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THE INFLUENCE OF PARATHYROID AND SEX HORMONES ON THE PACEMAKER AND CONTRACTILE ACTIVITY OF THE FROG ISOLATED HEART

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Abstract

The impairment of the hormonal regulation of the calcium systemic and cellular metabolism is one of the forcible causes of the cardiovascular system disorders. Calcium homeostasis maintenance depends on the status of the calcium-regulating system (parathyroid hormone - thyrocalcitonin – vitamin D_3), but, anyway, the participation of the sex hormones in its regulation is not excluded.

The aim of the present investigation is to reveal the effects of parathyroid and sex hormones on the pacemaker and contractile activity of the heart.

By the method of opto-electronic regulation of the heart contractions in the dynamic regimen a comparative analysis of the influence of the parathyroid hormone 1-34 active fragment (1-34 PTH) of both female (β -estradiol) and male (testosterone) sex hormones on the contraction frequency and amplitude of the frog's isolated heart was conducted. The independent effects of the mentioned hormones, as well as their combined influence (1-34-PTH/estradiol and 1-34-PTH/testosterone) on the dynamics of the frequency and amplitude feature changes of the heart contraction were investigated.

The monitoring of the obtained results points at the positive chronotropic and inotropic-stabilizing effects of 1-34-PTH. It was revealed that the sex hormones possess positive cardiotropic effect as well, but it is more expressed in β –estradiol action. In spite of the positive cardiotropic effect of testosterone, the latter, nevertheless, sharply contracts the viability of the frog's isolated heart as it stopped already at the 20th minute of the experiment whereas 1-34-PTH and estradiol supported the heart functioning for more than 50-60 minutes.

The combined influence of 1-34-PTH with estradiol reveals the modulating effect of PTH which somehow smoothes out the positive chronotropic-inotropic shifts observed in β –estradiol's action. In this way it prevented excessive activation of the heart contractions ("phenomenon of enraged heart pacification"). In case of the combination with testosterone the mentioned fragment of parathyroid hormone displays cardio-protecting activity which was expressed when supporting the viability of the isolated heart. Thus, 1-34-PTH corrects sharp changes of the functional indices of the heart observed under the influence of sex hormones and makes their shifts practically identical both in time and direction.

Keywords: *heart, parathyroid hormone, estradiol, testosterone.*

Introduction

At present one of the leading causes of death among 50-60-year-old people are cardio-vascular disorders [*WHO, 2011*]. For this reason, not only new methods of preventing and treating the mentioned diseases should be looked for, but the risk factors of their development should be revealed as well. So, it will be reasonable to conduct a more multilateral experimental and clinical investigation. The traditional factors, leading to the development of cardiac pathologies, are dysfunctions of the nervous, endocrine, renal systems, electrolytic indices of blood, age changes of the heart muscles,

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changes of the basic indices of hemodynamics, etc. It is well known that the hormonal regulation disturbances of the calcium systemic and cellular metabolism are one of the forcible causes of the cardiovascular system disorders which bring to dysfunction of the conducting system, hypertrophy of the left ventricle, arterial and renal hypertension, myocardium and valve calcification, ischemia, fatal arrhythmia, aortic failure, etc [*Smogorzewski M et al., 1993; Ross G, Schluter K, 2005; Hargstrom E et al., 2009; Palmer S et al., 2011; Fischer E et al., 2014*]. Calcium constant maintenance depends on the state of calcium-regulating system: parathyrod hormone-thyriocalcitonin-vitamin D_3. A correlation is noted between the parathyroid hormone (PTH) level, its separate fragments, as well as parathyroid hormone-related peptide (PTH-pP) in blood and the degree of the cardiovascular system activity impairment [*Deftos L et al., 1993; Hara M et al., 1997; Schulter K, Piper H, 1998; Barleta G et al., 2000; Schulter K et al., 2000; Ogino K et al., 2002; Jansen J et al., 2003; Giannakoulas G et al., 2006; Gruson D et al., 2014; Zhao H et al., 2014*]. It is shown that some fragments of PTH have a cardio-protective and/or modulating effect on the functional activity of the heart. E.g. the 1-34 fragment of the PTH increases the heart rate and supports its contraction amplitude, which is especially manifested in heart ischemia [*Deftos L et al., 1993; Arakelyan K et al., 2007; Gruson D et al., 2014*].

