Fundamental researches

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THE ROLE OF PARASYMPATHETIC AUTONOMIC REGULATION IN ENSURING OF RATS' RESISTANCE IN THE MODEL OF MULTIPLE ORGAN DYSFUNCTION SYNDROM

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To assess contribution of autonomic regulation in multiple organ dysfunction syndrome (MODS) survival ensuring and to test hypothesis about possible correction of clinical course by modulating the activity of parasympathetic influences we performed experiments on rats' model of the MODS. It was determined that nonresistant animals differentiated by less intensity of parasympathetic regulation response. It was revealed that stimulation of cholinergic system decrease lethality in rats, and inhibits the power of high frequency regulatory effects on the heart rate.

KEY WORDS: multiple organ dysfunction syndrome model, heart rate variability, N-cholinoreceptors

РОЛЬ ПАРАСИМПАТИЧНОЇ ВЕГЕТАТИВНОЇ РЕГУЛЯЦІЇ У ЗАБЕЗПЕЧЕННІ СТІЙКОСТІ ЩУРІВ В МОДЕЛІ СИНДРОМУ ПОЛІОРГАННОЇ НЕДОСТАТНОСТІ

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Для оцінки вкладу вегетативної регуляції у забезпеченні виживаності при синдромі поліорганної недостатності та для перевірки гіпотези щодо можливої корекції клінічного перебігу шляхом модулювання активності парасимпатичних впливів, виконані експерименти на щурах в умовах моделі поліорганної недостатності. Встановлено, що нестійки тварини відрізняються меншою реакцією з боку парасимпатичної регуляції. Показано, що стимуляція холінергічної системи знижує летальність щурів, та призводить до пригнічення потужності високочастотних регуляторних впливів на серцевий ритм.

КЛЮЧОВІ СЛОВА: модель синдрому поліорганної недостатності, варіабельність серцевого ритму, Н-холінорецептори

РОЛЬ ПАРАСИМПАТИЧЕСКОЙ ВЕГЕТАТИВНОЙ РЕГУЛЯЦИИ В ОБЕСПЕЧЕНИИ УСТОЙЧИВОСТИ КРЫС В МОДЕЛИ СИНДРОМА ПОЛИОРГАННОЙ НЕДОСТАТОЧНОСТИ

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Для оценки вклада вегетативной регуляции в обеспечение выживаемости при синдроме полиорганной недостаточности и для проверки гипотезы о возможности коррекции клинического течения путем модулирования активности парасимпатических влияний, выполнены эксперименты на крысах в условиях модели полиорганной недостаточности. Установлено, что неустойчивые животные отличаются меньшей реакцией со стороны парасимпатической регуляции. Показано, что стимуляция холинергической системы снижает летальность крыс, и приводит к угнетению мощности высокочастотных регуляторных влияний на сердечный ритм.

КЛЮЧЕВЫЕ СЛОВА: модель синдрома полиорганной недостаточности, вариабельность сердечного ритма, Н-холинорецепторы

INTRODUCTION

Multiple organ dysfunction syndrome (MODS) frequently described as consequences of systemic inflammatory response (SIR) may develop in the most different diseases, which characterized by relatively high lethality [1]. The most different links take participation in its formation, including autonomic nervous system, which role remaining not studied enough [2]. Previously findings about influence of autonomic regulation on rats' survival in the model of multiple organ dysfunction syndrome, made us to investigate the possibility of systemic inflammatory response correction by modulation of autonomic circuits of regulation activity.

Considering recently appeared in the papers data about possible involvement of N-cholinoreceptors in regulation of functional activity of lymphocytes, macrophages and other cells participating in inflammation we suppose that the parasympathetic activation may decrease the systemic inflammation and so decrease the lethality of rats in the experimental model of multiple organ dysfunction syndrome.

OBJECTIVE

The aim of the present study was assessment of the parasympathetic autonomic regulation role in survival of rats in MODS model.

MATERIALS AND METHODS

Experiments were performed on the 78 Wistar lab male rats with 220-250 g body weight, which kept in the standard vivarium conditions. Multiple organ dysfunction syndrome was simulated by cecal ligation and puncture (CLP) procedure [3]. It was performed under combined anesthesia with ether inhalation and intraperitoneal injection of sodium thiopental (75 mg/kg).

