langendorff perfused hearts obtained from Wistar rats, we demonstrated different calcium concentrations ($[Ca^{2+}]_o$) in Krebs–Henseleit buffer to be able to affect FFR in these hearts.

Results. There was found that the transient responses have biphasic structure, where first beat (B_1) and extremum beat (B_{ex}) can be found. The transient response after step pacing period change can be rather long (sometimes more than 180–300 s); and one should be careful when choosing the duration of pacing protocols. The negative FFR (at 60 s — B_{60s}) is easily modulated by external $[Ca^{2+}]_o$ and the parameters of force transient response B_1 and B_{ex} are more sensitive (then B_{60s}) to experimental conditions. The increase of intracellular Ca^{2+} with the help of Ouabain (50 uM) does not affect chrono-inotropic relations in perfused heart of rats.

Conclusion. Calcium concentration in perfusion solution was found to be able to affect the dynamics of force transient response after step change in pacing frequency and change the resulting sign of FFR. Intracellular calcium does not significantly change the sign of chrono-inotropic relations.

Key words: cardiac contractility, force-frequency relationships, isolated heart, force transient response.

Healthy hearts respond to enhancement of pacing rate by an increase of contractility exhibiting positive force-frequency relations (FFR). In failing hearts, rhythm acceleration leads to no change or progressive decline in force that reflects blunted or negative staircase [1–2]. For heart failure patients, even an easy exercise can evoke such a situation when the heart is unable to satisfy the enhanced systemic demands [3].

The phenomenon of FFR was first described as a positive 'Treppe' or staircase by Bowditch in 1871 in frog hearts. Since that time similar relations were shown in rabbit, dog, sheep and guinea pig cardiac preparations [3–4]. In many experimental works, hearts from small rodents, such as rats or mice showed the negative force-frequency relations that can be explained by specific intracellular Ca^{2+} circulation in these species [5–6]. Inadequate perfusion under fast rates can cause ischemia and hence negative force-frequency relationship [7, 8]. In some studies on rat cardiac preparations and even whole hearts the positive force-frequency relationships were reported [9–12].

Extracellular calcium strongly affects cardiac contractility and is able to change the sign of chronoinotropic relation in the heart [4, 12]. Calcium saturation and even calcium overload under high $[Ca^{2+}]_o$ (over 2.5 mM) were reported by some authors [13, 14]. Other workers [4] showed the increased ability of sarcoplasmic reticulum to accumulate Ca^{2+} when $[Ca^{2+}]_o$ is reduced.

The heart contractility response to the step change in pacing rate is always accompanied by transient changes in force and $[Ca^{2+}]_i$ [5, 7, 12]. However this transient

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response has generally been ignored and force-frequency response has been considered as the only steady-state condition.

The aim of the investigation is to study force-frequency relationships in rat heart under different experimental conditions using force transient response analysis, and show extracellular calcium to change the dynamics of force transient response, and therefore the chronoinotropic relations in the heart.

Materials and Methods. The protocols of the present study was approved by the Board of Ethics of Nizhny Novgorod Medical Academy (Russia) and Academia Sinica (Taiwan) and performed according to Guidelines for the Use and Care of Laboratory Animals of USA National Institutes of Health [15].

Langendorff preparation. 15 males of Wistar rats (250–300 g) were anesthetized with Zoletyl (10 mg/kg, intraperitoneally), heparinized (500 IU, intraperitoneally), then the hearts were rapidly excised and arrested in an ice-cold Krebs-Henseleit buffer (KHB) containing (in Mm): NaCl 118; KCl 4.7; $CaCl_2$ 2.4; $MgSO_4 \cdot 7H_2O$ 1.5; KH_2PO_4 1.2; $NaHCO_3$ 20 and glucose: 10. Then the hearts were quickly mounted on a classical Langendorff perfusion apparatus (Radnoti, USA) and perfused with an oxygenated (95% O_2, 5% CO_2) KHB at 37°C with pH at 7.3–7.4. Gravitational retrograde perfusion under a pressure of 80 cm of water was performed. To reduce spontaneous activity of hearts the sinus node was cut; atrioventricular node was damaged by an injection of formalin (0.05 ml 10%). To simulate calcium overload [16] Ouabain (Sigma, USA) was used.

