Peculiarities of the Effect of Parathyroid Hormone on the Relaxation of Rat Myocardium

T. A. Barabanova and S. K. Churina

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Contraction of rat myocardium is recorded in a regime of physiological loading that simulates the function of the intact myocardium. Addition of parathyroid hormone accelerates relaxation of the rat myocardium in the control solution and in a solution containing norepinephrine or high calcium concentrations. In a regime of isometric loading the addition of parathyroid hormone inhibits relaxation. At a low Ca concentration in the medium, parathyroid hormone has no effect on the rate of relaxation.

Key Words: *myocardium; relaxation; parathyroid hormone*

Calcium is the key ion determining the contractile function of the myocardium via coupling excitation and contraction in cardiomyocytes. Contractility of the myocardium depends on the level of free calcium ions in excited cardiomyocytes, and this level in turn directly depends on the blood concentration of Ca^{2+}. Among the factors regulating the blood calcium concentration a crucial role is played by parathyroid hormone (PTH), which is considered to be a specific Ca-regulating hormone [5]. In hypocalcemia the blood concentration of PTH rises, leading to a rapid drop of excretion of Ca^{2+} in the urine and its mobilization from the bone tissue. Moreover, PTH also acts, albeit more slowly, through modulation of the synthesis of a 1,25-$(OH)_2D_3$ metabolite in the kidneys, which augments the adsorption of Ca^{2+} in the intestine [4].

PTH is thought to be able to affect the transmembrane Ca^{2+} currents in cardiomyocytes, thereby modulating the electromechanical coupling. For instance, in the ventricular myocardium of frogs and adult rats PTH steadily lowers the amplitude and duration of the action potential, this being accompanied by a negative inotropic effect [1].

Laboratory of Experimental and Clinical Cardiology, I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg (Presented by A. N. Klimov, Member of the Russian Academy of Medical Sciences)

It has been shown that the parameters of contraction and relaxation sometimes closely correlate [3]. Since PTH produces an inotropic effect, its influence on relaxation may be assumed to be at least partially mediated through modulation of the contraction force. This poses the problem of isolating the purely relaxing (loosening) effect of PTH, a problem which can be solved by studying the effect of PTH on contraction and relaxation under strictly controlled mechanical conditions, i.e., at a fixed length and load, which may be achieved in a regime with the physiological loading sequence allowing for the effect of mechanical variables.

MATERIALS AND METHODS

The experiments were carried out on thin (diameter 0.2-0.3 mm) papillary muscles from the right ventricles of rats. The cardiac muscle preparations were promptly excised and placed in physiological saline of standard composition (pH 7.0-7.2). The experiments with myocardium of warmblooded animals were performed in a solution with a temperature of 28°C. Stimulation was performed with suprathreshold pulses of 1-2 msec duration using wide plate electrodes applied longitudinally.

One end of the muscle was attached to a force transducer and the load was applied to the

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other end clamped to the lever of a precision ergometer. In the regime with the physiological loading sequence changes in force and length were recorded simultaneously. In this regime the muscle contracted at first isometrically until the force became equal to the applied load and then against a variable load proportional to the magnitude and rate of shortening (auxotonic shortening). After the muscle attained the end-systolic length (L_{es}), it relaxed isometrically to the preload state and then extended linearly. The servosystem and details of the experiment were described previously [2].

First, series of contractions for different afterloads were recorded in the standard solution (7.5×10^{-8} M) and then the procedure was repeated in solutions with various concentrations of calcium (1.25 and 5 mM) and norepinephrine (NE, 10^{-6} M).

RESULTS

The addition of PTH to the control Krebs solution prolonged the characteristic relaxation time t_{30} corresponding to the relaxation of an isometrically contracted muscle to 30% of the maximal contraction amplitude. For instance, 3 min after the addition of PTH, t_{30} was increased by 10%, while after 5 and 10 min the increase was 11 and 12%, respectively, and practically did not differ from that observed after 3 min (Fig. 1, *a*). Thus, PTH added to the control Krebs solution prolongs t_{30}, i.e., the drop in contractility is accompanied by a decrease in the relative relaxation rate.

In the hypercalcium medium PTH reduced the characteristic relaxation time t_{30} by 10-12% both 5 and 10 min after addition, i.e., apart from the drop of contractility we observed an increase in the relative rate of isometric relaxation (Fig. 1, *b*).

