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MECHANISM OF BACTERIAL LIPOPOLYSACCHARIDE EFFECT ON THE FUNCTIONAL ACTIVITY OF THE HEART IN VITRO. CORRECTION OF ITS EFFECTS BY THE CALCIUM REGULATING HORMONE SYSTEM

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ABSTRACT

Bacterial lipopolysaccharides belong to the group of pathogen-associated molecular patterns such as alarmins. They can cause various dysfunctions of the cardiovascular system, including arrhythmia, myocardial hypertrophy, myocarditis, and heart failure.

The damaging effect of lipopolysaccharides on the myocardium can be a result of their direct action or ensured by uncontrolled secretion of endogenous alarmins and cytotoxic shock.

Lipopolysaccharides are perceived by specific caspases of host cells and activate the release of interleukins, and in maximum stimulation they lead to necrosis and/or pyroptosis of cells.

The mechanism of the action of bacterial lipopolysaccharide on the functional activity of the heart was studied in vitro using an isolated frog heart model. By the method of pharmacological blockade, the participation of calcium and potassium channels, phosphodiesterase and Na^+-K^+-ATPase in the mechanism of the lipopolysaccharide influence on the inotropic and chronotropic activity of the heart was revealed.

In our experiments, we observed a dose-dependent negative effect of lipopolysaccharide on the inotropic and chronotropic functions of isolated frog heart, as well as a significant reduction in its viability.

It is assumed that the mechanism of the action of lipopolysaccharide on the heart involves calcium and potassium ions, Na^+-K^+-ATPase and cAMP.

The study investigated the role of the calcium-regulating hormonal system (including parathyroid hormone, parathyroid hormone-related protein, calcitonin, and vitamin D_3) in preventing disturbances in the functional parameters of the isolated heart induced by bacterial lipopolysaccharide.

The protective role of these hormones in preserving the pacemaker and contractile functions of the heart under the influence of bacterial lipopolysaccharide has been shown. A particularly significant effect is exerted by vitamin D_3, parathyroid hormone and parathyroid hormone-related protein, which maintain the functional activity of the isolated heart for a long time.

KEYWORDS: *bacterial lipopolysaccharide, heart, calcium-regulating hormonal system, calcium and potassium channels, cAMP, Na^+-K^+-ATPase.*

INTRODUCTION

Lipopolysaccharide (LPS) is an important component of outer membrane of gram-negative bacteria. Bacterial lipopolysaccharides belong to the group of pathogen-associated molecular patterns

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such as alarmins. They can cause various dysfunctions of the cardiovascular system, including arrhythmia, myocardial hypertrophy, myocarditis, and heart failure [*Bowman J et al., 2017*]. The damaging effect of LPS on the myocardium can result from its direct action or from the uncontrolled secretion of endogenous alarmins of damage-associated molecular patterns [*Gallucci S, Matzinger P, 2001*]. Endogenous and exogenous pro-inflammatory factors are releasing during mechanical, hypoxic, necrotic, pathogen-induced cell damage and are triggers of innate and adaptive immune reactions [*Yang D et al., 2017*]. The immune responses are aimed at protecting host cells through neovascularization, repair and regeneration of damaged cardiac tissue [*Lai S et al., 2019*]. Alarmins play a key role in the processes of stem cell proliferation and neo-angiogenesis. At the same time, excessive activation of alarmins causes a cytokine storm and leads to cell death [*Yang D et al., 2017; Russo A et al., 2021*]. Bacterial lipopolysaccharides are perceived by specific caspases in host cells and activate the release of interleukins. However, under conditions of maximum stimulation they can lead to necrosis and/or pyroptosis of cells [*Rathinam V et al., 2019*]. Lipopolysaccharide promotes the release of high mobility group box 1 (HMGB1), an alarmin associated with damage-associated molecular patterns, into the extracellular space. It accelerates the degradation processes in hepatocytes through the activation of caspase-11 [*Deng M et al., 2018*]. High mobility group box 1 has a multifunctional effect on the cardiac tissue: on the one hand, alarmin is involved in the processes of modulating the inflammatory process in the myocardium [*Germani A et al., 2007; Raucci A et al., 2019*], and on the other hand, it is a powerful pro-inflammatory cytokine that accelerates ischemic damage of tissue (Janus face) [*Zhai C et al., 2012; Kang R et al., 2014*]. Secretion of the HMGB1 is realized by activation of calcium-calmodullin-dependent protein kinase mechanism [*Zhai C et al., 2012*]. While the effects of HMGB1 during the acute phase of heart tissue injury are generally considered cytoprotective, its prolonged action might be more negative [*Raucci A et al., 2019*]. Elevated serum levels of HMGB1 in LPS-induced sepsis have been associated with negative inotropic effects. Treatment of feline cardiac myocytes with HMGB1 resulted in decreased sarcomere shortening and a reduction in the height of the peak Ca2+ transient, possibly due to the decreased calcium levels [*Tzeng H et al., 2008*].