The autonomic regulation was assessed by heart rate variability (HRV) spectral analysis with the using of the computer-based cardiograph «Cardio Lab» (KhAI-Medica, Ukraine) with signal discretization frequency of 500 Hz. Spectral power of parasympathetic influence (HF) was calculated in the range from 0.6 to 3.0 Hz and compared with power of sympathetic influence (LF) in the range from 0.08 to 0.6 Hz.

In the 1st group of animals (34 rats) we assess survival rate and association of survival with individual changes of autonomic regulation in response to single-dose intramuscular injection of phenylephrine (2 mg/kg) and in response to development of MODS after two hours of animal's induction to the model.

In the 2nd group of animals (44 rats) we assess survival rate in condition of N-cholinoreceptors stimulation. For this purpose, animals expose to intravenously injections of acetylcholinesterase inhibitor – neostigmine (0.5 mg/kg) and M- cholinergic antagonist - atropine (1 mg/kg). Such injections we performed by caudal vein access at 30 min before CLP and every 2 hours after induction to MODS model. Total duration of pharmacological exposure was 8 hours.

All statistical calculations were performed using Statistica 6.0 (Stat Soft Inc., USA). Spectral characteristics in tables and text are presented as median (Me), minimal (Min) and maximal values (Max), lower (Qi) and upper (Q_u) quartiles. Significance of differences between spectral characteristics was assessed by Mann-Whitney test.

RESULTS AND DISCUSSION

As it was expected, in spite of strictly standardized conditions of experiment, rats were characterized by different resistance to the induction of the experimental model of MODS. On the basis of findings about animals survival rate in the 1st group we subdivided animals into group of highly resistant rats (survived during 10 days - 3 animals) and low resistant rats (died within 3 days - 32 animals).

The power spectral indexes changes in response to pharmacologic sympathetic stimulation in the subgroups of high- and low resistant rats are presented below (table).

In the subgroup of highly resistant animals adrenoreceptors stimulation by phenylephrine lead to increase of the autonomic regulative influences power. In the selected by us range of the low frequencies (LF) the value of spectral power is increasing in 5-fold and of the high frequencies (HF-range) this value is increasing in 4.4 fold. As a result, in highly resistant rats, pharmacologic adrenoreceptors stimulation did not change significantly the values of sympathovagal balance - relation of spectral powers in the low and high frequencies range.

Table

Power spectral indexes of rats' HRV before and after stimulation of α- adrenoreceptors

Power spectral indexes		highly resistant rats (n=3)		low resistant rats (n=31)	
		before	after	before	after
LF, ms^2	Me	0.11	0.54*	0.10	0.50*
	Min- Max	0.01-0.53	0.09 - 2.02	0.01 - 0.50	0.03 - 2.13
	Q_i- Q_u	0.06-0.20	0.32 - 1.24	0.07 - 0.19	0.41 - 1.17
HF, ms^2	Me	1.35	5.92*	1.54	4.50*
	Min- Max	0.32 - 3.23	0.28 - 7.83	0.46 - 4.50	0.43 - 6.95
	Q_i- Q_u	0.99 - 1.89	3.47 - 6.35	1.01 - 2.09	3.82 - 4.93

Note: * - intragroup differences are significant at $p < 0.05$.

In the subgroup of low resistant animals, the response of sympathetic regulation to stimulation of α-adrenoreceptors corresponds with so in rats of compared subgroup - the power of influences in the low frequency range made increase in 5-fold. However, the HF spectral power of HRV in the succumbed rats in response to injection of phenylephrine made increase only in 2.9-fold. At the same time as in the group of survived rats the value of sympathovagal balance is not changed after phenylephrine injection – 0.10 (0.01-0.33) before phenylephrine and 0.11 (0.01-0.60) after pharmacologic stimulation.

The group of low resistant rats demonstrated 2-fold increase in the power of sympathetic influences as compared with initial values - up to 0.20 ms^2 (0.11-0.42), after 2 hours of CLP ($p < 0.05$). Nevertheless, the value of spectral power in the range of high frequencies, reflective mainly of parasympathetic influences, had tendency to decrease – 1.12 ms^2 (0.50-2.73). As a result the sympathovagal balance in this subgroup has mount to 0.28 (0.10-2.73), which was significantly more than initial values ($p < 0.05$).