Under conditions of NE-induced activation of adenylate cyclase, 5 min after the addition of PTH we observed a 25% drop of the characteristic relaxation time t_{30}, i.e., the relative rate of relaxation was decreased. By the 10th min the drop of t_{30} still constituted 25-30%. Thus, in the NE-pretreated muscle PTH markedly increased the relative rate of isometric relaxation (Fig. 1, *c*).

Thus, in the isometric regime the addition of PTH to the control solution inhibited relaxation, while in the hypercalcium medium and under conditions of NE-induced activation of the adenylate cyclase system the addition of PTH increased the relative rate of isometric relaxation. In a hypocalcium medium PTH had no effect on the rate of isometric relaxation of the myocardium.

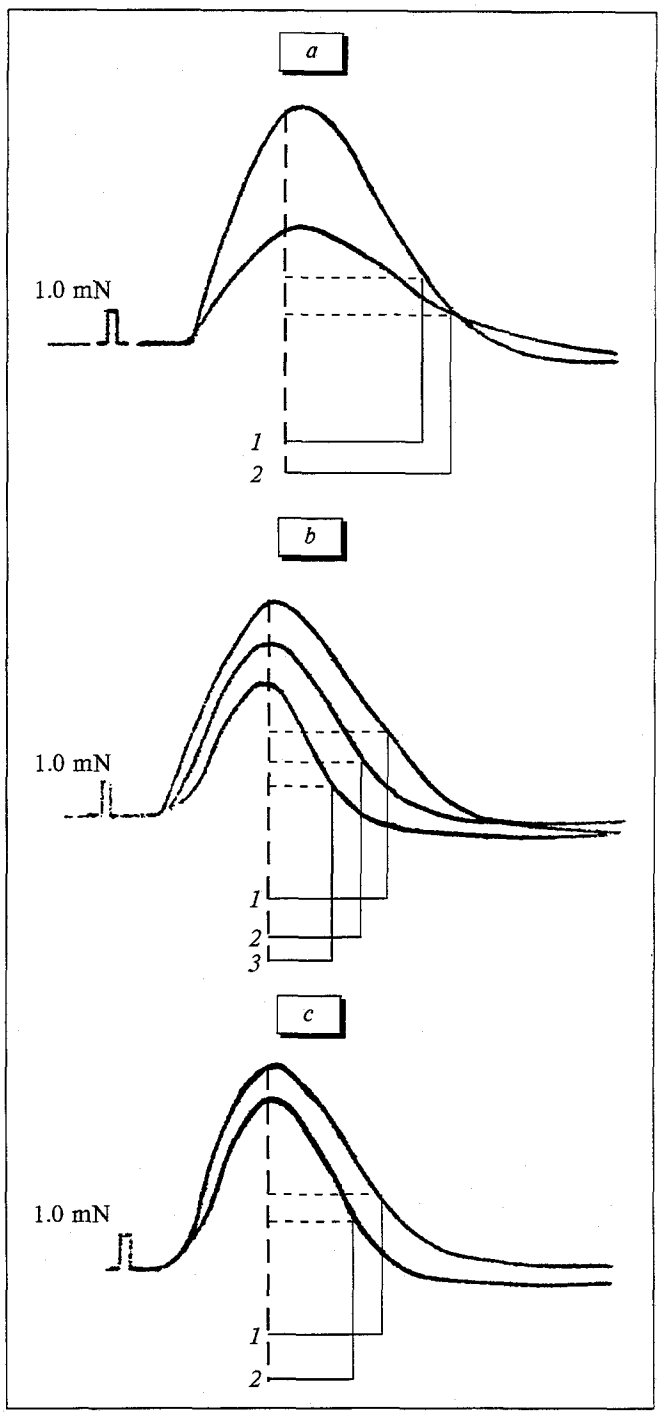

Fig. 1. Superposition of isometric contractions of papillary muscles from rat myocardium. Shifts of t_{30} are depicted. Effect of PTH in the control solution (*a*), in the hypercalcium medium (*b*), and after treatment with NE (*c*); Contraction before the addition of PTH (*1*), 5 min after the addition of PTH (*2*), and 10 min after the addition of PTH (*3*).