Recently the attention of the clinicians and physiologists is directed to other components of the hormonal system, e.g. sex hormones, which have a direct or indirect influence on the calcium metabolism and, related to it, on the impairment of the heart activity [*English K et al., 2001; Vitale C et al., 2009; Morris P, Channer K, 2012; Hirokawa M et al., 2016*].There are data that in women in pre-menopause period calcium homeostasis shifts in blood and, as a consequence cardio-vascular system pathologies (heart failure, calcification of the valves, hypertrophy of the ventricles, atherosclerosis, etc) are less expressed than in men whereas in the post-menopause period the morbidity rate increases [*Tofovic S, 2010; Austin E et al., 2013; Sivasinprasasn A et al., 2016*]. It is clinically and experimentally proved that the female sex hormones have a protecting role in the development of the cardiovascular risk factor, it is also shown that mortality issue due to heart diseases is less observed than in men [*Baldi A et al., 2004; Cao X et al., 2015; Sivasinprasasn A et al., 2016*]. In our previous clinical investigations [*Arakelyan K et al., 2007*] we have shown that the shifts of calcium, inorganic phosphate, sodium, potassium, and especially, PTH and PTH-rP concentrations in blood were unidirectional both in males and females but they were more expressed in women than in men suffering from heart failure. There is single information in the literature that in order to prevent the coronary heart vessels from calcification in osteoporosis it is recommended to restrict the duration of estradiol hormonal therapy [*Manson J et al., 2007*], besides, cardio-protecting effect of testosterone but not β-estradiol is a topic of discussion, for example, in chronic cardio-toxicity caused by doxorubicine introduction [*Morris P, Channer K, 2012*].

In spite of the numerous investigations devoted to the problem of the development, prevention and treatment of cardiovascular disorders, some aspects of this problem, however, remain insufficiently studied. E.g., there aren't enough indications on the effects of sex hormones on the pacemaker and contractile activity of the heart, moreover, there aren't any data on the combined influence of PTH and sex hormones on its activity, though it is well known that complex effect activates the calcium metabolism in the organism. In some investigations [*Neer R et al., 2001; Zhou M et al., 2001; Miao Q et al., 2012*] is shown that estrogens decrease the risk of developing osteoporosis in the bone matrix through PTH and PTH-rP - depending mechanism, but C.Greenberg and co-authors (1987) show that estrogenic therapy of women in the post-menopause period brings to dose-depending increase of the immune-reactive PTH level in blood plasma.

Taking into account the above mentioned and comparing the results of our previous works [*Arakelyan K et al., 2007; Ter-Markosyan A et al., 2012; 2014; 2015*] with those, presented in the bibliography, we raised a problem to study the changes of the pacemaker and contractile activity of the heart in combined effect of 1-34 active fragment of PTH with β-estradiol or testosterone.

Material and methods

As an investigation material the frog's isolated heart located in a special chamber of the recording photoelectric device was used (Fig.1) The chamber contained Ringer solution for cold-blooded animals (incubation environment), to which was added physiological concentration of PTH 1-34 active fragment (10^{-10}M) and β-estradiol (10^{-6}M)/ testosterone (10^{-8}M) of "Sigma".

The recording of the heart contractile activity was carried out with the help of the mentioned device by the principle of dispersing the light flow. The heart contraction changed the angular distribution of the light beam and brought to the corresponding change in the indices of the photo-receiver.