Thus, analysis of survival demonstrated that resistance of rats in the model of MODS was related to properties of their autonomic response. Highly resistant animals characterizes by more pronounced response of parasympathetic regulation to pharmacologic stimulation as compared with low resistant rats.

In response to CLP (development of an acute bacterial inflammation in the abdominal cavity and organ dysfunction), the highly resistant rats respond with balanced increase of HRV spectral power in both investigated ranges, sympathetic and parasympathetic links of autonomic regulation.

Subgroup of low resistant animals in response to the MODS model characterized by less pronounced activation of sympathetic influences in the absence thereof significant changes in the tone of parasympathetic link of regulation. In the total, we find marked shift of sympathovagal balance toward domination of sympathetic influences in the low resistant animals.

The results of experiment in the 1st group of rats confirm presumption about possible suppression of systemic inflammatory response through N-cholinergic anti-inflammatory pathway during parasympathetic activation [4]. These results was the basis to perform experiments on the animals of 2nd group with purpose to investigate the possibility of increasing resistance of rats in the MODS model by modulating the activity of different links of autonomic regulation.

Despite of the long-continued cholinergic stimulation of 2nd group rats by combination of neostigmine and atropine injections the index of 3-day lethality in the MODS model didn't differ significantly from 1st group of rats – 93.2 % и 91.2 %, accordingly ($p = 0.371$). At the same time, the comparative analysis of time curves of rat's deaths in these groups demonstrates following features (fig. 1).

Under cholinergic stimulation, there were no any deaths during the first 15 hours after induction rats to the model. In the compared group almost 12 % of animals died. On the background of the neostigmine almost 32 % of rats survived 34 hours, whereas until this term in the compared group died 88.2 % of animals ($p < 0.001$).

The results are the evidence of long-continued pharmacological stimulation of the cholinergic system affects status and character of response of different parts of autonomic nervous system. All spectral indices of HRV in rats of this experimental group were lower than in other one. Intensity of these effects not equal for sympathetic and parasympathetic parts of regulation Thus, after 2 hours of induction of animals to the MODS model the indices of spectral power of HRV in the range of low frequencies (sympathetic influences) were about equal in rats of 1st and 2nd experimental groups.

