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Research Article

Influence of experimental heart failure therapy with different generations of β-adrenergic blockers on Cardiac Electrical Activity (ECG) and Autonomic Regulation of Heart Rhythm (ARHR)

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Abstract

Abstract: In the complex treatment of chronic heart failure (CHF) β-adrenoblockers (carvedilol, nebivolol, bisoprolol, metoprolol) are used, which due to their pharmacological properties increase the survival rate of patients, improve cardio- and haemodynamic parameters, metabolism. However, modern realities in medicine require creation of new more effective and safe β-adrenoblockers. In this regard, the potential drug Hypertirl is of interest. **The aim** was to evaluate the cardioprotective effect of 1-(b-phenylethyl)-4-amino-1,2,4-triazolium bromide (Hypertril) on cardiac electrical activity and autonomic regulation of heart rhythm in a model of CHF in comparison with β-adrenoblockers of different generations (Nebivolol, Carvedilol, Bisoprolol, Metoprolol).

Materials and methods: Chronic heart failure was induced by 14-day administration of doxorubicin in a cumulative dose of 15 mg/kg to white mongrel rats weighing 190–220g (total 85). The investigated drugs were administered after doxorubicin course for 30 days: Hypertril at an experimentally substantiated dose of 3.5 mg/kg, Metoprolol succinate 15 mg/kg, Nebivolol 10 mg/kg, Carvedilol 50 mg/kg, Bisoprolol 10 mg/kg. At the end of drug administration under thiopental anaesthesia (40 mg/kg), electrocardiogram (ECG) and autonomic regulation of heart rhythm (ARHR) were analysed using a computer analyser CardioCom-2000plus (KAI-Medica, Ukraine). The results of the study were calculated using a standard statistical package "STATISTICA for Windows 6.0" (StatSoftInc., №AXXR712D833214FAN5), "SPSS 16.0" and "Microsoft Office Excell 2003".

Results and discussion: Hypertril administration resulted in a negative chronotropic effect, normalisation of atrial (P) and ventricular (R) spike amplitude, ST segment inversion below isoline, increased amplitude of ventricular myocardial repolarisation T. Myocardial repolarisation spike were observed, as well as normalised the duration of atrial (P) and ventricular depolarisation phase (QRS complex) to the intact value and restored the duration of electrical diastole (TR interval). Hypertril reduced systolic and diastolic myocardial dysfunction in animals with CHF. Hypertril restored autonomic mechanisms of heart rhythm regulation and balanced activity of sympathetic and parasympathetic parts of autonomic nervous system in the control of cardiac function.

Conclusion: The obtained results demonstrated the undoubted advantage of the new original molecule (Hypertril) over basic β-adrenoblockers (Metoprolol, Nebivolol, Carvedilol and Bisoprolol) and experimentally justify further in-depth study to create on its basis a drug for the treatment of CHF.

Keywords

chronic heart failure, cardiac bioelectrical activity, β-adrenoblockers

Introduction

Chronic heart failure (CHF) is one of the key clinical and economic problems for the healthcare systems of most developed countries in the world. It is characterized by a high prevalence and an unfavorable prognosis (Bozkurt et al. 2021). The overall mortality rate in CHF varies depending on the severity of the condition, ranging from 15% to 50%. The annual mortality rate for patients in functional classes III and IV reaches 40% and more than 60%, respectively, with sudden death rates accounting for 50% or more of the total mortality (Urbich et al. 2020). In the complex treatment of CHF, β-adrenoblockers are used, which reduce the sympatho-adrenal load on the heart, reduce myocardial oxygen demand, inhibit the hyperproduction of reactive oxygen species, protect cardiomyocyte membranes (Konyakhin 2009). This class of drugs improves the survival rate of patients, effectively increase the ejection fraction, reduce the mass and sphericity of the left ventricle of the heart, slow down the reversion of cardiac remodelling (Wenningmann et al. 2019). Despite the fact that a number of β-adrenoblockers have been described to have cardioprotective, anti-ischaemic and antioxidant properties, the molecular and cellular mechanisms of these effects have not been completely revealed (Bien et al. 2007). Such β-adrenoblockers as metoprolol, carvedilol, bisoprolol and nebivolol have found clinical application in CHF. From the point of view of evidence-based medicine, the efficacy in CHF is confirmed only for some representatives of this class of drugs. In addition, the presence of adverse reactions β-adrenoblockers limit their widespread use in the clinic (Watanabe et al. 2001). In this context, the development of new drugs with selective β_1-blocking action and the determination of the choice of different forms of these drugs in the comprehensive treatment of CHF is of scientific interest. As a result of targeted research, a compound (1-(b-phenylethyl)-4-amino-1,2,4-triazolium bromide) was created, which is called "Hypertril". It exhibits β_1-blocking, vasodilatory, antihypertensive, and cardioprotective effects and belongs to the IV class of toxicity (LD50 is 683.4 mg/kg for intragastric administration in rats) (Mazur et al. 2019). There are several groups of β-adrenoblockers depending on selectivity to adrenoreceptors, affinity of binding to receptors, etc (Mazur et al. 2010).