By binding to calcium-specific G-receptors, LPS increases calcium load, induces autophagy of cardiomyocytes [*Wang H et al., 2013*] and disrupts cardiac electrophysiological parameters by suppressing calcium-regulated potassium current [*Yücel G et al., 2017*]. Activation of Toll-like receptors-4 by LPS promotes the production of norepinephrine by intra-cardiac structures and leads to myocardial hypertrophy [*Yang D et al., 2022*]. Lipopolysaccharide also activates macrophages, dendritic cells, CD4-markers and can cause the atherosclerosis and vasculitis [*Gorabi A et al., 2022*]. It is believed that LPS enhances secretion of TNF-α, thereby causes cardiac depression [*Bowman J et al., 2017*]. It has been shown that any inflammatory process is accompanied by uncontrolled secretion of pro-inflammatory alarmins, resulting in myocardial damage and chronic heart failure. This, in turn, causes impaired tissue perfusion and is involved in the mechanism of multiorgan damage [*Van Linthout S, Tschöpe C, 2017*].

The cardiac effects of LPS depend not only on the dose (low concentrations enhance and high concentrations suppress cardiac activity), but also on the experimental model (*in vivo* or *in vitro*) [*Kuo F et al., 2023*]. Intraperitoneal administration of LPS to animals resulted in a significant prolongation of the HR and QT intervals, as well as a shortening of the QRS complex on the electrocardiogram. These effects were mitigated by concurrent administration of LPS and 1-34 active fragments of parathyroid hormone (PTH) [*Harutyunyan K et al., 2022 a; b*]. Negative T wave in ECG, tachycardia and decreased arterial pressure is observed in 8-hour exposure to LPS [*Blad S et al., 2008*]. Variability of ECG in LPS-exposure depends on age: the effect of LPS (increased PR, QT intervals, decreased heart rate) was more prominent in old mice [*Esfahani N et al, 2021*]. The cardio-protective effects of calcium-regulating hormones in cardiomyopathy, myocarditis and chronic heart failure was shown in our previous studies [*Arakelyan K et al., 2007; Adamyan S et al., 2021; Harutyunyan K et al., 2023*] and confirmed in numerous literature sources [*Brown S et al., 2017;*

Goltzman D et al., 2018]. Mobilization of CD34+/CD45, increased neovascularization and reparative properties of cardiomyocytes were observed when PTH was administered directly into the infarction zone in mice [*Zaruba M et al., 2008*]. The activation of inotropic and chronotropic functions by parathyroid hormone and parathyroid hormone-related protein (PTH-rP) is discussed in other studies [*Brown S et al., 2017*]. Several mechanisms have been proposed relating vitamin D deficiency to cardiovascular risk factors such as renin-angiotensin-aldosterone system activation, abnormal nitric oxide regulation, oxidative stress or altered inflammatory pathways [*De la Guía-Galipienso F et al., 2021*]. Another mechanism of antimicrobial action of vitamin D involves down-regulating the production of cytotoxic factors and regulating iron metabolism in cells [*Bilezikian J et al., 2020*]. The cardioprotective effects of these hormones are realized through the involvement of Ca^{2+}, K^{+}, Na^{+} ions, protein kinases A and C, Na^{+}-K^{+}-ATPase, Na^{+}-Ca^{2+}-exchanger, etc. in the process [*Eisner D et al., 2017; Ter-Markosyan A et al., 2017; Goltzman D et al., 2018*]. One of the most severe outcomes of sepsis is cardiac dysfunction, characterized by a decrease in ventricular ejection fraction, leading to reduced perfusion of peripheral and central organs such as the heart, kidneys and central nervous system [*Valeanu L et al., 2021*]. Maintaining the contractile function of the heart, its left ventricular ejection fraction and other hemodynamic parameters, significantly increases patient survival in conditions of cytotoxic attacks.