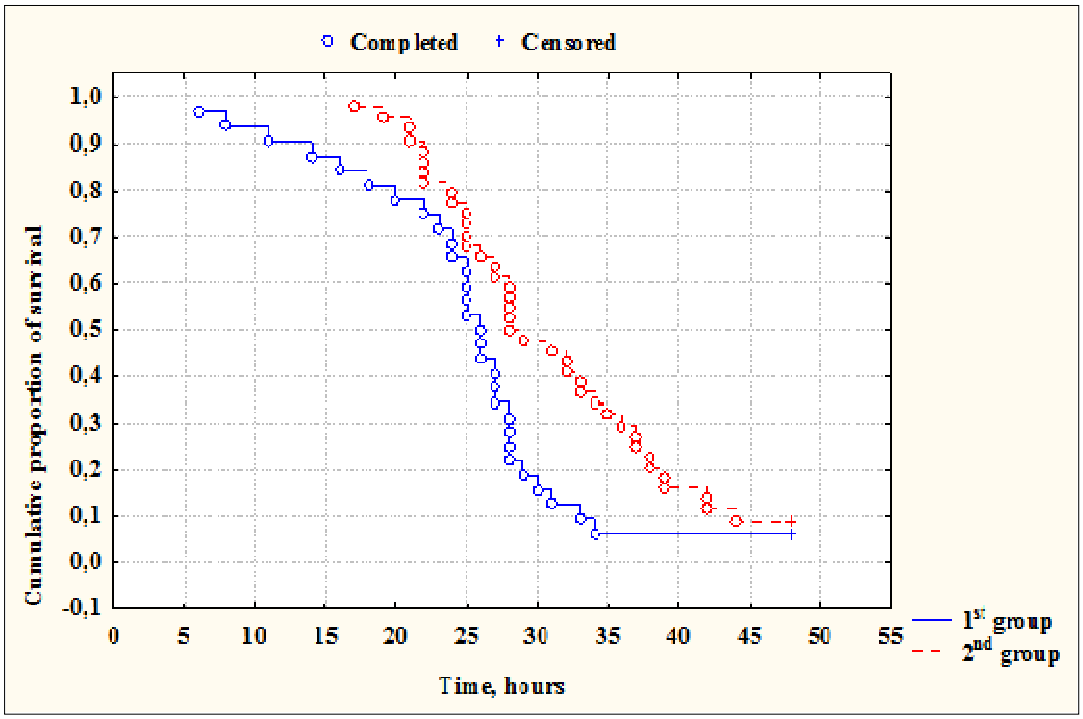

Fig. 1 Kaplan-Meier cumulative proportion of survival in rats' MODS model without (1st group, n=34) and with pharmacologic stimulation of cholinergic system (2nd group, n=44)

Neostigmine has more pronounced effects on the power of high frequencies of heart rhythm regulations (fig. 2). Mean values of spectral power index in rats with pharmacological stimulation of cholinergic nervous system were significantly ($p < 0.01$) lower (4.72 ± 1.61 ms^2), then in rats of compared group of investigation (5.89 ± 1.77 ms^2).

Our findings about ability of acetylcholinesterase inhibitor to decrease intra-experimental lethality of rats in MODS model is matched with results of other investigators, which were gained in other diseases models. Thus, it is known that stimulation of choline-receptors increases survival of rats after intraperitoneal injection of bacteria [4]. Experiments on the guinea pigs proved the ability of N-cholinoblockers to intensify of anaphylactic shock and, conversely, inhibition of acetylcholinesterase prevent from development of shock [5].

With no relation to intensity and direction of effects of stimulation of the parasympathetic regulation demonstrated in different studies it is noteworthy that unity of settled opinion about the cause-and-effect relations between factors of systemic and local inflammation on the one hand and the status of different parts of the autonomic nervous system on the other.

Applied scheme of long-continued selective pharmacologic stimulation of N-cholinoreceptors, in our experiments, leads to significant inhibition of the power of high frequency regulatory influences on the heart rate. The same direction but less pronounced influences on the index of the spectral power of regulation in low frequency range (that reflect as is well known sympathotonic influences) was registered by us.

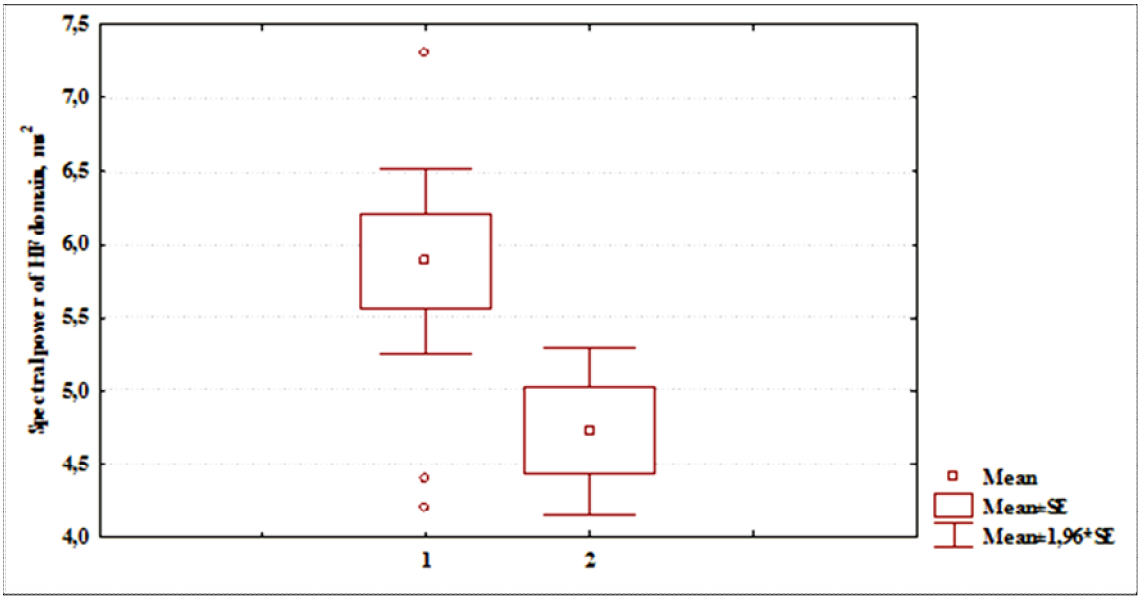

Fig. 2. HF spectral power of HRV in rats after 2 hours of induction to MODS model, without (1) and under (2) pharmacological stimulation of cholinergic system.