Chamber pressure record. A latex balloon (about 3 mm in diameter) filled with water was placed into the left ventricular cavity of the isolated heart and inflated to simulate an end-diastolic pressure of approximately 10–20 mmHg. Changes in left ventricular pressure (LVP) in iso-volumetric regimen were registered by connecting the balloon to a strain gauge transducer (MPX5050D, Freescale, USA) by means of a polyethylene catheter. Maximal left ventricular pressure (LVP_{max}) was determined from the time course of the measured LVP. Similarly, the maximum contraction force ($+dP/dt_{max}$) or index of contractility was obtained from the maximum of the time derivative of the measured LVP signal. The signals were recorded by a digital data acquisition system NI-6221 (National Instruments, USA) which was controlled by PowerGraph Professional software (version 3.3.7, Russia) with a 4 KHz sampling rate.

Pacing. The hearts were paced at the endocardium of interventricular septum using custom-made stainless mini-hook electrodes. Programmed electrical stimulations were sent to the hearts by an isolated stimulator (Model 2100, A-M Systems, USA) controlled by a computer. The form of a single electrical stimulation was a rectangular monophasic current pulse, 1 ms in duration, and the amplitude being 2–3-fold the diastolic threshold current. To produce a staircase, increasing and decreasing protocols were used. The period of pacing (T) was changed every cycle (every 60 s) by step of 20 ms (increased or decreased).

Data processing. The data were statistically analyzed using non-parametric Mann-Whitney test to compare groups, one-way ANOVA test for repeated measurements,

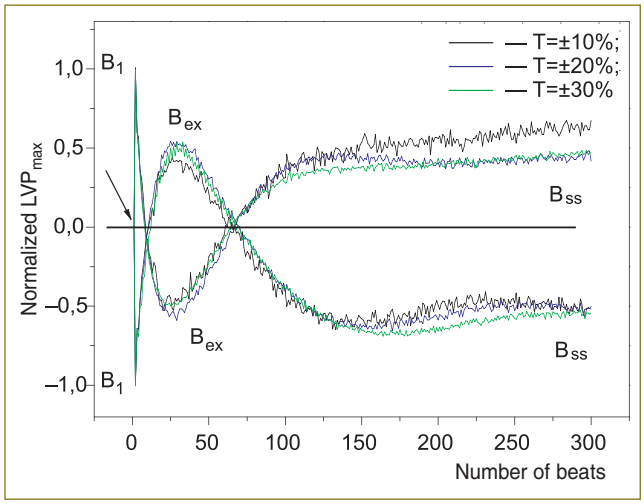

Fig. 1. Representative examples of contractility responses of Langendeorff perfused rat hearts to the switch of pacing periods $[Ca^{2+}]_o$=2.5 mM. Normalized systolic pressure LVP_{max} versus beats, switches from 150 ms by 10, 20 и 30% to both directions

