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# The role of the autonomic nervous system in stress cardiomyopathy

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## ABSTRACT

**Aim.** To identify the role of the autonomic nervous system in stress cardiomyopathy in an experimental model of Takotsubo syndrome.

**Materials and methods.** The study was carried out on 120 female Wistar rats. Stress modeling was performed by immobilizing animals on the back for 24 hours. Intact rats were used as controls. The rats were decapitated after termination of immobilization under general anesthesia with ether. Stress cardiomyopathy (SCM) was quantified by accumulation of $^{99m}$Tc pyrophosphate radiopharmaceutical ($^{99m}$Tc PP) in the myocardium. The pharmacological agents used included the ganglionic blocker hexamethonium, administered five times at a dose of 20 mg / kg; guanethidine (50 mg / kg) administered subcutaneously once a day for three days, the last injection was performed 24 hours before immobilization; the muscarinic receptor antagonist atropine methyl nitrate (1 mg / kg); the $\alpha_1$-AR (adrenergic receptor) antagonist prazosin (2 mg / kg); the $\alpha_2$-AR antagonist yohimbine, administered at a dose of 2 mg / kg; the $\beta_1$-AR antagonist nebivolol (1.2 mg / kg); the $\beta_2$-AR antagonist ICI 118,551 (0.3 mg / kg); and the $\beta_3$-AR antagonist L-748337 (0.1 mg / kg).

**Results.** Three-day administration of guanethidine caused a decrease in the degree of $^{99m}$Tc-PP accumulation in the heart by 35.9%. Hexamethonium did not affect the degree of SCM. The blockade of the muscarinic receptor caused an increase in accumulation of $^{99m}$Tc-PP by 26.5%. Inhibition of $\alpha_1$-AR did not affect SCM. The blockade of α2-AR caused a 2.2-fold increase in the accumulation compared with stress control. The blockade of $\beta_1$-AR reduced $^{99m}$Tc-PP accumulation by 2.5 times. The blockade of $\beta_2$-AR by ICI 118,551 increased the degree of $^{99m}$Tc-PP accumulation by 34.6%. Inhibition of $\beta_3$-AR had no effect on SCM.

**Conclusion.** The adrenergic system and $\beta_1$-adrenergic receptor play an important role in the development of SCM. The parasympathetic nervous system ensures resistance of the heart to stress.

**Key words:** stress, heart, autonomic nervous system, Takotsubo syndrome, adrenergic nervous system.

**Conflict of interest.** The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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**Conformity with the principles of ethics.** The study was approved by the Ethics Committee of Cardiology Research Institute, Tomsk NRMC (Protocol No. 192 of 18.12.2019).

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# Роль вегетативной нервной системы в стресс-индуцированном повреждении сердца

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**РЕЗЮМЕ**

**Цель.** Оценка роли вегетативной нервной системы в стресс-индуцированном повреждении сердца в экспериментальной модели синдрома такотсубо.

**Материалы и методы.** Исследование выполнено на 120 самках крыс линии Вистар. Каждая группа животных состояла из 12 особей. Моделирование стресса осуществляли с помощью иммобилизации животных на спине в течение 24 ч. В качестве контроля использовали интактных особей. Крыс декапитировали после прекращения иммобилизации под общим эфирным наркозом. Количественную оценку стресс-индуцированного повреждения сердца (СИПС) осуществляли по аккумуляции радиофармпрепарата $^{99m}$Tc-пирофосфата ($^{99m}$Tc-ПФ) в миокарде.

Фармакологические агенты вводили внутрибрюшинно: ганглиоблокатор гексаметоний вводили пятикратно в дозе 20 мг/кг; гуанетидин (50 мг/кг) – подкожно 1 раз/сут в течение 3 сут, последнюю инъекцию делали за 24 ч до иммобилизации. Остальные препараты (антагонист М-холинорецепторов атропина метилнитрат (1 мг/кг); антагонист $\alpha_1$-адренорецепторов (АР) празозин (2 мг/кг); антагонист $\alpha_2$-АР йохимбин (2 мг/кг); антагонист $\beta_1$-АР небиволол (1,2 мг/кг); антагонист $\beta_2$-АР ICI 118,551 (0,3 мг/кг); антагонист $\beta_3$-АР L-748337 (0,1 мг/кг)) вводили 2 раза/сут с интервалом 12 ч.

**Результаты.** Трехдневное введение гуанетидина вызвало уменьшение степени аккумуляции $^{99m}$Tc-ПФ в сердце на 35,9%. Гексаметоний не оказал влияние на степень СИПС. Блокада М-холинорецепторов вызвала усиление аккумуляции $^{99m}$Tc-ПФ на 26,5%. Ингибирование $\alpha_1$-АР не оказало влияния на СИПС. Блокада $\alpha_2$-АР вызывала усиление аккумуляции в 2,2 раза по сравнению со стресс-контролем. Блокада $\beta_1$-АР снизила степень аккумуляции $^{99m}$Tc-ПФ в 2,5 раза. Блокада $\beta_2$ АР ICI 118,551 увеличила степень аккумуляции $^{99m}$Tc-ПФ на 34,6%. Ингибирование $\beta_3$-АР не оказало эффекта на СИПС.