As a source of radiation, the semi-conducting laser (MOD HLDPM10-650-3) was used and in order to estimate the light intensity – flint photodiode FD-256. The signals of the photo-receiver underwent an analogous-digital transformation after intensification and entered ECM. The discretization time in analogous-digital transformation was 10 *ms*. According to the worked out LabView program pack the recorded signals were visualized on the display screen, stored in ECM and later underwent analysis for estimating the amplitude and rate characteristics of the frog's heart contractile activity.

The indices of the amplitude heart contractions in the experimental series were compared with the control ones, the initial significance index of which was considered to be 100%. The heart contraction rate is presented in graphs in absolute values (*beats/min*), and in histograms – by % from control indices. Recording of the mentioned parameters of the heart activity was carried out in a non-stop manner during 1 hour (in some experiments even more), but in order to make their identification simpler we restricted ourselves by comparing the effects of the hormones' influence at the 10th and 40th minutes. Due to the average duration of the experiments we judged of the isolated heart's viability.

The experiments were carried out in the following succession: recording the amplitude and rate of the heart contraction

- √ in Ringer's solution (1st series, control);
- √ under the influence of 1-34PTH (2nd series);
- √ under the influence of β-estradiol (3rd series***);***
- √ *by combining* ***(***one step introduction) the influence of β-estradiol and 1-34 PTH (4th series);
- √ under the influence of testosterone (5th series)
- √ by combining (one step introduction) the influence of testosterone with 1-34 PTH (6th series).

In order to work out the obtained data and make up curves the program pack "Origin 8.5" was implemented.

Correlating analysis of the amplitude and rate distribution of the recorded signals under the influence of different combined hormones (correlation coefficient – R) and statistical procession of the results by using White's non-parametric criteria were conducted [*Ivanov Yu, Pogoreliuk O, 1990*]. According to the mentioned criteria reliable difference of selection $p<0.05$ in $W>1.5$; $p<0.02$ in $W>3.5$; $p<0.01$ in $W>5.5$. For R value there is a high correlation between the selections if $R=0.75$-1.00, average - $R=0.4$-0.75 and low/absence - $R\leq0.4$.

Results

In the control series of the experiments the contraction amplitude and rate of the frog's isolated heart located in the incubation Ringer solution significantly decreased: at the 10th and 40th minutes

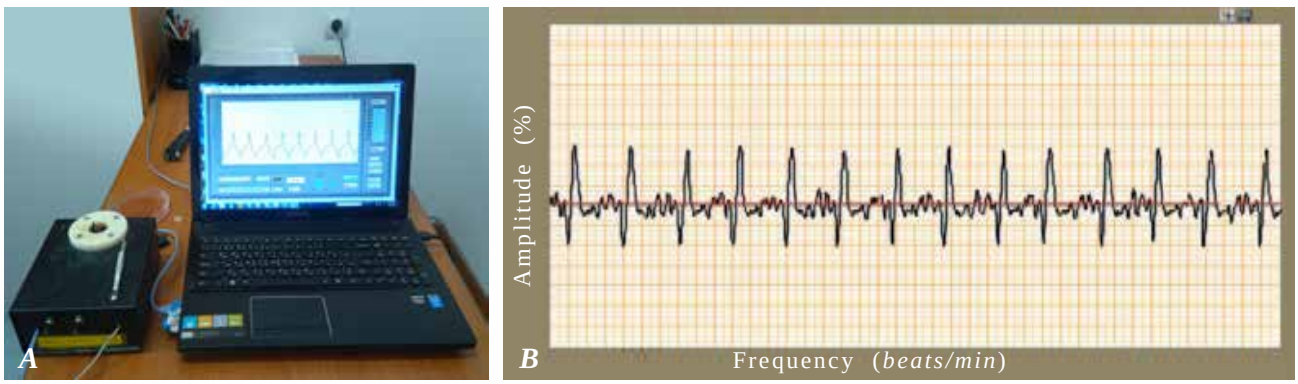

Figure 1. *Device for investigating the contractile activity of the frog's isolated heart*
A – General shape of the device, B – an example of recording the contractile activity of the frog's isolated heart.

the average amplitude of the heart contraction correspondingly was 30% and 23% of the initial level (Fig. 2A) and the rate of heart contractions was 35 и 20 beats/min (Fig 2B). The maximal duration of the experiments thanks to which was judged about the viability the heart was within 20-40 minutes.