The Scientific and Production Association "Farmatron," together with the Scientific and Technological Complex "Institute of Single Crystals" of the National Academy of Sciences of Ukraine, has developed methods for the synthesis and standardization of the substance "Hypertril" and the technology of parenteral solutions (Certificate No. 2, Series 020213). According to the decision of the State Expert Center of the Ministry of Health of Ukraine, the first phase has been successfully completed, and the second phase of clinical trials for the injection solution of Hypertril is underway. The aforementioned progress determines the prospects for studying oral forms of Hypertril as a promising agent in the comprehensive treatment of CHF.

The aim of the research

To evaluate the cardioprotective effect of 1-(b-phenylethyl)-4-amino-1,2,4-triazolium bromide (Hypertril) on cardiac electrical activity and autonomic regulation of heart rhythm in the model of CHF in comparison with β-adrenoblockers of different generations (Nebivolol, Carvedilol, Bisoprolol and Metoprolol).

Materials and methods

Animals

The experiments were carried out on 85 white outbred rats weighing 190–220 g, obtained from the vivarium of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine and the Institute of Physiology. A.A. Bogomolets of the Academy of Medical Sciences of Ukraine. The duration of the quarantine (acclimatization period) for all animals was 14 days. During quarantine, each animal was examined daily (behavior and general condition), animals were observed twice a day in cages (morbidity and mortality). Before the start of the study, animals that met the inclusion criteria in the experiment were divided into groups using the randomization method. Throughout the experiment, the animals were observed, death was recorded, and their appearance was described. All manipulations were carried out in accordance with the provisions on the collection of animals for biomedical experiments (Strasbourg 1986, as amended in 1998) and the "European Applicable Protection of Vertebrate Animals used for Experimental and Scientific Purposes". The protocols of experimental studies and their results were approved by the decision of the Commission on Bioethics of ZSMU (Protocol No. 3 dated March 22, 2021).

Experimental model

Doxorubicin model was used to reproduce chronic heart failure (Khloponin 2009). The doxorubicin pharmacological model of CHF could be considered as the most effective, leading to the development of severe and progressive CHF in most animals. The use of doxorubicin (intraperitoneally

at a cumulative dose of 15 mg/kg, divided into 6 injections for 14 days) leads to a decrease in left ventricular myocardial contractility, its eccentric remodeling, and the formation of progressive CHF in rats (Khloponin 2009; Ryzhov et al. 2017; Belenichev et al. 2023).

Drugs and pharmacological agents

The study used Doxorubicin "Ebeve" 50 mg/25 ml (EBEWE Pharma Ges.mbH Nfg. KG, Austria. All preparations were administered intragastrically once a day in the form of a suspension of 1% starch mucus for 30 days after a 14-day administration of doxorubicin – Hypertril at an experimentally substantiated dose of 3.5 mg/kg (Mazur et al. 2019), Metoprolol succinate – 15 mg/kg (Chekman et al. 2013; Sidorov 2013), Nebivolol 10 mg/kg (Cosentino et al. 2002), Carvedilol 50 mg/kg (Chen and Hong 2016), Bisoprolol 10 mg/kg (Watanabe et al. 2003). In a 45-day survival study involving rats, the intact group demonstrated a perfect survival rate, with all 10 rats completing the study successfully. In contrast, the control group displayed a lower survival rate, with only 6 out of 20 rats surviving. However, when was treated with CHF+Hypertril, the survival rate significantly improved to 95%, as 19 out of 20 rats survived. Among the treatment groups, those receiving CHF in combination with Metoprolol succinate and Bisoprolol both exhibited promising results, with 12 out of 20 rats surviving in each group. The CHF+Carvedilol group demonstrated slightly lower survival rates, with 10 out of 20 rats surviving, while the CHF+Nebivalol group with 16 out of 20 rats successfully completing the study. The following substances were used in the work: Hypertril substance (Scientific and technological complex "Institute of Single Crystals" of the National Academy of Sciences of Ukraine), Metoprolol succinate tablets (Astra Zeneca UK Ltd, Sweden), Nebivolol tablets (Teva Pharmaceutical Industries, Ltd, Israel), Carvedilol tablets (Salutas Pharma GmbH, Germany), Bisoprolol tablets (Teva Pharmaceutical Industries, Ltd, Israel).

Anesthesia

At the end of the experiment, in animals under anesthesia (sodium ethaminal, 40 mg/kg), the heart was taken and weighed, and blood was taken from the abdominal artery with a syringe for biochemical studies.

Physiological methods

In all groups of animals on the 45th day of the experiment under thiopental anaesthesia (40 mg/kg) electrocardiogram (ECG) and autonomic regulation of heart rhythm (ARHR) were analysed using a computer analyser Cardio-Com-2000plus (KAI-medica, Ukraine).

When analysing ECG, the parameters of clips and intervals were calculated as the average value of their amplitude or duration module, respectively, in all recorded leads.