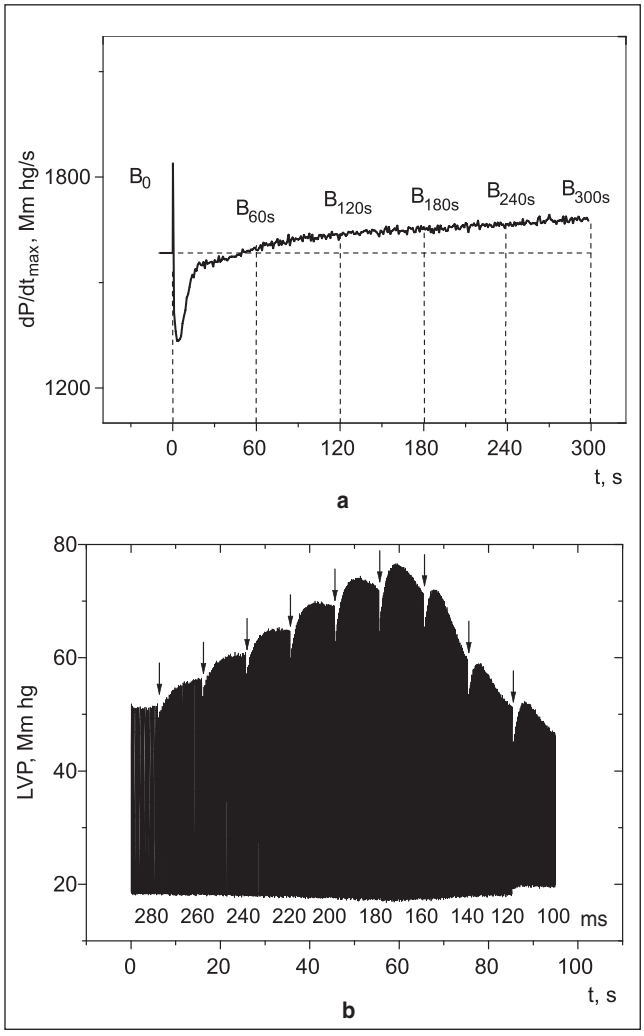

Fig. 2. The effect of stimulation duration to response of contractility *a* — left ventricular dP/dtmax, switch 120–140 ms, duration 300 s, $[Ca^{2+}]_o$=1 mM; *b* — staircase of LVP, switches 280–100 ms with step 20 ms every 10 s. Arrows show the switch points

Wilcoxon paired test to compare dependent parameters. P<0.05 was considered significant.

Results.

Contractility response to the heart rate change (transient). Fig. 1 depicts the two opposite responses of normalized LVP to deceleration and acceleration of pacing rate. When stimulation period is changed, steady state contractility is not quickly achieved; biphasic response is always observed. For example, when the period is increased, the amplitude of the first beat (B_1) is always bigger comparing to the background level (B_0) of contractility. After the intense first beat contractility (LVP_{max}) quickly runs down to the some minimal (extreme) level (B_{ex}), which is usually lower than previous B_0, and then rises again (Fig. 1). The fall down of the force below previous B_0 is characterizes by the so-called negative *overshoot* (B_{ex}–B_0). After transient phases contractility goes to the appropriate steady state level common for this new pacing rate (Fig. 1). For the shortening of period of stimulation (T) one can observe the anti-symmetric response to that depicted above (Fig. 1).

The dependence on pacing duration. In order to estimate the influence of pacing duration on chronoinotropic relations we conducted experiments with different pacing duration protocols. It is shown in Fig. 2, *a* that even at stimulation during 180–300 s (up to 5 min) with one frequency at low $[Ca^{2+}]_o$=1mM some trend in contractility appears. If pacing rate was changed every 10 s, every switching will coincidence with force transient development and result could be different (positive or negative) depending on frequency. On Fig. 2, *b* one can see it: when period of stimulation is switched to the acceleration of rhythm, it is easy to get 'staircase' of positive chronoinotropic relation in a range of periods from 280 to 160 ms (with step 20 ms). Under the stimulation with period from 140 to 100 ms the reduction of contractility after B_{ex} is getting faster resulting in negative force-frequency relationships. But if one looks at the 60 s protocols, FFR will be negative at the entire range of periods (Fig. 3, *a*).

Duration of 60 s was chosen as minimal time required for contractility to be near steady state conditions in a short time scale, and instead steady state contractility B_{ss} parameter we used 60 s contractility parameter B_{60s}.

The effect of calcium concentration in solution. To reveal the effect of calcium extracellular concentration on FFR there were used KHB with different Ca^{2+} concentrations. It has been established that calcium concentration in most carbonate buffers is slightly elevated (2.5 mM) because