When 10^{-10}M of 1-34 PTH active fragment was introduced into the incubation field (2nd series) a tendency to the average heart contraction amplitude increase was noted, composing 110% (at the 10th minute) and 120% (at the 40th minute) which exceeded the control indices by 90% and 97% correspondingly (fig.3A). At the same time was noted growth of pacemaker activity of the heart composing on average 50 and 63beats/min (Fig.3B). The data obtained allow to testify to the positive chronotropic and inotropic-stabilizing effects of PTH.

The introduction of 10^{-6}M estradiol into the incubation field brought to a more expressed positive inotropic-chronotropic effect compared to PTH. At the 10th minute of the experiment the average amplitude of the heart contraction was 180% and at the 40th minute – 220%. By the reliable degree $p<0.02$ indices exceeded the control level by 160% and 200%, and 1-34 PTH – by 70% and 100% correspondingly (Fig3A, 4A, Table).

The heart contraction rhythm frequency observed under the influence of β-estradiol was also significant – 65and 90b/min (Fig.3B and 4B). The heart viability was on average 50-60 minutes and in some experiments even longer. The degree of the indices' reliability during the 10 minutes was $p<0.05$, and during the subsequent time intervals – $p<0.02$ (Table).

However, more interesting were the results of combined influence of estradiol with 1-34 active fragment of PTH. The following was revealed: sharply expressed positive inotropic effect, particularly of estradiol, was amended under the influence of 1-34PTH. In this connection, the positive

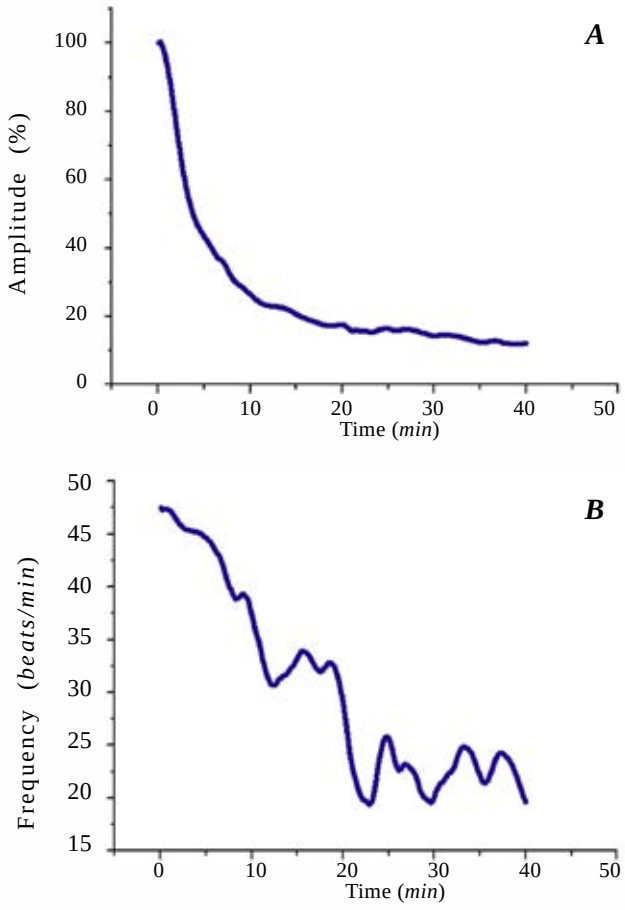

FIGURE 2. *Control. Dynamics of average amplitude (by % of the control) (A) and rate changes of the heart contraction (frequence beats/min) (B) in the incubation Ringer solution*