In addition, ECG in the 2nd standard lead was recorded for 5 minutes (at least 1000 cardio intervals) to study autonomic regulation of heart rhythm (ARHR) The method allows to estimate the functional state of activity of sympathetic (adrenergic) and parasympathetic (cholinergic) departments of the autonomic nervous system on the sinus node (Kolesnik et al. 2014). The determined heart rate variability (HRV) parameters were:

1) heart rate (HR, min-1);
2) mode value (Mo, sec) – duration of RR interval, which occurs more frequently in the studied electrocardiogram segment;
3) mode amplitude (AMo, %) – the percentage of cardiac intervals that correspond to the mode value in the total sample;
4) variation range of cardiac intervals (ΔX, sec) – the difference between the highest and the lowest values of RR duration in the sample;
5) SI – stress index (Baevsky index, stress-index), which reflects the stress of regulatory mechanisms in heart rhythm control and is determined by the formula: SI =AMo/(2-ΔX-Mo);
6) APR (Adequacy of Processes Regulation) – the index of adequacy of regulation processes, which reflects the correspondence between the activity of sympathetic link of autonomic nervous system and the level of sinus node functioning, is determined by the formula: APR =AMo/Mo;
7) TP (total power) or PSD (power spectral density), ms2 – total level of activity of regulatory systems, characterises not only periodic, but also non-periodic processes; increase of the index is considered as a manifestation of growth of activity of autonomous circuit of heart rhythm regulation, and decrease is considered as a sign of suppression of activity of autonomous circuit. We used the discrete Fourier transform methods for HRV analysis and PSD measurement. Typically bands of HRV spectral analysis are VLF (the very-low-frequency spectral band), LF (the low-frequency spectral band), HF (the high- frequency spectral band), and PSD as the sum of VLF, LF, and HF bands;
8) ULF, ms2 – power of ultra low-frequency range of the spectrum, characterises the state of electrolyte-metabolic level of autonomic regulation of heart rhythm, partially connected with thermoregulation processes;
9) VLF, ms2 – power of very low-frequency spectrum range, characterises the state of energy-metabolic level of vegetative regulation of heart rhythm, which implies the severity of ergotropic influences of suprasegmental department of vegetative nervous system;
10) LF, ms2 – power of low-frequency spectrum range, is a marker of sympathetic influences and/or sympathetic and parasympathetic mechanisms conjugated with baroreflex regulation of heart

rhythm; characterises absolute level of sympathetic vasomotor centre activity, reflects involvement in regulation of intrasystem level of control;

11) HF, ms2 – power of high-frequency spectrum, reflects periodic strengthening of tonic activity of the vagus nerve (parasympathetic influences) on the heart, synchronised with the rhythm of respiratory movements;
12) LF/HF index of vagosympathetic interaction power spectrum, characterises the relative predominance of subcortical sympathetic nerve centre activity over the activity of parasympathetic channel of heart rhythm regulation;
13) IC – centralisation index, characterises the relative predominance of the central circuit of regulation over the autonomous circuit, reflects the degree of centralisation of cardiac rhythm control of heart rhythm, is determined by the formula: IC = (LF+ VLF) / HF;
14) M±m – mean ± S.E.M. (standard error of the mean).

All calculations were carried out using the original computer programme.

Statistical methods

The results of the study were calculated using a standard statistical package "STATISTICA for Windows 6.0" (StatSoftInc., №AXXR712D833214FAN5), "SPSS 16.0" and "Microsoft Office Excell 2003". To determine the presence and nature of the relationship between numerical variables, a regression analysis procedure was used using linear, logarithmic, power, exponential, polynomial (second and third degree) models, achieving an independent (according to the Durbin-Watson criterion), normal distribution of residuals (at the same time as skewness and kurtosis values were used for the goodness of fit criterion. Distribution normality was assessed using the Shapiro-Wilk test. The data were presented as an average value. The significance of negativity between the mean values was determined by Student's t-test (in the case of a normal distribution). The Mann-Whitney U-test was used in the case of a distribution that is negative compared to normal or analysis of ordinal variables. To compare independent variables in more than two samples, analysis of variance (ANOVA) was used with a normal distribution or the Kruskal-Wallis test for a distribution that was negative from normal.

Results

The analysis of myocardial electrical activity in the control group of rats under light anesthesia, recorded in the second standard lead, exhibited a regular heart rhythm. The R wave showed higher amplitude compared to other ECG waveforms, and the ST segment was slightly below the baseline (-0.006±0.009 mV). In terms of ECG structure (Table 1), the duration of the atrial electrical systole (P wave) accounted for 15.111±0.209%, the ventricular systole (QRS complex) was 18.182±0.419%, and the

Table 1. Effect of drugs on ECG characteristics in standard leads (M±m).