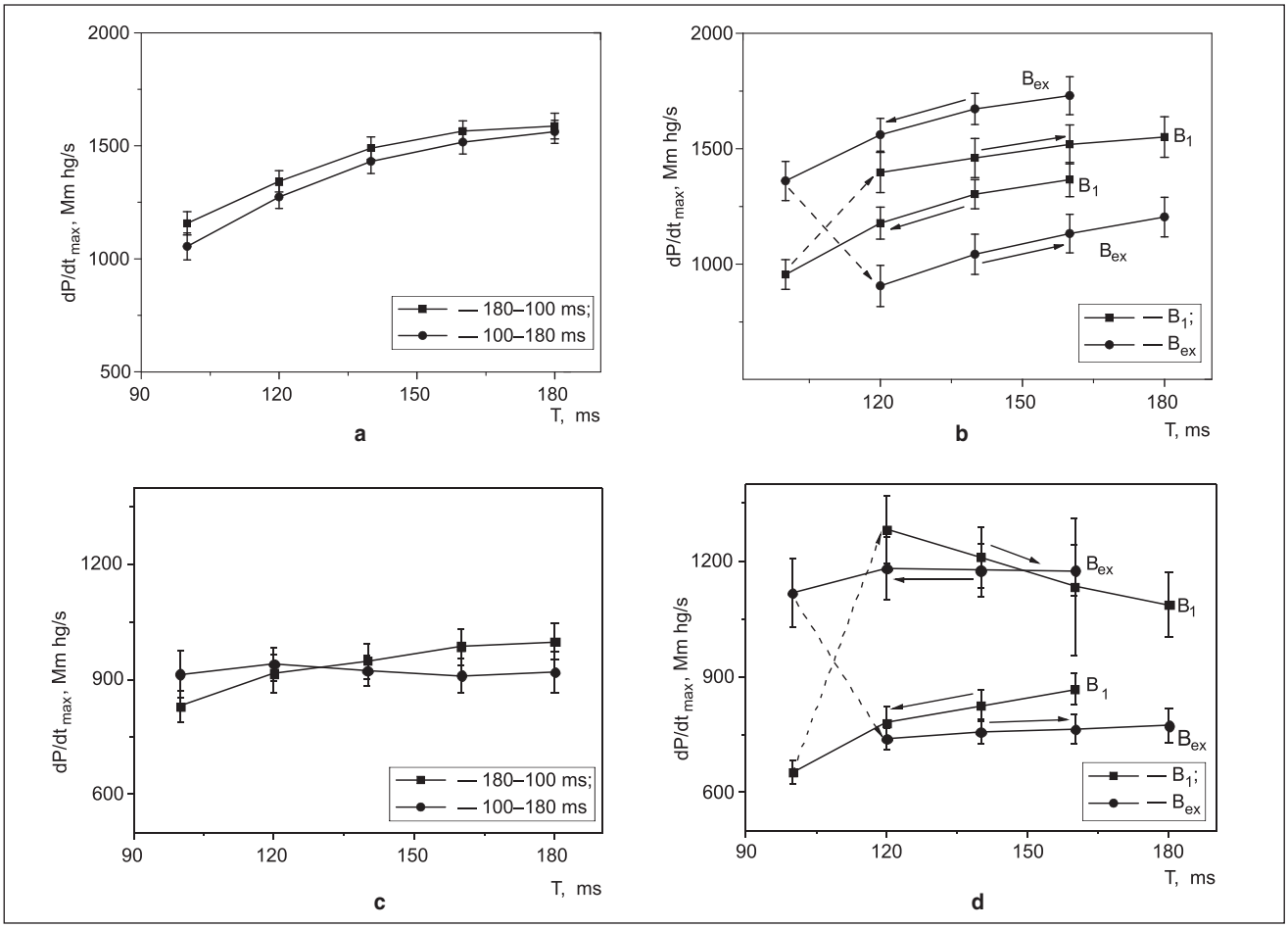

Fig. 3. Force-period relations of Langendorff perfused rat heart by KHB with $[Ca^{2+}]_o$=2.5 mM и 1 mM upon pacing period T change in a range of 100–180 ms. *a* — LV contractility (dP/dtmax) at 60 s of pacing (B_{60s}) $[Ca^{2+}]_o$=2,5 мМ; *b* — LV contractility of the first beat (B_1) extreme beat (B_{ex}) with $[Ca^{2+}]_o$=2.5 mM; *c* — LV contractility (dP/dtmax) at 60 s of pacing (B_{60s}) $[Ca^{2+}]_o$=1 mM; *d* — of the first beat (B_1) extreme beat (B_{ex}) with $[Ca^{2+}]_o$=1 mM. Arrows show the direction of period change