Despite the availability of numerous literature data on the effects of LPS on cardiac function parameters, the mechanism of its action has not been thoroughly studied. This research aimed to study the mechanism of LPS action on functional parameters such as pacemaker activity and heart rate amplitude using an isolated animal heart model (*in vitro*) and assess the role of the calcium-regulating hormonal system in correcting any shifts in these parameters induced by LPS.

MATERIAL AND METHODS

Model experiments were performed on isolated hearts of frogs (*Rana temporaria*). The maintenance and use of experimental animals adhered to the provisions of the Institutional Committee of Bioethics. All stages of experiments were directly controlled by the Ethical Committee of Yerevan State Medical University. The choice of isolated organ methods allows for studying the mechanisms of direct influence of biologically active substances in vitro, without interference from other factors such as neural or hormonal and etc. [*Olejnikova V et al., 2015*]. The frog's heart is a convenient object of study due to the presence of multiple ion channels (voltage-gated and mechano- sensitive, ligand-sensitive). At the same time, being a cold-blooded animal, frog metabolism is energy efficient, so its heart can be contracted for a long time [*Burggren W, Warburton S, 2007*].

For *in vitro* studies, a frog's heart was surgically removed after euthanizing the animal with ether. The isolated heart was then placed in a photoelectric device chamber with Ringer's solution for cold-blooded animals (0.65% NaCl, 0.018% KCl, 0.02% $CaCl_2$, 0.03% Na_2HPO_4, 0.01% NaH_2PO4, pH = 7.2, t = 18°C).

In the first series of experiments the dose-dependent cardiotropic effects of LPS (Sigma, USA) were studied, identifying the most effective concentration of substance. For this purpose, 10 *μg/ml*, 20 *μg/ml* or 100 *μg/ml* dose of LPS were introduced into the incubation medium with Ringer's solution where the isolated heart was located.

In the second series of experiments a pharmacological analysis of the mechanism of LPS action on the pacemaker and contractile functions of the isolated heart was carried out, using a calcium channel blocker (10^{-5} *M* verapamil (Sigma, USA)), a potassium channel blocker (10^{-3} *M* aminopyridine (Sigma, USA)), phosphodiesterase (10^{-4} *M* theophylline (Sigma, USA)) and Na^{+}-K^{+} pumps (10^{-3} *M* ouabaine (Sigma, USA)) in dynamics. The comparative analysis of alone effects of LPS and effects of LPS with blockers was done. A comparative analysis was conducted to evaluate the effects of LPS alone and in combination with blockers.

In the III series of experiments LPS was combined with one of the calcium-regulating hormones. 1-34 active fragment of PTH (Sigma, USA), 1-34 active fragment of parathyroid hormone-related protein (Sigma, USA), vitamin D_3 (Sigma, USA) or calcitonin (CT) (Sigma, USA) were added in different sets of experiments and final concentrations of mentioned hormones in the

incubation medium were close to physiological, 10^{-10} *M*. A comparative analysis was conducted to evaluate the effects of LPS alone and in combination with calcium-regulating hormones.

The registration of heart functional activity (pacemaker rhythm, amplitude of heart contractions) was performed using a specific photoelectric device (LLC BioArt, Armenia), which operates based on the principle of dispersion of the luminous flux. Heart contraction changed the angular distribution of the light beam and led to corresponding changes in the indices of the photodetector.

The semiconductor laser (MOD HLDPM10–650–3, Huey Jann Electronics, China) was used as a radiation source, and a FD–256 silicon (Si) photodiode (Russia) was used to estimate the light intensity. After amplification, the photo-detector signals were subjected to analogue-to-digital conversion (Takfly communications co. Ltd, China) and stored. The sampling time for analogue-to-digital conversion was 10 *ms*. The custom-developed software (in the LabView environment) allowed visualization and subsequent analysis of the recorded signals. The amplitude and frequency of the frog's heart contractile activity were evaluated. Averaged amplitude and time-frequency data of the contractile activity of the heart were plotted for each series of studies (15-20 animals in each group). To standardize the amplitude distributions of heart functional activity, the initial registration values were set at 100%. The frequency of contractions is expressed in absolute values. The analysis of the recorded signals was carried out in two stages. First, to assess the changes in the amplitude of the heart contractions in dynamics, all values of the investigated parameters were compared with the initial contraction amplitude. Second, to analyze the frequency of heart contractions, the signals were normalized by the calculated current amplitude of contractions and their peak values were distinguished by the amplitude discrimination method.