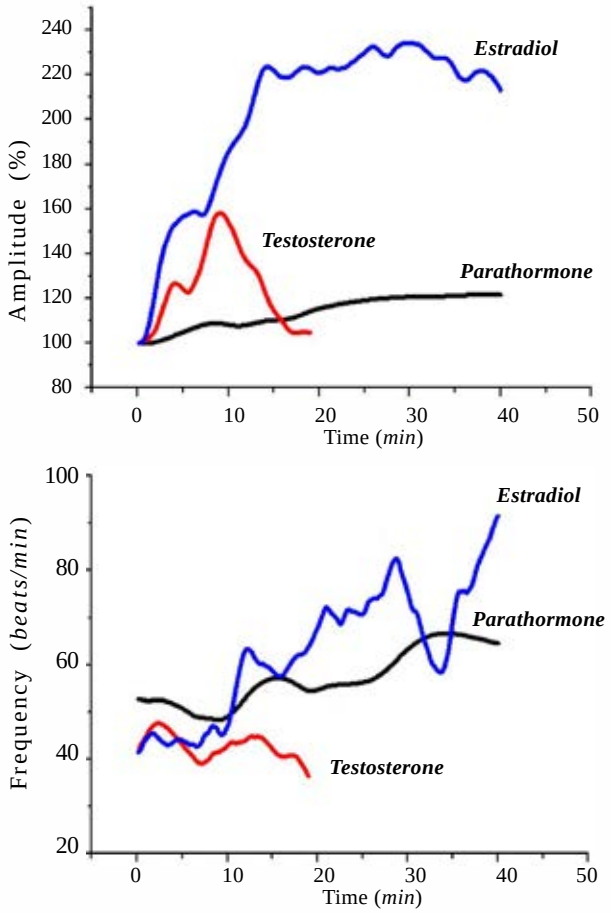

FIGURE 3. *Dynamic changes of the average amplitude (by% of control) (A) and rate (frequence, beats/min) (B) of heart contractions under the influence of PTH, estradiol and testosterone*

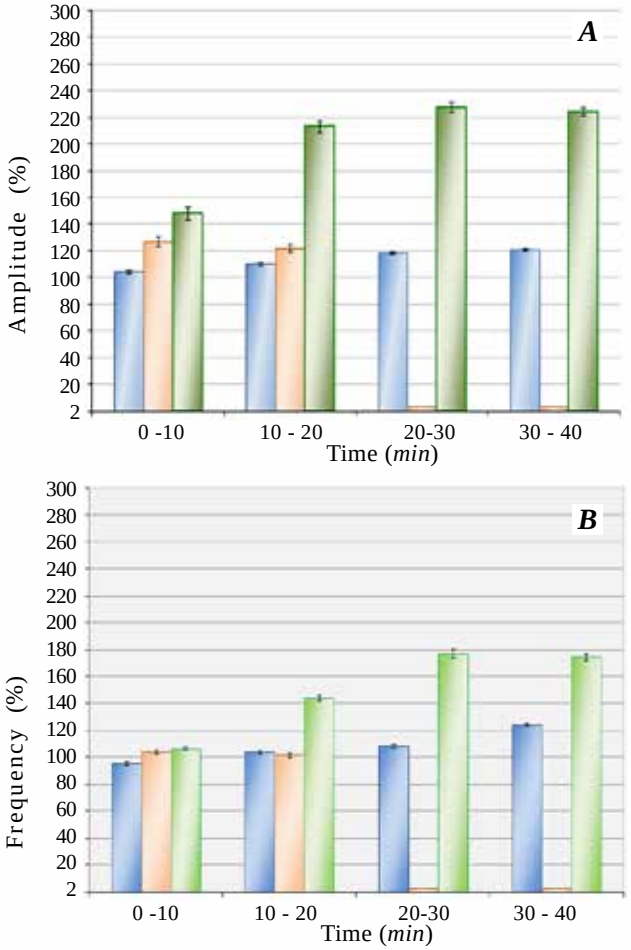

Figure 4. *Histogram of the average amplitude (A) and frequency (B) changes of the heart contractions (by percentage compared to the control) under the influence of parathormone, estradiol and testosterone during different time intervals*