Ca^{2+} concentration in rat plasma was found to be about 1 mM [17, 18]. Fig. 3, *a* shows the result of applying protocols of stimulation with constant delta of period as a negative chronoinotropic relation of B_{60s} which was insensitive to direction of the period change. Note that dP/dt_{max} is changing almost linearly with the change of a period. In this group, when an interval was decreased from 180 ms to 100 ms, B_{60s} dropped by 27% ($p<0.001$). When the pacing interval was increased from 100 to 180 ms, a similar relation was observed: force elevated upon an increase of pacing period by 48% returning almost to the same value ($p=0.0002$). No significant difference was found between these protocols using ANOVA test. Fig. 3, *b* depicts chronoinotropic relations in rat heart perfused by normal KHB during force transient when pacing period is changed: the first beat (B_1) and the extreme beat (B_{ex}). Note that for all FFR parameters used, the negative relation is preserved.

Fig. 3, *c* demonstrates the chronoinotropic relations for KH solution containing 1 mM of Ca^{2+}. Under conditions of low calcium in solution hearts develop smaller force that can be predictable. For example, B_{60s} was smaller by 40% at the T=180 ms when compared to the group Ca^{2+} 2mM ($p<0.0001$). FFR was close to zero in these experiments (Fig. 3, *c*). Force transient parameters showed complicated relations (Fig. 3, *d*). When the rate was accelerated (arrow to the left) chronoinotropic relations for B_{ex} were close to zero, while B_1 showed negative relations (Fig. 3, *d*). When pacing rate was decelerated, B_{ex} was independent of the period, but B_1 showed positive relations. Thus, the transient FFR parameters seemed to be more sensitive to the external calcium (Fig. 3, *d*).

***Ouabain effect*.** Perfusion of rat hearts with KHB Ouabain (50 uM) resulted in negative FFR (Fig. 4, *a*) as with regular KHB without Ouabain. Perfusion with Ouabain was accompanied by ventricular tachyarrhythmias observed almost in all the hearts in pacing conditions. ANOVA test did not reveal any significant difference in chronoinotropic relations between control KHB and perfusion with Ouabain. However, if transient period is considered in details (Fig. 4, *b*), one can see that Ouabain changes the relative value of the overshoot for both protocols and decelerates the whole process.