Our findings allow us to suggest that revealed influence of the cholinergic stimulation on the animals' survival in the experimental MODS realizes through the cytokine system. Thus, it is well known ability of cytokines to cause not only local but also distant effects on almost all organs and systems including tissues of central nervous system. This respectively would accompany with alterations in intensity and character of reflective responses, hormone and other biologic regulators synthesis [6]. On the other hand, electrical stimulation of vagal nerve inhibits synthesis of tumor necrosis factor in mice [4].

It must be underlined that revealed relations of the autonomic regulation status with factors of inflammation, is not specific for multiple organ dysfunction syndrome. Similar on direction but different in intensity patterns was descrybed in studies of toxic injuries [7], in oncology clinics [8], in diabetes [9], and others.

CONCLUSIONS

Thus, our findings clarify understanding about the role of neurohumoral regulation in resistance of the body to the experimental model of multiple organ dysfunction syndrome.

Long-continued pharmacologic stimulation of N-cholinoreceptors decrease lethality in rats' model of multiple organ dysfunction syndrome, and significantly inhibits the power of high frequency regulatory influences on the heart rate.

PROSPECTS FOR FUTURE STUDIES

Our findings are justifying the reasonability of clinical approbation of medications, which effect on the autonomic nervous system and stimulate of its cholinergic link in patients with multiple organ dysfunction syndrome.

REFERENCES

1. Knox, D. B. Phenotypic clusters within sepsis-associated multiple organ dysfunction syndrome / D. B. Knox, M. J. Lanspa, K. G. Kuttler, et al. // Intensive Care Med. — 2015. — V. 41, № 5. — P. 814 22.
2. Scheff, J. D. On heart rate variability and autonomic activity in homeostasis and in systemic inflammation / D. S. Jeremy, G. Benjamin, A. C. Siobhan et al. // Math Biosci. — 2014. — V. 252 — P. 36 44.
3. Yehya, N. Cecal ligation and puncture accelerates development of ventilator-induced lung injury. / N. Yehya , Y. Xin , Y. Oquendo et al. // Am J Physiol Lung Cell Mol Physiol, — 2015. — V. 308, № 5. — P. L443-L451.
4. Wang H. Nicotinic acetylcholine receptor 7 subunit is an essential regulator of inflammation / H. Wang, M. Yu, M. Chain // Nature. — 2003. — V. 421, — P. 384 388.

5. Холинергическая регуляция анафилактического шока. Влияние С-реактивного белка. / Г. И. Нежинская, П. Г. Назаров, Н. Р. Евдокимова [и др.] // Цитокины и воспаление. — 2004. — Т. 3, № 1. — С. 44 48.
6. Симбирцев А. С. Цитокины: классификация и биологические функции / А. С. Симбирцев // Цитокины и воспаление. — 2004. — Т. 3, № 2. — С. 16 22.
7. Зинкович И. И. Роль вегетативной регуляции в развитии аллоксан-индуцированного сахарного диабета у крыс / И. И Зинкович., И. А Хрипаченко., Л. А Кеденко., А. В Савустьяненко // Ендокринологія. — 2005. — Т. 10, № 1. —С. 35 40.
8. Fulda S. Signaling through death receptors in cancer therapy / S. Fulda, K. M. Debating // Cur. Open. Pharmacology. — 2004. — V. 4, № 4. — P. 327 332.
9. Irigoyen, M. C. Autonomic nervous system, inflammation, and diabetes: mechanisms and possible interventions / M. C. Irigoyen, D. E. Casarini, M. Morris, N. Montano // Exp Diabetes Res. — 2012. — V. 2012 — P. 894157.