**Заключение.** Симпатоадреналовая система и, в частности, $\beta_1$-адренорецепторы играют важную роль в развитии СИПС. Парасимпатическая нервная система обеспечивает устойчивость сердца к стрессу.

**Ключевые слова:** стресс, сердце, вегетативная нервная система, синдром такотсубо, адренорецепторы.

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## INTRODUCTION

In 1974, G. Johansson et al. revealed the existence of stress cardiomyopathy (SCM) in a study carried out on pigs [1]. However, it was only in 1990 that a group of Japanese cardiologists first verified SCM in humans, calling it Takotsubo syndrome (TS), because the heart of these patients resembled an octopus trap – "takotsubo" [2]. This condition is characterized by dilatation of the left ventricle of the heart, chest pain, an increase in the level of myocardial necrosis markers, contractile dysfunction of the left ventricle,

and ECG changes (prolongation of the QTc interval, T wave inversion, elevation or depression of the ST segment) [3].

Takotsubo syndrome is a serious illness with mortality comparable to that of acute ST-segment elevation myocardial infarction (STEMI) [4]. This is explained by insufficient knowledge about the mechanism of SCM formation and, consequently, a lack of pathogenetically substantiated therapy. It is assumed that the autonomic nervous system plays an important role in the pathogenesis of SCM [5]. The literature indicates the important role of hyperactivation of the sympathoadrenal system in the pathogenesis of TS. In the study by A. Vaccaro et al., it was shown that in TS patients in the subacute period, the activity of the sympathetic nervous system is increased [6]. The level of adrenaline in the blood plasma of patients with TS in the subacute period is higher than 100 days or 12 months after hospitalization [7, 8]; in TS patients, the level of norepinephrine in the blood serum is also elevated [9]. The results of the above-mentioned studies confirm that the development of TS is accompanied by an increase in the activity of the sympathetic system. It is important to note that TS is characterized by higher serum concentrations of catecholamine than in patients with acute coronary syndrome [10]. It is also worth noting that methods of radionuclide scanning reflect the pathophysiological changes occurring in the body during certain pathological processes [11, 12] and with high sensitivity allow to assess the damage to cardiomyocytes *in vivo* [13].

The aim of this study was to assess the role of the autonomic nervous system in stress cardiomyopathy using an experimental model of Takotsubo syndrome.

## MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Cardiology Research Institute, Tomsk NRMC. The study was carried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes.

The study was carried out on 120 female Wistar rats. Stress was simulated by immobilizing the animals on their backs for 24 hours. The animals were randomly divided into groups of 12 animals each in accordance with the pharmacological agent used. To assess the effect of immobilization, a stress control group consisting of 12 animals was introduced. 16 hours before immobilization, the rats were deprived of food while maintaining access to water. The rats were fixed with standard plastic "clamps" manufactured by DKS JSC (Tver, Russia) at the upper and lower extremities, which constrained their movement, but did not cause pain. As our studies have shown, such an effect causes formation of Selye's three phases of general adaptation (involution of the thymus and spleen, hypertrophy of the adrenal glands, and the appearance of stomach ulcers) [14]. As controls, we used 12 intact animals. The rats were decapitated after the termination of immobilization under general anesthesia with ether.

The quantitative assessment of SCM was carried out by accumulation of the radiopharmaceutical $^{99m}$Tc-pyrophosphate ($^{99m}$Tc-PP) in the myocardium according to the method proposed by D.G. Miller and S. Mallov [13]. The radiopharmaceutical, obtained using a TEKCIS technetium-99m generator (France), was injected intravenously at a dose of 150 MBq / kg 30 minutes after the termination of immobilization. 100 minutes after the injection, the animals were decapitated under ether anesthesia. The incorporation of $^{99m}$Tc-PP into the myocardial tissue was calculated as a percentage of the administered dose per 1 gram of tissue. After removal from the chest, the heart was washed from the blood by perfusing it with normal saline (10 ml) through the aorta. Radioactivity was recorded using a Philips Forte gamma camera (Philips, Netherlands).