Statistical analysis of the obtained data was performed by using "Origin 8.5" software, according to Student's t-test. The average value of investigated parameters for each 10-minute interval were compared to those obtained in the first time interval (1-10 *min*) (p_1). the investigated parameters in different 10-minute intervals were compared to the parameters of the control (I series) or under the influence of LPS alone (II and III series) (p_2).

Results

The goal of the first series of experiments was to determine the effective dose of LPS action on the heart *in vitro*. A significantly negative shift in inotropic parameters was observed at a dose of 10 μg/ml LPS, characterized by statistically significant changes ($p1<0.01$, $p2<0.001$) in subsequent intervals (Table 1). At a concentration of 20 *μg/ml*, LP dose of 100 *μg/ml*, LPS increased the strength of heart contractions but slowed down the pacemaker rhythm. Based on these findings, we determined that using a dose of 10 *μg/ml* would be appropriate for the subsequent series of experiments.

In the second series of experiments, LPS was administered together with one of the blockers indicated in the "Materials and Methods" section. When 10 *μg/ml* of LPS was injected alone into the incubation medium, the amplitude of contractions of the isolated heart was reduced by an average of 76% from the initial level, and the frequency of the pacemaker rhythm decreased from 48 to 42 *beats* per *minute* (Table 2). The viability of the isolated heart decreased from 40 *minutes* (in control) to 20 *minutes* (under the influence of LPS) and the isolated heart contractions stopped.

A significant positive shift in the heart rate and amplitude was observed when LPS was combined with verapamil or theophylline, compared to the effects of LPS alone (Table 2). The combination of LPS with aminopyridine did not result in significant changes in these parameters during the first 20 *minutes*. However, when combined with ouabain, LPS caused a negative shift. The chronotropic index significantly decreased under the influence of LPS combined with verapamil or ouabain, but was maintained for a long time (50 *minutes* or more) under the influence of LPS and theophylline. The effect of LPS combined with aminopyridine was similar to the effect of LPS alone. The obtained results suggest the involvement of Ca2+-, Na+-K+-ATPase-, and cAMP-dependent mechanisms to varying degrees in the cardiotropic effects (heart contraction amplitude, pacemaker activity) of lipopolysaccharide.

In our previous studies [*Ter-Markosyan A et al., 2017*] and in literature references [*Wang T, 2016; Meyer R et al., 2007; Schavinski A, 2021*], it has been shown that the protective/modulatory effects of calcium-regulating hormones (1-34 PTH, 1-34

TABLE 1.

The dose-dependent impact of lipopolysaccharide on the functional activity of isolated heart in dynamics

Substance \ Average value		Interval 1-10min	11-20min	21-30min	31-40min	41-50min
Amplitude (%)						
Control n=15	%	102.31±6.31	83.78±8.03	66.62±7.37	29.81±1.74	
	p_1		>0.05	<0.01	<0.001	
LPS 10 *μg/ml* n=16	%	57.22±7.8	25.04±3.87	-	-	-
	p_1		<0.01	-	-	-
	p_2	<0.001	<0.001	-	-	-
LPS 20 *μg/ml* n= 15	%	82±5.42	49.15±2.46	38.44±22.01	-	-
	p_1		<0.001	>0.05		
	p_2	<0.05	<0.001	>0.05		
LPS100 *μg/ml* n=15	%	180.78±26.88	182.66±31.82	185.01±39.21	156.44±45.17	157.42±57.04
	p_1		>0.05	>0.05	>0.05	>0.05
	p_2	<0.01	<0.01	<0.01	<0.01	
Rate (beat/min)						
Control n=15	%	50.81±5.75	47.46±5.58	38.27±4.84	43.01±4.17	
	p_1		>0.05	>0.05	>0.05	
LPS 10 *μg/ml* n=16	%	48.33±9.43	42.83±3.37			
	p_1		>0.05			
	p_2	>0.05	>0.05			
LPS 20 *μg/ml* n=15	%	50.11±3.93	42.31±7.99	49.72±21.92		
	p_1		>0.05	>0.05		
	p_2	>0.05	>0.05			
LPS 100 *μg/ml* n=15	%	48.1±6.93	45.5±10.47	30.45±3.98	25.49±3.65	23.8±3.99
	p_1		>0.05	<0.05	<0.01	<0.01
	p_2	>0.05	>0.05	>0.05	<0.01	