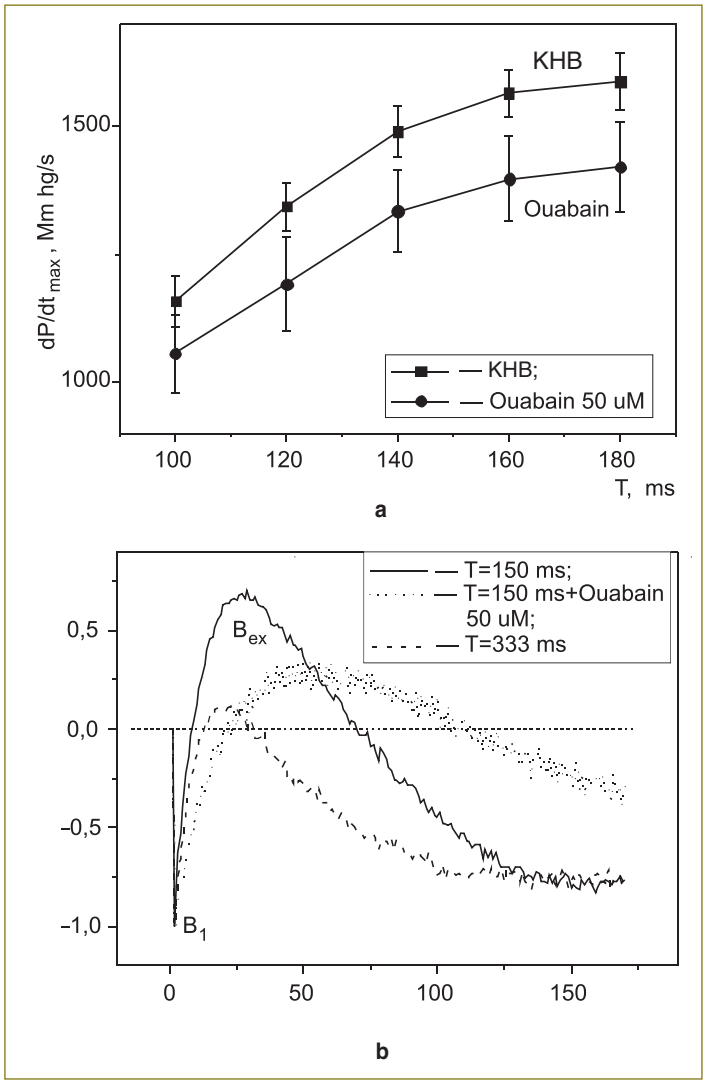

Fig. 4. The effect of perfusion with Ouabain (50 uM): a — force-frequency relationship for KHB alone and with Ouabain; b — left ventricular pressure versus beats, transient upon switch in period 150–105 ms

Discussion. However Bowditch discovery was made more than 140 years ago, the mechanisms of negative force-frequency relationships are still the question under discussion. Our experiments on isolated Langendorff perfused hearts of rats showed that when using standard KHB perfusion solution with $[Ca^{2+}]_o$=2,5 mM, the negative chronoinotropy is observed (Fig. 3, *a*). The sign change is possible when calcium concentration in solution is reduced (Fig. 3, *c*) that is in agreement with other studies [4, 12].

We first showed that under reduced calcium conditions the transient response can last more than 3–5 min resulting in non-steady pressure in the chambers of heart even on 120 or 180 s of perfusion (Fig. 2, *a*). Other authors demonstrated less time for heart preparations from rats and rabbits for steady state to be reached, 5–15 s and 45 s respectively [12]. From this point, some potential mistakes appear which connected to the appropriate duration of pacing protocols. For example, in the study of Henry [9] the switches of frequencies were done every 10 s resulting in positive staircase in isolated rat heart. We repeated the experiment and obtained the similar positive staircase (Fig. 2, *b*); however, when longer pacing protocols were used (60 s) negative FFR were observed. Note that in our experimental conditions hearts were perfused adequately with measured coronary flow about 8–15 ml/min being in normal range for rats. Moreover, instability in contractility parameters was more prominent in experiments with low calcium when coronary flow was even bigger (data not shown).

Classic concept of force-frequency relations is related to steady state conditions under heart pacing. We suppose that usage of the first beat (B_1) or extreme beat (B_{ex}) contractility can give some additional information about regulation process inside the heart. We first showed that the behavior of parameters B_1

and B_{ex} is similar to that observed using B_{60s} (Fig. 3, *b*). Note that under low calcium in KHB the first beat and extreme beat contractility parameters show the different dynamics. B_1 shows the positive and negative FFR upon acceleration as well as deceleration; as B_{ex} keeps being independent from the period of stimulation and direction of period change (Fig. 3, *d*). When perfused by standard KHB with $[Ca^{2+}]_o$=2.5 mM hearts demonstrate symmetric responses to opposite way changes of pacing periods (Fig. 1), while under low calcium conditions (1 mM) transients are different at different stimulation protocols (acceleration and deceleration). It is clear that Ca^{2+} homeostasis in the cell is connected to the extracellular $[Ca^{2+}]$, probably via Na^+/Ca^{2+} exchanger work which is very sensitive to outside calcium $[Ca^{2+}]_o$ [19].

Finally, we proposed that increasing calcium concentration in the cell we can affect chronoinotropic relation in the heart. However, experiments with Ouabain showed that calcium overload (which is obvious by enhanced ventricular arrhythmia susceptibility [16]) was not followed by change in FFR sign. Thus, the increase of intracellular Ca^{2+} has no significant effect on chronoinotropic relations.

Conclusion. Calcium concentration in perfusion solution was found to be able to affect the dynamics of force transient response after step change in pacing frequency and change the resulting sign of force-frequency relations. Intracellular calcium does not significantly change the sign of chronoinotropic relations.

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