For a better evaluation of the relaxation ability of the myocardium under the physiological regime in the control Krebs solution we used the relationship between L_{es} and t_{30} as a measure of the mechanical function. Figure 2, *a* depicts the L_{es}—t_{30}

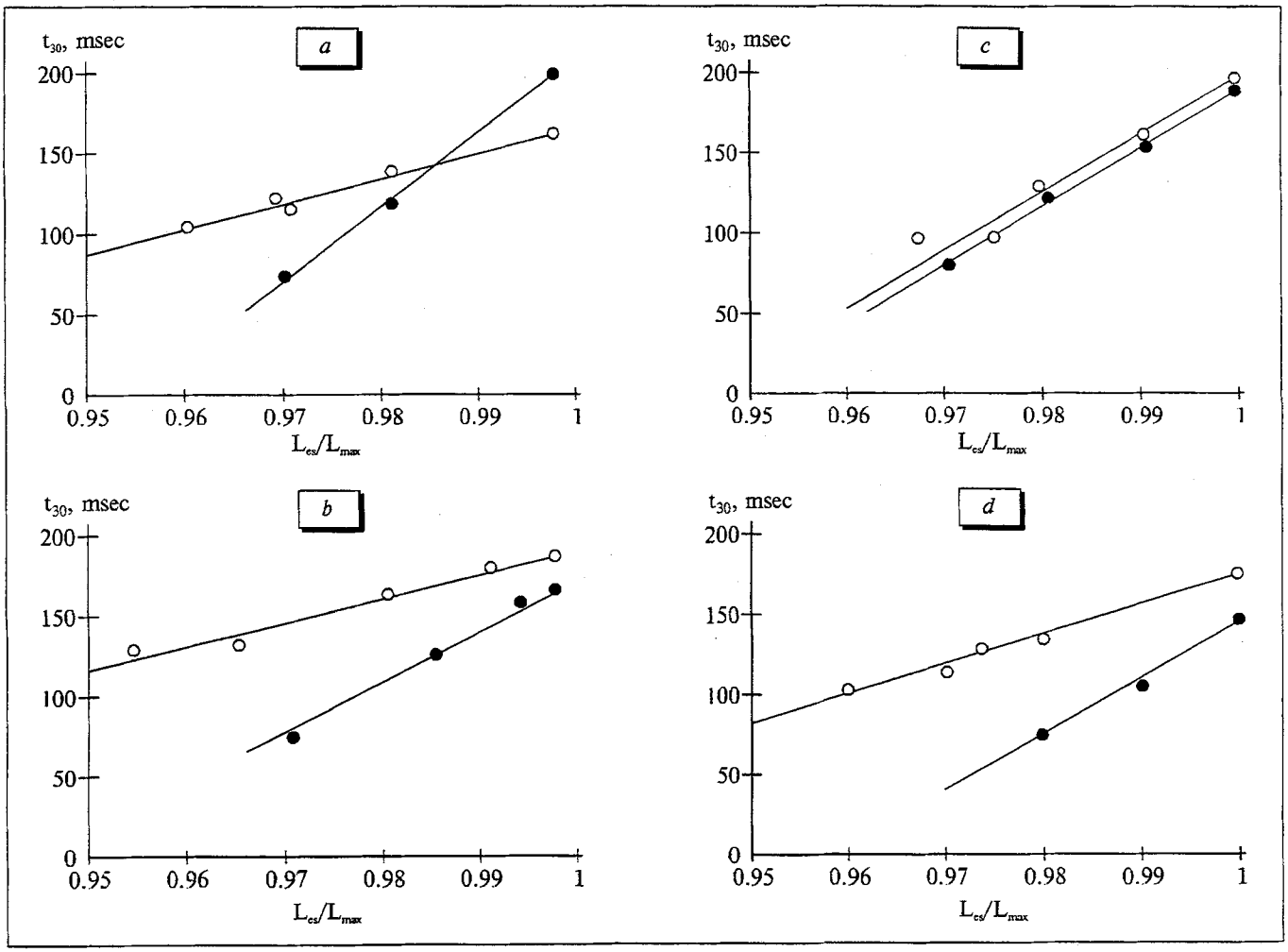

Fig. 2. Effect of PTH on characteristic relaxation time t_{30} of rat ventricular myocardium in the control solution (*a*), hypercalcium medium (*b*), hyporcalcium medium (*c*), and against the background of NE–pretreatment (*d*). Open circles: t_{30} without PTH, dark circles: in the presence of PTH.

dependences for the rat myocardium preparations before and after the addition of PTH to the control solution. The ratio of the end-systolic load to the maximal force of isometric contraction was plotted as the abscissa and the characteristic relaxation time t_{30} (msec) as the ordinate. Under a constant stimulation frequency (3 sec) the addition of PTH to the perfusate increases the slope of the L_{es}—t_{30} dependence. PTH acts in a such way that for any L_{es} of the muscle, the relative relaxation rate surpasses that developed in the absence of PTH (Fig. 2, *a*). Consequently, the relaxing effect of PTH manifests itself in a more rapid relaxation for the same value of L_{es}. The "intrinsic" relaxing effect of PTH is more pronounced at lower L_{es} values.