Notes: *p_1 – index of statistical significance indicating the shift of the investigated parameter in the different intervals compared to the same parameter at 1-10 min; p_2 – index of statistical significance indicating the shift of the investigated parameter in the different intervals compared to the control. The absence of the heart contractions is noted "–". Colored cells show prolonged vitality of the isolated heart compared to the control*

PTH-rP, vitamin D_3, calcitonin) on the heart are mediated through calcium ions and cAMP-dependent mechanisms. Considering the key role of these pathways in the negative effects of LPS on the heart, the third series of experiments assessed the extent to which calcium-regulating hormones could prevent disturbances in the functional activity of the heart.

LPS induced cardiac arrest within 11-20 minutes of the experiment (Table 3). Calcium-regulating hormones prevented the LPS-induced negative shift in the amplitude of heart contractions but had varying effects on the pacemaker rhythm. Under LPS attack, 1-34 PTH maintained the inotropic index and caused a biphasic change in heart rate. Vitamin D_3 sustained both the pacemaker rhythm and heart contraction amplitude for an extended period. 1-34 PTH-rP had late positive inotropic and chronotropic effects, while calcitonin did not significantly affect the amplitude or pacemaker rhythm. The longest maintenance of isolated heart viability (up to 60-70 *minutes*) was observed when LPS was combined with either vitamin D_3 or 1-34 PTH.

DISCUSSION

Functional parameters (automatism, rhythm, electromechanical coupling, energy supply, etc.) of the heart largely depend on the balance of extra-

Table 2.

The dose-dependent impact of lipopolysaccharide on the functional activity of isolated heart in dynamics

Substance \ Average value / Interval		1-10min	11-20min	21-30min	31-40min	41-50min
Amplitude (%)						
LPS 10 *μg/ml* n=16	%	57.22±7.	25.04±3.87			
	p_1		<0.01			
LPS + verapamil n=20	%	86.74±8.52	41.89±3.99	28.39±5.31	23.03±4.4	
	p_1		<0.001	<0.001	<0.001	
	p_2	<0.05	<0.01			
LPS + theophylline n=17	%	65.3±6.18	37.49±9.06	20.38±6.84	17.48±5.82	4.95±1.25
	p_1		<0.05	<0.001	<0.001	<0.001
	p_2	>0.05	>0.05			
LPS + ouabaine n=15	%	42.25±4.7	5.65±2.08			
	p_1		<0.001			
	p_2	>0.05	>0.001			
LPS +aminopyridine n=16	%	66.16±6.7	39.04±9.3	44.76±14.17		
	p_1		<0.05	>0.05		
	p_2	>0.05	>0.05			
Rate (beat/min)						
LPS 10 *μg/ml* n=16	%	48.33±9.43	42.83±3.37			
	p_1		>0.05			
LPS + verapamil n=20	%	43.19±3.42	31.76±3.54	31.76±6.18	29.77±6.82	
	p_1		<0.05	>0.05	>0.05	
	p_2	>0.05	<0.05			
LPS + + theophylline n=17	%	51.14±2.79	42.67±2.45	36.63±5.15	39.44±7.9	56.13±4.96
	p_1		<0.05	<0.05	>0.05	>0.05
	p_2		>0.05			
LPS + ouabaine n=15	%	44.01±3.04	24.83±2.2			
	p_1		<0.001			
	p_2	>0.05	<0.001			
LPS +aminopyridie n=16	%	48.84±4.2	43.91±3.7	37.28±3.9		
	p_1		>0.05	>0.05		
	p_2		>0.05			

Notes: *p_1 – index of statistical significance indicating the shift of the investigated parameter in the different intervals compared to the same parameter at 1-10 min;p_2 - index of statistical significance indicating the shift of the investigated parameter in the different intervals compared to alone action of LPS. The absence of the heart contractions is noted "–".Colored cells show prolonged vitality of isolated heart compared to the control*

cellular calcium influx, its release from the sarcoplasmic reticulum during the systole, and its outflow from the cytosol during the diastole [*Eisner D et al., 2017*].