ECG parameters	Research series			
	Intact(1)	CHF(2)	CHF + Metoprolol(3)	CHF + Hypertril(4)
HR, min-1	378±4	497±6[1,3,4,5,6,7]	437±6[1,2,4]	400±2[1,2,3,5,6,7]
		Amplitude, mV		
P	0.048±0.004	0.019±0.005[1,4,5,6,7]	0.032±0.003[1,4,6,7]	0.061±0.004[2,3,5]
R	0.371±0.027	0.202±0.013[1,4,5,6,7]	0.192±0.01[1,4,5,6,7]	0.359±0.033[2,3]
T	0.114±0.009	0.066±0.015[1,3,4,5,6,7]	0.059±0.011[1,4,5,6,7]	0.148±0.010[1,2,3]
ST	-0.006±0.009	0.103±0.008[1,3,4,5,6,7]	0.029±0.011[1,2,4,6,7]	-0.013±0.008[2,3]
		Cardiac cycle duration, ms		
RR	159.9±1.7	122.5±1.6[1,3,4,5,6,7]	137.1±1.8[1,2,4]	149.4±1.0[1,2,3]
	CHF(2)	**CHF + Bisoprolol(5)**	**CHF + Nebivolol(6)**	**CHF + Carvedilol(7)**
HR, min-1	497±6[1,3,4,5,6,7]	425±9[1,2,4]	431±10[1,2,4]	424±10[1,2,4]
		Amplitude, mV		
P	0.019±0.005[1,4,5,6,7]	0.044±0.005[2,4]	0.055±0.008[2,3]	0.064±0.010[2,3]
R	0.202±0.013[1,4,5,6,7]	0.359±0.033[2,3]	0.398±0.033[2,3]	0.318±0.040[2,3]
T	0.066±0.015[1,3,4,5,6,7]	0.148±0.010[1,2,3,4]	0.152±0.025[2,3]	0.165±0.022[1,2,3]
ST	0.103±0.008[1,3,4,5,6,7]	-0.022±0.034[2]	-0.021±0.021[2,3]	-0.058±0.039[2,3]
		Cardiac cycle duration, ms		
RR	122.5±1.6[1,3,4,5,6,7]	145.4±4.4[1,2]	142.9±3.1[1,2]	143.3±4.4[1,2]
SDNN	1.547±0.192[1]	1.726±0.181	1.800±0.245	1.957±0.223
		Interval duration, % of cardiac cycle duration RR		
P	17.956±0.364[1,3,4,5,6,7]	12.282±0.827[1,2,4]	13.186±0.316[1,2,4]	13.209±0.729[1,2]
PQ	8.538±0.440[3,4,5,6,7]	12.196±0.935[1,2,4]	11.005±0.994[2,4]	11.046±0.756[1,2,4]
QRS	21.520±0.642[1,3,4,5,7]	14.317±0.943[1,2,3,4,6]	22.917±0.557[1,2,3,4,5,7]	16.626±0.826[2,3,6]
T	30.280±0.967[1,3,4,5,6,7]	22.724±1.291[2,3,4,6]	17.685±0.816[1,3,4,5,7]	22.952±1.183[2,3,4,6]
TP	21.704±1.240[1,4,5,6,7]	38.478±1.685[1,2,3,4]	35.204±1.623[2,3]	36.164±1.579[2,3]

Note: reliability of changes PST<0,05 in relation to intact animals ([1]), animals with CHF ([2]), during treatment with Metoprolol ([3]) and Hypertril ([4]), Bisoprolol ([5]), Nebivolol ([6]), Carvedilol ([7]).

electrical diastole (TR interval) was 32.886±1.536% of the cardiac cycle duration. The heart rate of individual animals ranged from 317 to 451 beats per minute, with an average of 378±4 beats per minute, indicating normal cardiac function in resting conditions for rats. Hence, the effect of light anesthesia had minimal impact on heart function.

The induction of heart failure in rats resulted in an increased heart rate by 31.5% and a shortened RR interval (duration of the cardiac cycle) by 23.8% compared to the control group. ECG recordings of animals with heart failure exhibited a 59% reduction in the amplitude of P waves, as well as more than 40% reduction in R and T wave amplitudes, indicating a decreased contractile function of the myocardium (inotropic dysregulation) and a potential decrease in stroke volume. In terms of ECG structure (Table 1), the duration of the atrial systole (P wave) and ventricular systole (QRS complex) significantly increased by approximately 18% ($p<0.05$), accompanied by a 22% prolongation ($p<0.001$) of the ventricular repolarization phase (T wave). These findings likely indicate moderate conduction slowing in the atria and ventricles, as well as delayed depolarization and repolarization processes in the myocardial cells under the development of experimental heart failure. The absence of changes in the PQ interval ($p=0.5$) indicated preserved dromotropic function of the atrioventricular connection. However, the duration of the electrical diastole (TR interval) decreased by almost 34% ($p<0.001$). Considering that the diastolic interval is crucial for adequate intracardiac hemodynamics and resynthesis of macroergic molecules in myocardial cells, its shortening exacerbates myocardial energy deficit and impairs cardiac function. An elevation of the ST segment above the baseline by 0.103±0.008% was also observed, suggesting the occurrence of myocardial ischemia processes. Therefore, based on the ECG data, the utilized heart failure model leads to ischemic myocardial damage and the development of persistent systolic and diastolic dysfunction.

The development of CHF in experimental animals was also accompanied by dysregulation of vegetative function of the heart, which were accompanied by a significant, about 4-fold, decrease in spectral power of heart rhythm TPW with a 5-fold increase in stress index SI. The absolute power of high-frequency component HF, which characterizes the influence of parasympathetic regulatory component on HRV, decreased nearly 3-fold and the absolute power of low-frequency component LF, which characterizes the influence of sympathetic regulatory component on HRV, decreased 2.5-fold. At the same time, the balance of sympathetic and parasympathetic regulation LF/HF decreased in 2 times (PST<0,001) on a background of the decrease of spectral power of heart rhythm. The obtained data testify to the fact that CHF development in experimental animals was accompanied by compensatory predominance of parasympathetic regulation of cardiac cycle by vagus nerve in response to tachycardia and decreased spectral power of ARHR (Table 2).