Notes: *Blue column - PTH, yellow - testosterone; green- estradiol*

inotropic shift observed in combined influence of the mentioned hormones, was significantly inferior to the independent effect of estradiol. Let's compare: the index at the 10th minute was only 150% by the reliability $p<0.02$ degree, and at the 40th – 130%. To speak figuratively, so-called ("phenomenon of enraged heart pacification" occurred. (Fig.5, Table).

This phenomenon is likely to be the consequence of the modulating influence of 1-34 active fragment of PTH on the heart activity which is expressed under the pressure of excessive activation of the heart contractions caused by estradiol, though PTH itself, as mentioned above, also possesses positive chronotropic and inotropic- stabilizing effect. The mechanism, lying on the basis of that effect, is related to the calcium homeostasis regulation in myocytes. It is well-known that PTH regulates calcium homeostasis in cells [*Kostyuk P et al., 1990; 1992*]: e.g. in case of calcium surplus in neurons' cytoplasm the hormone limits and, on the contrary, in case of its deficiency it increases its entering into the cell [*Ter-Markosyan A, 1996; Khudaverdyan D, Ter-Markosyan A, 1998; Ter-Markosyan A, Khudaverdyan D, 1992; 1998; 2000*]. A tendency of the analogous modulating effect of 1-34 PTH was observed in the dynamics of pacemaker potentials' frequency changes. At the 10th and 40th minutes the indices were 25 and 42 *b/m* (Fig.4B and 5B) correspondingly whereas the data of the estradiol influence itself in the mentioned terms were much higher (Fig.3B). Besides, the estradiol's own chronotropic influence was accompanied by expressed arrhythmia which, however, was somehow smoothed over

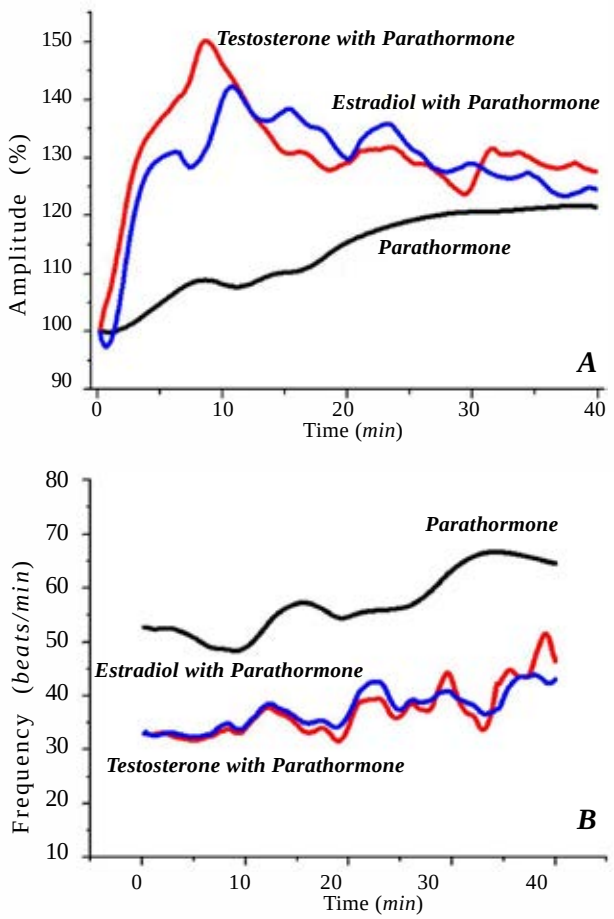

Figure 5. *Dynamics of the changes of the average amplitude (in % from the control) (A) and frequency (beats/min) (B) of the heart contraction under the influence of parathormone, combined influence of parathormone with testosterone and parathormone with estradiol.*