The effect of LPS on the cardiac pacemaker activity is carried out by a Ca^{2+}-dependent mechanism, which is confirmed by a more pronounced negative chronotropic effect observed in the presence of verapamil (Table 2). At the same time, verapamil somewhat neutralized the negative inotropic shift of LPS.

The obtained results coincide with the literature data [*Sermsappasuk P et al., 2008*], indicating a reduction in the negative inotropic effect in LPS-

TABLE 3.

The dose-dependent impact of lipopolysaccharide on the functional activity of isolated heart in dynamics

Substance \ Average value		Intervals 1-10min	11-20min	21-30min	31-40min	41-50min	51-60min	61-70min	71-80min
Amplitude (%)									
LPS 10 *μg/ml* *n=16*	%	57.22±7.8	25.04±3.87						
	p_1	p<0.001	p<0.001						
LPS + PTH *n=15*	%	65.27±4.54	42.7±2.73	26.34±3.63	17.98±2.56	13.25±1.85	11.7±2.2	10.34±1.8	
	p_1		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
	p_2	>0.05	<0.01						
LPS + PTH rP *n=18*	%	68.72±5.46	43.52±8.2	31.79±7.85	35.83±10.3	30.91±11.9			
	p_1		<0.05	<0.001	<0.01	<0.01			
	p_2	>0.05	<0.05						
LPS + Vitamin D_3 *n=20*	%	75.94±4.6	69.7±10.5	60.57±9.67	52.82±7.97	49.98±11.46	42.57±11.8	37.1±12.7	42.7±16.7
	p_1		>0.05	>0.05	<0.05	<0.05	<0.05	<0.01	>0.05
	p_2	<0.05	<0.001						
LPS +Calcitonin *n=15*	%	65.9±3.86	34.47±5.26	24.51±4.46	18.04±3.9	15.62±2.18			
	p_1		<0.001	<0.001	<0.001	<0.001			
	p_2	>0.05	>0.05						
Rate (beat/min)									
LPS 10 *μg/ml* *n=16*	%	48.33±9.43	42.83±3.37						
	p_1		>0.05						
LPS + PTH *n=15*	%	36.81±4.35	29.46±3.79	25.17±4.49	23.08±5.14	26.9±4.66	32.09±3.2	31.8±3.72	
	p_1		>0.05	>0.05					
	p_2	>0.05	<0.05						
LPS + PTH rP *n=18*	%	45.37±5.74	31.16±3.2	25.6±6.82	37.84±9.6	37.65±9.07			
	p_1			<0.05	<0.05				
	p_2		>0.05	<0.05					
LPS + Vitamin D_3 *n=20*	%	44.35±2.48	37.56±3.65	33.74±4.38	29.66±4.19	26.9±5.24	24.52±5.5	27.2±5.2	28.9±6.9
	p_1		>0.05	<0.05					
	p_2	>0.05	>0.05						
LPS +Calcitonin *n=15*	%	49.83±3.1	41.99±3.03	37.04±3.1	36.21±2.01	31.13±1.69			
	p_1		>0.05	<0.01					
	p_2	>0.05	>0.05						

Notes: *p_1 – index of statistical significance indicating the shift of the investigated parameter in the different intervals compared to the same parameter at 1-10 min; p_2 - index of statistical significance indicating the shift of the investigated parameter in the different intervals compared to the alone action of LPS. The absence of the heart contractions is noted "–".Colored cells show prolonged vitality of isolated heart compared to the control*

perfused rat hearts and a reduced mortality rate in septic mice receiving verapamil for therapeutic purposes [*Rattis B et al., 2021*].