Thus, the complex of revealed changes demonstrates the disturbance of cardiac electrical work (according to ECG – disturbance of automaticity, conduction and contractility) and neurogenic mechanisms of its regulation (according to HRV – decentralization of neurogenic regulation circuits, reduction of spectrum power and increase of stress index) can in this case be considered as a manifestation of systolic and diastolic dysfunction in animals with experimental CHF.

Table 2. Effect of drugs on spectral characteristics of autonomic regulation of heart rhythm (ARHR) (M±m).

ARHR indicators	Research series			
	Intact (1)	**CHF (2)**	**CHF + Metoprolol (3)**	**CHF + Hypertril (4)**
PSD, ms^2	4.007±0.495	0.984±0.185 [1,4,5]	1.585±0.301 [1,4]	2.920±0.395 [2,3,5,6,7]
ULF, ms^2	2.057±0.343	0.373±0.074 [1,4]	0.664±0.180 [1]	1.283±0.266 [2,5,6,7]
VLF, ms^2	1.565±0.168	0.615±0.167 [1,4]	0.721±0.130 [1,4]	1.333±0.154 [2,3]
LF, ms^2	1.353±0.243	0.528±0.126 [1,4]	0.549±0.182 [1,4]	1.623±0.264 [2,3,5,6,7]
HF, ms^2	0.572±0.097	0.203±0.037 [1,4]	0.329±0.075 [4,5,6,7]	0.663±0.099 [2,3,5,6,7]
LF norm, %	70.884±1.318	50.984±4.370 [1,4,5,6,7]	60.878±4.862 [4,5,6]	71.441±0.684 [2,3,5]
HF norm, %	29.115±1.318	49.015±4.370 [1,4,5,6,7]	39.121±4.862 [4,5,6]	28.558±0.684 [2,3,5]
LF/HF (norm)	2.624±0.176	1.404±0.240 [1,4,5,6,7]	2.002±0.284 [5,6,7]	2.545±0.080 [2,5]
	CHF (2)	**CHF + Bisoprolol (5)**	**CHF + Nebivolol (6)**	**CHF + Carvedilol (7)**
PSD, ms^2	0.984±0.185 [1,4,5]	1.785±0.341 [1,2,4]	1.150±0.177 [1,4]	1.328±0.15 [1,4]
ULF, ms^2	0.373±0.074 [1,4]	0.442±0.112 [1,4]	0.420±0.141 [1,4]	0.464±0.129 [1,4]
VLF, ms^2	0.615±0.167 [1,4]	0.978±0.231 [1]	0.970±0.326	1.114±0.299
LF, ms^2	0.528±0.126 [1,4]	0.387±0.044 [1,4]	0.310±0.070 [1,4]	0.300±0.057 [1,4]
HF, ms^2	0.203±0.037 [1,4]	0.139±0.011 [1,3,4]	0.150±0.016 [1,3,4]	0.128±0.012 [1,3,4]
LF norm, %	50.984±4.370 [1,4,5,6,7]	74.328±0.262 [1,2,3,4]	72.390±2.414 [2,3]	72.450±2.927 [2]
HF norm, %	49.015±4.370 [1,4,5,6,7]	25.671±0.262 [1,2,3,4]	27.610±2.414 [2,3]	27.550±2.927 [2]
LF/HF	1.404±0.240 [1,4,5,6,7]	2.884±0.038 [2,3,4]	2.820±0.262 [2,3]	3.200±0.487 [2,3]
IC	0.231±0.043 [6]	0.350±0.059 [3,4]	0.630±0.172 [1,2,3,4]	0.457±0.125 [3]
SI	42154±4752 [1,4,5,6,7]	19144±803 [1,2,3,4]	19477±3648 [1,2]	20537±608 [1,2,3,4]
APR	739±43 [1,4,5,6]	501±27 [2,3,7]	537±43 [2,3]	624±40 [3,5]

Note: reliability of changes PST<0,05 in relation to intact animals ([1]), animals with CHF ([2]), during treatment with Metoprolol ([3]) and Hypertril ([4]), Bisoprolol ([5]), Nebivolol ([6]), Carvedilol ([7]).

Table 3. Ranking of β-adrenoblockers of different generations (Carvedilol, Bisoprolol, Nebivolol, Metoprolol, Hypertril) by efficacy in experimental CHF.