Table

Comparative indices of nonparametric criteria of White (W) and correlation R for the amplitude and frequency of the heart contraction under the influence of PTH and estradiol, PTH and testosterone, PTH and testosterone/estradiol

	Amplitude (%)				Frequency (%)			
	Time intervals in minutes for analysis (*min*)				Time intervals in minutes for analysis (*min*)			
	0-10	10-20	20-30	30-40	0-10	10-20	20-30	30-40
PTH, estradiol								
W	4.3	5.0	5.2	5.1	2.3	3.9	4.5	5.2
P	<0.02	<0.02	<0.02	<0.02	<0.05	<0.02	<0.02	<0.02
R	0.89*	0.73*	0.92*	-0.91	-0.41	0.47	0.89*	-0.53
PTH, testosterone								
W	3.7	4.3	-	-	3.8	4.4	-	-
P	<0.02	<0.02			<0.02	<0.02		
R	0,92*	-0.89	-	-	0.85*	-0.3	-	-
PTH+testosterone, PTH+estradiol								
W	2,5	2,1	1.9	4.7	1,4	2,1	2.2	1.04
P	<0.05	<0.05	<0.05	<0.02	>0.05	<0.02	<0.02	>0.05
R	0.95*	0.82*	0.87*	0.3	0.84*	0.95*	0.63	0.83*

***Note:** * Indices with high degree of correlation are marked*

by combining it with 1-34 PTH. The influence of 10^{-8} M testosterone on the heart activity was expressed by the increase of the heart contraction amplitude as well. At the 10th minute the index was 160% with p<0.02 degree reliability (Table) vs 30% in the control and 107%- under the PTH influence (Fig.3A). However, after already 20 minutes was observed sudden cardiac arrest. The heart contraction rate index changed in the following way: maximal shift – 50*b/min* (p<0.02) was recorded soon after the testosterone introduction, but at the 10th minute it went back to the initial value- 40*b/min* with further tendency to decrease and cardiac arrest – at the 20th minute (Fig.3, table).

The results of the experiments of the combined influence of testosterone with 1-34 fragment of PTH on the pacemaker and contractile activity of the heart are also of special attention. Although it was revealed that the contraction amplitudes observed at the 10th minute didn't differ from the testosterones' own effect, the heart functioning in these conditions became longer up to 50-60 minutes (Fig. 5, 6). The data obtained testify not only to the modulating, but also to the cardio-protecting effect of 1-34 PTH. Pacemaker potential rate, despite their arrhythmic oscillation, was within 32-35 b/min both at the 10th and 40th minutes, practically coinciding with the combined effect of PTH and estradiol (Fig. 5B, 6B). The reliability degree of the heart contraction amplitude and frequency indices was p <0.02 (Table).

On the whole, when analyzing the graphs and histograms presented in figures 5 and 6 we should note the identity and unidirectional of the changes of the heart activity functional parameters in combined influence of 1-34 PTH with estradiol or testosterone both in time and in character which once more proves the modulating and protecting effects of 1-34 active fragment of PTH on the heart activity.

Discussion

The data obtained have revealed a significant activation of the pacemaker rhythm and contractile ability of the heart under the influence of β-estradiol. The effect of calcium metabolism intensification by estradiol is shown in the works [*Zhou H et al., 2001*].