There is an opinion [*Zhang H et al., 2007; Zheng H et al., 2011*], that an excessively high level of Ca^{2+} in the cytosol enhances the production of cytokines and leads to the death of cardiomyocytes. The activation of calcium-sensitive receptors by lipopolysaccharide causes calcium overload, disrupts the electromechanical coupling of the heart and reduces the left ventricular ejection fraction [*Joulin O et al., 2009; Wang H et al., 2013*]. It has been shown that LPS causes hyperpolarization and reduces the contractility of striated muscle tissue [*Vacassenno R et al., 2023*], but increases the contractility of smooth muscle cells of

isolated uterine [*Zhang L et al., 2015*]. Inhibition of L-calcium channels by verapamil or nifedipine prevents the penetration of the genomes of certain viruses (Ebola, SARS-CoV-2) into cardiac cardiomyocytes and reduces the release of endolysosomal apoptotic enzymes [*Sakurai Y et al., 2015; Moccia F et al., 2020*]. The protective effect of verapamil, which reduces neurotoxicity in models of dopaminergic structures by blocking calcium current in LPS-induced sepsis, is subject to debate [*Liu Y et al., 2011*].

Numerous literature references [*Semmler J et al., 1993; Jin S, Conti M, 2002*] indicate that phosphodiesterase inhibitors increase cAMP levels and thereby suppress the synthesis and release of cytokines in tumor cells, improve mitochondrial respiration in septic cardiomyocytes [*Neviere R et al., 2016*] and prevent organ dysfunction caused by LPS attack [*Jin S, Conti M, 2002; Flemming S et al., 2014*]. This assumption is supported by a comparative analysis of the curves showing the effects of LPS alone and LPS combined with theophylline, which revealed identical shifts in both amplitude and frequency during the initial 30 minutes (Table 2). Comparing these findings with existing literature data suggests that an elevation in cytosolic cAMP levels in cardiomyocytes mitigates the negative effects of LPS on heart functional activity.

The activation of potassium channels is considered one of the early stages of LPS signaling in macrophage host cells [*Seydel U et al., 2001*]. The pharmacological blockade of K^+-channels with aminopyridine delayed repolarization in cardiomyocytes, expanded the action potential plateau and increase calcium-current [*Bellou A et al., 2016*], but it may also cause long-QT syndrome [*Li X et al., 2017*]. In our experiments, no significant differences in the functional parameters of cardiac activity were found under the influence of LPS alone or when combined with aminopyridine.

The most acute functional disorders of the heart were identified when LPS and ouabaine were introduced into the incubation medium. The functional parameters of the heart were sharply suppressed, and the heart viability was reduced to 11-20 *minutes*. Similar results are presented in the literature [*Orellana A et al., 2016; Kobayashi M et al., 2017*]. The authors believe that ouabaine enhances macrophage infiltration and facilitates the release of IL-1β, while also inducing cardiac dysfunction in mice receiving LPS. The phenomenon of pathological cardiac hypertrophy development when Na^+-K^+-ATPase is inhibited by lipopolysaccharide and ouabaine has been described [*Wang L et al., 2010*]. At the same time, Wang C et al. (2018) recommend the use of ouabaine to improve lung parameters in LPS-induced lung tissue damage in mice. Ouabaine, widely used in the treatment of chronic heart failure and arrhythmia, has a very controversial effect and numerous side effects [*Whitbeck M et al., 2013*].

Thus, based on the results of these experiments and data presented in the literature, it is assumed that Ca^{2+}-ions, cAMP and Na^+-K^+-ATPase participate in the effects of lipopolysaccharide on the pacemaker and contractile activity of the heart.

Our previous studies showed the protective effect of the calcium-regulating hormonal system (1-34PTH, 1-34PTH-rP, vitamin D_3) in cases of chronic heart failure and cardiomyopathies of various etiologies [*Adamyan S et al., 2021; Ter-Markosyan A et al., 2021; Harutyunyan K et al., 2023*]. Present study showed a significant increase in the inotropic index and long-term maintenance of cardiac pacemaker activity when LPS was combined with all calcium-regulating hormonal system hormones, with the exception of calcitonin (Table 3). The cardioprotective effects of these hormones are mediated through the involvement of Ca^{2+}, K^+, Na^+ ions, protein kinases A and C, Na^+-K^+-ATPase, Na^+-Ca^{2+}-exchanger, and others [*Eisner D et al., 2017; Ter-Markosyan A et al., 2017; Goltzman D et al., 2018*]. Maintaining the heart contractile function, its left ventricular ejection fraction and other hemodynamic parameters significantly increased the survival of patients in conditions of cytotoxic attacks [*Valeanu L et al., 2021*].