Indicator ranks	Intact	CHF	Metoprolol	Hypertril	Bisoprolol	Nebivolol	Carvedilol
Chronotropic function of the myocardium	3	1	1.5	2	1.5	1.5	1.5
Myocardial inotropic function	3.5	1	1.5	4	3.5	3.5	4
Autonomic regulation of heart rhythm	3.5	1	1.5	3.5	2	2	2

The administration of Metoprolol to animals with heart failure resulted in a negative chronotropic effect, reducing heart rate by 12% (P_{ST}<0.001). However, this value remained 16% higher (P_{ST}<0.001) compared to animals in the intact group. In the group receiving Metoprolol, a significant decrease in the level of ST segment elevation was observed (more than 3 times compared to untreated animals), indicating potential anti-ischemic effects of the drug. There was also a slight increase in the amplitude of the P wave (depolarization of the atria), although its magnitude remained lower (P_{ST}=0.01) compared to intact rats, along with a shortening of its duration (P_{ST}<0.001) both in relation to untreated animals and intact rats. Metoprolol did not affect the amplitude of the QRS complex (ventricular depolarization), but partially normalized the duration of ventricular systole (P_{ST}=0.005 compared to animals with heart failure). Metoprolol had minimal impact on the amplitude of the T wave (myocardial repolarization) and the duration of the electrical diastole (TR interval). Therefore, Metoprolol exerted a moderate negative chronotropic effect and had a significant impact on neither systolic nor diastolic dysfunction of the heart.

Introduction of Metoprolol to animals with CHF practically did not affect the spectral power of heart rate TPW (P_{ST} >0.05) and the stress index SI (P_{ST} >0.05), as well as the centralization index IC (P_{ST} >0.05) and the index of vagosympathetic interaction LF/HF (P_{ST} >0.05). In other words, the introduction of Metoprolol to animals with HF did not have a significant impact on the balance of autonomic mechanisms regulating heart rate in experimental pathology.

The administration of Hypertril had a negative chronotropic effect on animals with HF, leading to a decrease in heart rate (HR) by 20% (P_{ST} <0.001), which was only 6% higher than in intact animals (P_{ST} <0.001). After the administration of Hypertril, the electrocardiogram (ECG) showed normalization of the amplitude of atrial (P) and ventricular (R) waves (P_{ST} >0.05), while the amplitude of the myocardial repolarization wave T increased by 30% and exceeded the values in intact rats, which could be attributed to improved myocardial energy supply and more rational use of macroergs for the contractile function of the heart. Indirect confirmation of this assumption was the inversion of the ST segment below the isoline, reaching the levels of intact animals (P_{ST}>0.05). The administration of Hypertril normalized the duration of the depolarization phase of the atria (P wave) and ventricles (QRS complex) to intact values (P_{ST} >0.05) and restored the duration of the electrical diastole (TR interval, P_{ST} >0.05). At the same time, the duration of the atrioventricular segment PQ was shortened and became 40% shorter (P_{ST} <0.001) than in control rats. The obtained data apparently indicate that experimental therapy with Hypertril significantly reduced systolic and diastolic myocardial dysfunction in animals with HF.

The administration of Hypertril to animals with HF significantly increased the spectral power of heart rate TPW by 3 times (P_{ST} <0.001 compared to untreated animals and P_{ST} >0.05 compared to intact rats) and reduced the stress index SI by 2.6 times (P_{ST} <0.001), which, nevertheless, remained 1.8 times higher than in intact animals (P_{ST} <0.001). Hypertril in animals with HF contributed to an increase in the power of sympathetic (LF) and parasympathetic (HF) regulation of HRV to the levels of the intact group and normalized the index of vagosympathetic interaction LF/HF (P_{ST} >0.05 compared to control animals). Thus, the administration of Hypertril to animals with HF indicated a significant restoration of the autonomic mechanisms regulating heart rate and a balance in the activity of the sympathetic and parasympathetic branches of the autonomic nervous system in controlling heart function.

The administration of Bisoprolol had a negative chronotropic effect on rats with HF and reduced heart rate (HR) by 15% (P_{ST} <0.001). However, HR remained 12% higher (P_{ST} <0.001) than in the intact group of animals. The ECG showed normalization of the amplitude of atrial (P) and ventricular (R) waves (P_{ST} >0.05 compared to intact animals), while the amplitude of the myocardial repolarization wave T increased and was 1.5 times higher than the values in intact rats (P_{ST} <0.02). The administration of Bisoprolol reduced the amplitude of the ST segment, which became negative, and its amplitude, although lower compared to intact animals, did not show statistically significant differences (P_{ST} >0.05). The ECG showed a shortening of the duration of the atrial depolarization wave P, ventricular depolarization complexes QRS, and repolarization wave T, as well as an increase in the duration of the electrical diastole TR (P_{ST} <0.001 compared to untreated animals with HF and P_{ST} <0.02 compared to intact animals).

The administration of Bisoprolol to animals with HF led to an increase in the spectral power of heart rate TPW by 80% (P_{ST} <0.001), which remained 65% lower in absolute values than in the intact group (P_{ST} <0.001). Bisoprolol did not affect the indices of sympathetic (LF) and parasympathetic (HF) regulation of HRV and contributed to the restoration of the index of vagosympathetic interaction LF/HF (P_{ST} >0.05 compared to intact animals). The stress index SI decreased significantly during therapy with Bisoprolol in animals with HF but remained approximately three times higher (P_{ST} <0.001) than in intact animals.