In the hypercalcium medium PTH sharply increased the slope of the L_{es}—t_{30} dependence. Figure 2, *b* shows a monotonic rise in the relative rate of the isometric relaxation phase, i.e., parameter t_{30} decreased for any L_{es}. Thus, under conditions of an excess of Ca^{2+} ions in the extracellular medium PTH accelerates relaxation for any fixed L_{es} value.

In the hypocalcium medium PTH had no reliable effect on the L_{es}—t_{30} dependence (Fig. 2, *c*), so that the relative rate of isometric relaxation remained unchanged for any L_{es}.

Under conditions of NE-induced activation of adenylate cyclase the addition of PTH resulted in a monotonic rise in the relative rate of isometric relaxation, i.e., parameter t_{30} decreased for any L_{es} (Fig. 2, *d*).

Thus, our findings suggest that the loosening effect of PTH is due, not to inhibition of relaxation, as was demonstrated in the isometric regime, but to its acceleration. This is a radically new result. In hypercalcium medium and under conditions of NE-induced activation of adenylate cyclase the addition of PTH to the medium monotoni-

cally decreases the characteristic relaxation time t_{30} and increases the relative rate of isometric relaxation. In the hypocalcium medium, PTH has no effect on relaxation for any L_{es}. In our experiments due to the use of the physiological loading regime the effects of PTH were studied at fixed L_{es} values, which allowed us to observe the pure loosening effect of PTH.

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CCl_4 as Inductor of L-Arginine-Dependent Synthesis of NO

O. A. Kovalenko, N. I. Tarasova, V. D. Mikoyan, and A. F. Vanin

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The effect of CCl_4 on the generation of NO in mouse liver cells is studied *in vivo*. Injection of CCl_4 is shown to modulate the synthesis of NO by activating the NO-synthetase system. The experimental data suggest that O_2^- plays an essential role in the regulation of NO-synthetase system.

Key Words: *nitric oxide; NO-synthetase; CCl_4; antioxidants*

Many types of animal cells — macrophages, neutrophils, endothelial and muscle cells, fibroblacts, neurons, and hepatocytes — synthesize nitric oxide (NO) which, first acts as a transmitter and second, possesses a cytotoxic activity. NO in the cells forms from L-arginine due to the action of NO-synthetase (NOS). There are two isoforms of NOS differing in structure and mode of regulation. The constitutive NO-synthetase (c-NOS) permanently present in cells is regulated by changes in the concentrations of Ca^{2+} ions induced by hormones and neurostimulators and synthesizes NO in picomole amounts. Another isoform of NOS (i-NOS) can be induced in macrophages, smooth-muscle cells, etc. by exotoxins and cytokinins and produces a large amount (nanomoles) of NO over a long period of time [3]. The regulation of i-NOS has been little studied. It is assumed that transcription factor NF-κB, which controls the induction of the expression of various genes in inflammation, the immune response, and stress, also participates in the initiation of i-NOS synthesis [6,9]. This assumption is based on the following observations. First, both the activation of NF-κB and the production of i-NOS can be inhibited by antioxidants [6,8-10]. Second, reactive intermediate oxygen species, including O_2^-, produced in various electron transfer reactions, activate NF-κB [7]. Moreover, O_2^- generation is necessary for the initiation of NO synthesis in the cells exposed to various stimulating agents. It should be noted that the generation of O_2^- and production of NO are related in a very complex manner: an excess of O_2^- reduces the amount of detectable NO [5].

The aim of the present study was to investigate *in vivo* NO production in mouse liver cells induced by the chemical toxin CCl_4 rather than the usual biological agents (lipopolysaccharides, γ-interferon, tumor necrosis factor, etc.). This toxin induses a

N. N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow (Presented by Yu. A. Vladimirov, Member of the Russian Academy of Medical Sciences)

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