Intraperitoneal administration of LPS to animals caused disturbances in electrophysiological parameters (HR lengthening of HR and QT intervals, and shortening of the QRS complex), which were mitigated by the administration of the 1-34 active fragment of PTH [*Harutyunyan K et al., 2022*]. Patients suffering from cardiomyopathy exhibited an increase (within the physiological norm) in concentrations of 1-34 PTH, CT, ionized Ca, phosphates, and HMGB1 in the blood [*Harutyunyan K et al., 2023*]. Other authors have reported an

elevation in plasma PTH concentration and increased production of PTH-rP in animal hepatocytes during endotoxemia induced by LPS [*Funk J et al., 1997; Toribio R et al., 2005*].

The modulation of the immune response, inhibition of proliferation and release of lymphokines occurred in PTH-rP stimulation of T-lymphocytes [*White J, 2008*]. The involvement of PTH-rP in the down-regulation of the inflammatory process in the mouse mesangial cells was revealed [*Hochane M et al., 2018*]. Depending on the dose subcutaneous injections of PTH improved myocardial contractility, increased left ventricular systolic fraction, and reduced mortality in rats with non-ischemic cardiomyopathy [*Wu G et al., 2018*]. Mobilization of CD34+/CD45, increased neovascularization and reparative properties of cardiomyocytes were observed when PTH was administered directly into the infarction zone in mice [*Zaruba M et al., 2008*]. The increase in inotropic and chronotropic parameters by parathyroid hormone and its related protein is discussed in studies by other authors [*Brown S et al., 2017; Goltzman D et al., 2018*].

Vitamin D_3 is a unique factor for activating innate and adaptive immunity. The hormone suppresses the penetration of pathogens (SARS-COV-2) into the host cells, inhibits the production of cytokines and reduces the risk of developing an inflammatory process [*Bilezikian J et al., 2020; Driggin E et al., 2022*], normalizes cardiac activity and hemodynamic parameters in chronic heart failure [*De la Guía-Galipienso F et al., 2021*]. There is an opinion that vitamin D_3 deficiency can cause an escalation of cardiovascular dysfunction [*Bilezikian J et al., 2020; Harutyunyan K et al., 2023*].

The effect of calcitonin on the heart has been poorly studied. Limited data suggest a dose-dependent suppression of the inotropic and chronotropic activity of isolated rat cardiomyocytes by calcitonin, with more pronounced effects observed in atrial sections compared to ventricular sections [*Chiba S, Himori N, 1977*]. At the same time, systemic administration of calcitonin increased mean arterial pressure and caused arrhythmia in rats [*Peguero-Rivera A, Corder S, 1992*]. Extensive research concerns calcitonin gene-related peptide (CTG-rP), which is synthesized by the atrial cells in response to ischemic heart damage [*Kee Z et al., 2018; Wong W, 2020; Schavinski A et al. 2021*].

CTG-rP regulates the pacemaker rhythm by paracrine manner, preventing the development of fibrosis [*Moreira L et al.,* heart *2020*]. It induces hypocalcaemia, but increases L-calcium current by stimulating adenylyl cyclase in atrial cells [*Kee Z et al., 2018*]. The participation of CTG-rP in the protection of the myocardium from reperfusion injury and autophagy is assumed [*Schavinski A et al. 2021*]. CT, but not CTG-rP, was used in our experiments which had no significant effect on the heart rate amplitude and frequency.

Summarizing our results and comparing them with the literature data, it is assumed that the negative inotropic and chronotropic effects of LPS are realized mainly through calcium-, potassium- and cAMP-dependent mechanisms, and calcium-regulating hormones exhibit a protective effect and prevent the negative consequences of LPS attacks on the heart.

CONCLUSION

According to received data, bacterial lipopolysaccharide causes negative inotropic and chronotropic effects and significantly reduces the viability of isolated animal hearts. The mechanism of the cardiotropic effect of lipopolysaccharides involves calcium and potassium ions, Na^+-K^+-ATPase and cAMP. The calcium-regulating hormones protect the heart and promote the preservation of its contractile and rhythmogenic functions during LPS-attack. A particularly significant effect is exerted by vitamin D_3, PTH and PTH-rP, which maintain the functional activity of the isolated heart for a long time.

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