The administration of Nebivolol to rats with HF had a negative chronotropic effect, reducing heart rate (HR) by

13% (P_{ST} <0.001) while maintaining this parameter 14% higher (P_{ST}<0.001) than in the intact group of animals. Nebivolol led to the normalization of the amplitude of atrial (P) and ventricular (R) waves, as well as the repolarization wave T of the ventricular myocardium (P_{ST} >0.05 compared to intact animals) in rats with HF. However, the administration of the drug reduced the amplitude of the ST segment, which became negative, and its amplitude, although lower compared to intact animals, did not show statistical differences (P_{ST} >0.05). The administration of Nebivolol to animals with HF accelerated the depolarization time of the atria (P wave) and the ventricular repolarization complex (T), which were 13% and 29% shorter (P_{ST} <0.001), respectively, than in the intact group of rats. At the same time, the relative duration of the QRS complex did not differ from the values in untreated rats, while the time of atrioventricular conduction (PQ segment) and the duration of the electrical diastole TR were normalized to the intact level (P_{ST} >0.05).

The administration of Nebivolol to rats with HF did not affect the spectral power of heart rate TPW (P_{ST} >0.05), which remained 71% lower (P_{ST}<0.001) than in the intact group. Although the administration of Nebivolol to rats with HF did not have a statistical impact on the absolute spectral power indices of sympathetic (LF) and parasympathetic (HF) regulation of ARHR, there was an increase in the normalized power of sympathetic regulation (LF norm) and a decrease in the power of parasympathetic regulation (HF) of HRV (P_{ST} <0.001). This contributed to the normalization of the index of vagosympathetic interaction LF/HF (P_{ST} >0.05 compared to intact animals) and a significant increase in the index of centralization of regulatory systems IC (P_{ST}>0.05). The stress index SI decreased significantly during treatment with Nebivolol in animals with HF, but it remained 2.3 times higher (P_{ST} <0.002) than in intact animals.

The administration of Carvedilol had a negative chronotropic effect on rats with HF, reducing heart rate (HR) by 15% (P_{ST} <0.001) while maintaining this parameter 12% higher (P_{ST} <0.001) than in the intact group of animals. Carvedilol led to an increase in the amplitude of atrial (P) and ventricular (R) waves in animals with HF (by 3.3 times and 57%, respectively), as well as an increase in the amplitude of the myocardial repolarization wave T, which was 45% higher than in intact animals (P_{ST} <0.05). However, the administration of Carvedilol reduced the amplitude of the ST segment, which became negative, and its amplitude was comparable to that of intact animals (P_{ST} >0.05). Experimental therapy with Carvedilol contributed to the normalization of the duration of the electrical systole (QRS complex) and the repolarization time of the ventricles (T), as well as the duration of the electrical diastole of the heart (TR interval) (P_{ST}>0.05 compared to the intact group of rats). At the same time, there was a statistically significant reduction in the relative duration of the atrial electrical systole (P wave) and an increase in the atrioventricular conduction delay (PQ segment) compared to untreated (P_{ST} <0.005) and intact (P_{ST} <0.02) animals.

The administration of Carvedilol to rats with HF did not have a significant impact on the spectral power of heart rate TPW, which remained 67% lower (P_{ST} <0.001) than in the intact group and did not show statistical differences (P_{ST} >0.05) compared to untreated rats. The administration of Carvedilol to rats with HF did not statistically affect the absolute spectral power indices of sympathetic (LF) and parasympathetic (HF) regulation of HRV (P_{ST} >0.05). However, there was an increase in the normalized power of sympathetic regulation (LF norm) and a decrease in the power of parasympathetic regulation (HF) of HRV (P_{ST} <0.001), which contributed to the normalization of the index of vagosympathetic interaction LF/HF (P_{ST} >0.05 compared to intact animals). The stress index SI decreased significantly during treatment with Carvedilol in animals with HF but remained 2.4 times higher (P_{ST} <0.001) than in intact animals.

Principle: For ranking, we took the mean values of the values and calculated the modulus of the difference with the values of animals with CHF. In this case, the index in the CHF group was "0". The obtained results were sorted in ascending order, i.e., the more the indicator differed from "CHF", the higher rank it received. The sum of the ranks in each group was normalised by the total rank in the CHF group. The rank in the intact group of animals is also given for comparison.

Discussion

The β-adrenoblockers (Carvedilol, Bisoprolol, Metoprolol, Nebivolol) studied in this work are the main agents of standard therapy of heart failure (Belenichev et al. 2023), demonstrated a positive effect on ECG parameters and autonomic regulation of the heart after modelling of CHF by doxorubicin. The data obtained do not contradict various preclinical and clinical studies, but do not answer the question, what other mechanisms besides β-blocking are involved in cardioprotection in these β-adrenoblockers (Hayakawa et al. 2001; Octavia et al. 2012). In the treatment of CHF, β1-adrenoblocker metoprolol is used, which has a favourable effect on intracardiac and regional hemodynamics, normalizes myocardial oxygen regime, reduces the load on the heart. The highly selective β1-adrenoblocker Bisoprolol improves left ventricular ejection fraction, reduces mortality in CHF (Maisel and Somma 2016). However, metoprolol and Bisoprolol are ineffective and require additional combination in some forms of CHF. Metoprolol and Bisoprolol, as β-adrenoblockers without antioxidant and metabolitotropic properties, did not provide significant cardioprotection as with Carvedilol or Nebivolol (Chen and Hong 2016). It is known that carvedilol and Nebivolol due to additional metabolic and antioxidant, vasodilatory mechanisms have certain advantages in cardioprotection (Neilan et al. 2007) over "classical" β-adrenoblockers. Anti-apoptotic mechanisms of carvedilol are also known, associated with its antioxidative activity and regulation of AOS level (Belenichev et al. 2021), which also