The preparations were administered intraperitoneally two times: the first injection was carried out 30 minutes before immobilization and 12 hours after immobilization (except for guanethidine and hexamethonium). For pharmacological denervation, the ganglionic blocker hexamethonium chloride was used, which was injected five times at a dose of 20 mg / kg with an interval of 4 h 48 min [15]. Chemical sympathectomy was induced by administration of guanethidine. Guanethidine (50 mg / kg) was injected subcutaneously once a day for three days, the last injection was performed 24 hours before immobilization [16]. For the blockade of peripheral muscarinic receptors, atropine methyl nitrate was used, which was injected twice at a dose of 1 mg / kg [17]. Prazosin (2 mg / kg) was used to block $\alpha_1$-adrenergic receptors (AR) [18]. Yohimbine, an $\alpha_2$-AR antagonist, was administered at a dose of 2 mg / kg [19]. Nebivolol, a selective $\beta_1$-AR antagonist, was used at a dose of 1.2 mg / kg [20]. The selective $\beta_2$-AR antagonist ICI 118,551 was used at a dose of 0.3 mg / kg [21]. The selective $\beta_3$-AR

antagonist L-748337 was administered at a dose of 0.1 mg / kg [22].

Statistical processing of the obtained data was carried out using the Statistica 13 software (StatSoft Inc., USA, AXA001J575030FAACD-K). To assess the statistical significance of differences between the groups, the Mann – Whitney test was used. The results were expressed as the mean and the standard error of the mean ($M \pm SD$). In all cases, $p \leq 0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

As it can be seen from Figure 1, depletion of endogenous catecholamines after three-day administration of guanethidine reduced the $^{99m}$Tc-PP accumulation in the rat myocardium by 35.9% compared with the stress control. The observed fall indicates a decrease in SCM after immobilization stress. This fact confirms that endogenous catecholamines are involved in the development of stress-induced damage to the myocardium. The introduction of hexamethonium had no effect on the degree of accumulation (Fig. 1). It was found that when administered intravenously, the effect from hexamethonium (10 mg/kg) lasted 60 minutes [23]. It is possible that the absence of the effect of hexamethonium in our study is determined by a short-termed effect of hexamethonium. In addition, it is possible that the lack of the effect of hexamethonium is associated with the fact that the medication blocks both sympathetic and parasympathetic ganglia. This assumption is based on our hypothesis that activation of the sympathoadrenal system contributes to cardiomyopathy, and stimulation of the parasympathetic link of the autonomic nervous system increases cardiac resistance to SCM.

Our hypothesis was also confirmed in a series of experiments with the blockade of muscarinic receptors using atropine methyl nitrate (Fig. 1). When using this pharmacological agent, we observed an increase in the accumulation of $^{99m}$Tc-PP by 26.5% compared with the stress controls, which confirmed our hypothesis about the protective role of the activation of the parasympathetic nervous system. Our result is consistent with the data obtained by R.Q. Xue et al. [24]. Their study showed that stimulation of the vagus has a cardioprotective effect in myocardial damage after administration of isoproterenol. One of the mechanisms of adrenergic damage to the heart is calcium overload in cardiomyocytes, while acetylcholine attenuates calcium overload [25]. Therefore, activation of muscarinic receptors can inhibit SCM.

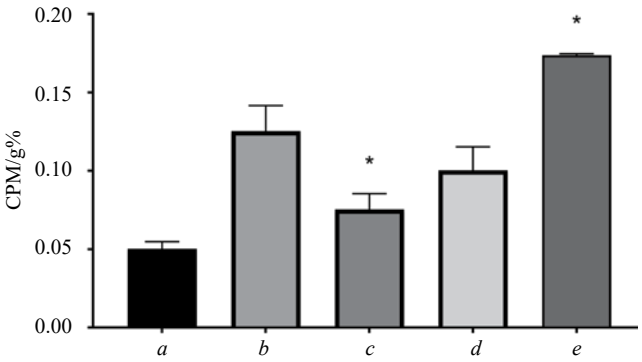


Fig.1. The degree of $^{99m}$Tc-pyrophosphate accumulation during stress: after depletion of endogenous catecholamines with guanethidine; after pharmacological denervation with hexamethonium; with blockade of the muscarinic receptor with atropine methyl nitrate: *a* – intact animals, *b* – stress controls; *c* – stress + guanethidine; *d* – stress + hexamethonium; *e* – stress + atropine methyl nitrate; **p* < 0.05 compared with the stress controls

During the experiments, data were obtained on the role of α-AR in SCM. As it can be seen from Figure 2, the blockade of $\alpha_1$-adrenergic receptors with prazosin did not affect the accumulation of $^{99m}$Tc-PP during stress. Blockade of $\alpha_2$-adrenergic receptors with yohimbine (Fig. 2) caused an increase in $^{99m}$Tc-PP by 220% compared with stressed animals. Yohimbine is known to block $\alpha_2$-ARs located on the sympathetic terminals, which leads to the release of norepinephrine [26]. Obviously, under stress, yohimbine causes an increase in the release of norepinephrine from the adrenergic nerve terminals that innervate the heart, which results in an increase in SCM.