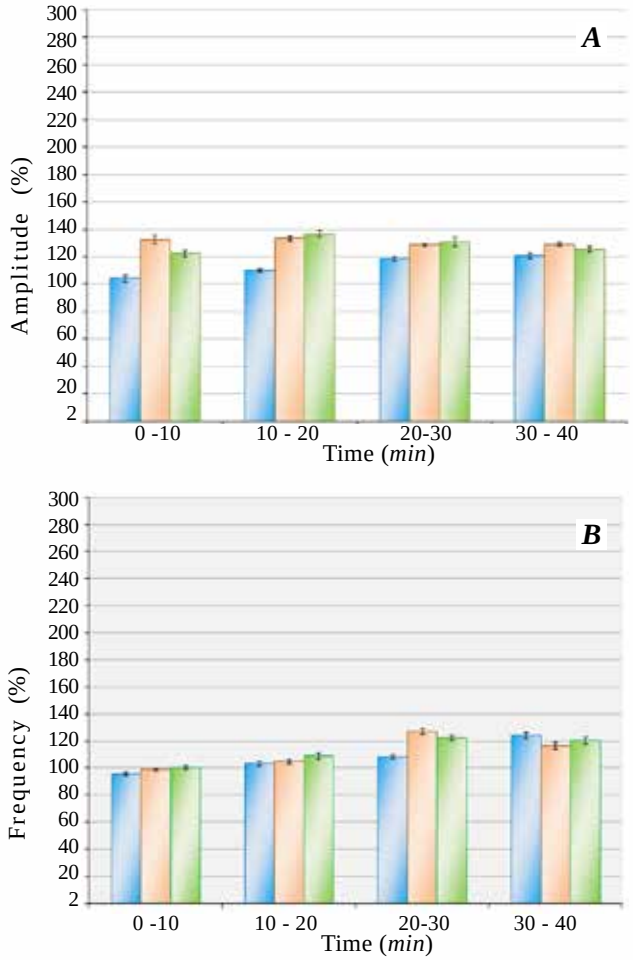

***FIGURE 6**. Histograms of heart contraction amplitude (A) and frequency (B) changes (in percentage compared to the control) in combined influence of parathormone with estradiol/testosterone during different time intervals*

***Notes:** Blue column - PTH, , yellow - testosterone; green- estradiol*

Miao Q et al., 2012]. Its positive effect on the regulation of calcium in the bone matrix in the development of postmenopausal osteoporosis, mediated by PTH and PTH-rR-dependent mechanism was noted [Greenberg C et al., 1987]. Earlier we have also mentioned that the shift of total and ionized calcium, phosphorus, sodium and potassium concentrations, as well as PTH and PTH-rP in blood plasma in men and women suffering from heart failure of I-IV group according to NYHA (New York Heart Academy) classification was not the same. Mainly, PTH and PTH-rP level in men increased only in the III and IV groups, and in women already in I group reaching the peak in the IV group [*Arakelyan K et al., 2007*]. When comparing the data obtained by us with those in the literature [*Manson J et al., 2007; Clarkson T, 2007; Cao X et al., 2015; Sivasinprasasn A et al., 2016;*], we can suppose that the cardio-protective effect of estradiol is realized by the calcium-dependent mechanism which can have a key importance for preventing cardiac failure development. In the present investigation we have revealed that when combining estradiol with 1-34 PTH the latter modulates the positive inotropic and chronotropic effects of estradiol, thus, preventing sharp shifts of the mentioned indices ("phenomenon of the enraged heart pacification"). The smoothing effect of PTH is particularly manifested on the heart activity rate index, which is likely to be related to its influence on the calcium current of the cell [*Ter-Markosyan A, 1996; Khudaverdyan D, Ter-Markosyan A 1998; 2000; Ter-Markosyan A, Khudaverdyan D, 1992; 1998*].1-34 active fragment of PTH displays not only modulating, but also protecting effect toward testosterone, expressed by intensification and long-lasting support of the heart activity.

CONCLUSION

On the base of the obtained data we can suppose that female sex hormones in a certain way interacting with the calcium-regulating hormonal system, mainly, 1-34 PTH, have a cardio-tropic effect on the heart. At the same time, in arrhythmic changes of the heart activity caused by testosterone, PTH itself has a modulating and cardio-protecting effect.

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