positively affects myocardial contractile function in CHF, progression of CHF. Positive effect of carvedilol on the main indices of central haemodynamics, which is a prerequisite for prevention of postinfarction remodelling processes, has been described. Nebivolol – β – adrenoblocker with NO-mimetic effect, showing the effect of peripheral vasodilator, reducing both pre- and postload on the heart is of particular interest from the point of view of treatment of CHF. The antioxidative, antiapoptotic action of Nebivolol is attributed to its positive effect on NO levels and bioavailability of this messenger, which plays a role not only in the regulation of cardiac muscle in norm and pathology, but is involved in apoptosis, cardiomyocyte antioxidative system, regulation of protective protein expression, compensatory energy shunts (Chekman et al. 2013). Numerous studies have shown that CHF is accompanied by significant disorders of myocardial nitroxidergic system. The dynamics of NO end metabolites in CHS shows that typical for endothelial dysfunction on the background of cardiovascular pathology is a decrease in the level of nitrite in blood and urine, indicating a depression of NO generation, which is associated with suppression of the endothelial NO synthase gene, lack of cofactors of NO synthesis, decrease in the amount of L-arginine and the main cofactor of NOS-tetrahydropterin, oxidation of very low density lipoproteins, increase in the local concentration of peroxynitrite in the vascular wall, decrease in antioxidant protection, increase in endogenous NO inhibitors (Trujillo et al. 2000).

Since NO is an important mediator of vasodilation, its deficiency plays a key role in the processes of arterial hypertension development, impaired arterial tone, decreased coronary reserve, left ventricular hypertrophy and formation of left ventricular diastolic dysfunction. Changes in the system of endogenous nitric oxide were revealed in patients with CHF. Cardiomyocytes express two types of nitric oxide synthases (NOS): eNOS and iNOS. The activity of eNOS is regulated through myocardial contractile state, whereas iNOS is induced by cytokines. In CHF, under the influence of AOS, eNOS expression decreases and the concentration of necessary NO synthase cofactors decreases, and NO deficiency is observed (Tassigny et al. 2008).

IL-1β, TNF-a, INF (interferon) stimulate NO synthesis in cardiomyocytes by induction of iNOS during eNOS deprivation and activation of oxidative stress production of cytotoxic forms of NO. Cytotoxic forms of NO have a direct toxic effect on the myocardium, activates the processes of interstitial growth and fibrosis, which increases the negative inotropic effect of NO on the myocardium and causes geometric remodelling of the heart (Ryzhov et al. 2017) In case of further increase in iNOS expression, cytokine-dependent NO production increases, leading to a decrease in contractility. The switch of fibroblast phenotype to myofibroblasts is caused by transforming growth factor beta-1 (TGF-b1) expression of which is regulated by AOS and NO, in particular peroxynitrite, and is associated with the beginning of smooth muscle alpha-actinin (a-SMA) and desmin expression (Youn et al. 2005). The data on the correlation between TGF-b1 expression and iNOS and NADPH-oxidase activity have been obtained (Gilleron et al. 2009). Therefore, the creation of drugs that have a stimulating effect on the synthesis of NO in patients with CHF is quite reasonable. A number of studies in recent years have revealed that, on the one hand, β – adrenoblockers have a favourable effect on the incidence of serious complications (including total and cardiovascular mortality) in patients with CHF, on the other hand, there is convincing evidence of the positive effect of certain β – adrenoblockers on NO synthesis in patients with cardiovascular diseases (Nicol et al. 2021). The mechanism of such an effect of Hypertril on ECG parameters in rats with CHF seems to be related not only to its β1-adrenoceptor action, but also, possibly, to additional mechanisms identified earlier – antioxidative, anti-ischaemic, and NO-mimetic (Liu et al. 2006). NO-mimetic mechanisms include increase of NO production due to eNOS expression and increase of its bioavailability (Chekman 2013). It is possible that Hypertril can improve myocardial bioelectic properties in CHF due to positive effect on morpho-functional indices of cardiomycytes and inhibition of apoptosis (Bak et al. 2021). It is also possible to assume that improvement of cardiac contractile function in CHF in case of Hypertril administration may be associated with improvement of myocardial energy metabolism and reduction of mitochondrial dysfunction. However, all this requires additional verification.

Conclusion

Thus, the obtained results demonstrated the undoubted advantage of the new original molecule (Hypertril) over the basic β-adrenoblockers (Metoprolol, Nebivolol, Carvedilol and Bisoprolol) and experimentally justify further in-depth study to create on its basis a drug for the treatment of CHF.

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