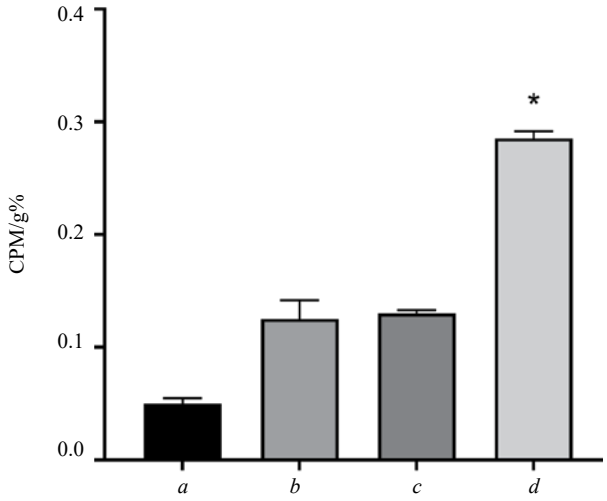


Fig. 2. The degree of accumulation of $^{99m}$Tc-pyrophosphate during the blockade of α-adrenergic receptors under stress: *a* – intact animals, *b* – stress controls, *c* – stress + prazosin, *d* –stress + yohimbine; E – stress + atropine methyl nitrate; **p* < 0.05 compared with the stress controls

During a series of experiments to determine the role of β-AR in SCM, data were obtained that the blockade of $\beta_1$-AR reduced the accumulation of $^{99m}$Tc-PP by 250% (Fig. 3). It is generally known that these receptors are associated with Gs proteins, activation of which leads to stimulation of adenylate cyclase and an increase in cAMP synthesis. The the $\beta_2$-AR antagonist ICI 118,551 has the opposite effect: after its administration, the accumulation of $^{99m}$Tc-PP in the myocardium increased by 34.6%. It is known that $\beta_2$-AR at high concentrations of catecholamines are able to switch from Gs-proteins to Gi/o-proteins [27]. It is also known that activation of these receptors along the pathway of Gi/o-protein stimulation causes inhibition of adenylate cyclase and a decrease in cAMP synthesis and has anti-apoptotic and cardioprotective effects [28, 29]. The activation of Gi/o-proteins most likely occurs in SCM. The increase in myocardial damage can be explained by the fact that the $\beta_2$-AR blockade impairs the physiological mechanisms of reducing the pathological effects of excessive $\beta_1$-AR activation. Inhibition of $\beta_3$-AR had no effect on the degree of $^{99m}$Tc-PP accumulation in the myocardium under stress (Fig. 3).

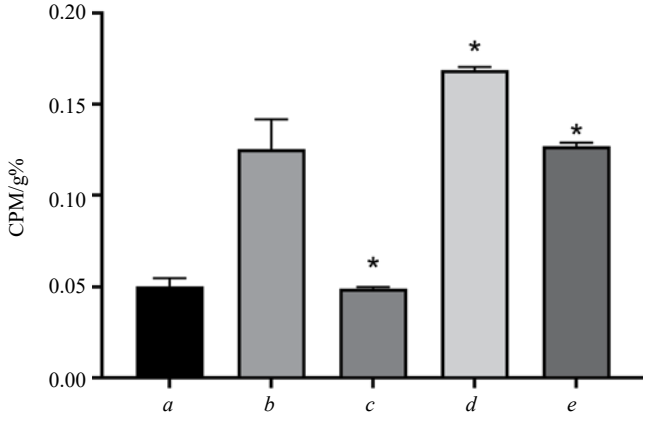


Fig. 3. The degree of $^{99m}$Tc-pyrophosphate accumulation during the blockade of β-adrenergic receptors in stress: A – intact animals, B – stress controls; C – stress + nebivolol; D –stress + ICI 118.551; E – stress + L-748337; * $p < 0.05$ compared with the stress controls

## CONCLUSION

Blockade of peripheral muscarinic receptors enhances SCM. $\alpha_1$-adrenergic receptors do not have a considerable impact on the pathogenesis of SCM. It was found that the blockade of $\alpha_2$-AR contributed to enhancement of SCM, most likely due to inhibition of pre-synaptic $\alpha_2$-AR. $\beta_1$-adrenergic receptors play a crucial role in the pathogenesis of SCM. The activation of $\beta_2$-adrenergic receptors by endogenous catecholamines limits SCM. $\beta_3$-adrenergic receptors do not significantly affect SCM. Consequently, the sympathoadrenal system and, in particular, $\beta_1$-AR contribute to the development of mechanisms that are involved in the development of SCM. The parasympathetic nervous system ensures resistance of the heart to stress.

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## Authors contribution

All authors contributed to conception and design, analysis and interpretation of data, substantiation of the manuscript, and critical revision of the manuscript for important intellectual content.

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