Effects of Cholinergic Agonists and Antagonists under Conditions of Spleen Denervation in Rats with Endotoxic Shock

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Model experiments on rats with endotoxic shock induced by intraperitoneal injection of LPS *Salmonella Typhi* strain ty-4441 (20 mg/kg) showed that crossing of the vagus nerve innervating the spleen increased HR, stimulated production of antibodies, and moderated serotonergic activity of splenocytes. Pharmacological correction of the shock with muscarinic receptor antagonist atropine and its combinations with anticholinesterase agent galantamine or muscarinic and nicotinic cholinoreceptor agonist choline alfoscerate 30 min before shock modeling moderated HR and normalized B cell functions and serotonin level in the spleen.

Key Words: *endotoxic shock; spleen denervation; cholinergic agonists; cholinergic antagonists; serotonin; B cells*

The cholinergic system is an integrating component in the regulation of systemic inflammatory reaction and development of polyorganic insufficiency during endotoxemia [3]. Activation of the cholinergic branch of CNS controlling the efferent pathways of the sympathetic and parasympathetic subdivisions of the autonomic nervous system plays an important role in moderation of inflammation [4,14]. Moreover, stimulation of α**7** nicotinic acetylcholine receptors [8] in cells of macrophage-monocyte lineage with acetylcholine or its precursor choline down-regulates the synthesis of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-18) [5]. The specific role of B cells in these processes was not examined, although they are transformed into plasma cells without proliferation upon contact with LPS, thereby providing LPS-neutralizing IgM-antibodies, which is noteworthy because the development of shock is mainly determined by production of IgM and IgG immunoglobulin series [9]. The content of serotonin in the spleen was not studied, although serotonin can play a role of a protective paracrine transmitter in elimination of the antigen substance [10].

Our aim was to study the effects of nicotinic and muscarinic agonists and antagonists on the heart rate (HR), production of antibodies, and serotonin concentration in denervated spleen in the rats with experimental endotoxic shock.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats (n=180) weighing 180-200 g obtained from Rappolovo Animal Breeding Department (Russian Academy of Sciences). The animals were maintained under vivarium (23°C) conditions under a 12-hour day-night cycle with food and water *ad libitum*.

The endotoxic shock was provoked by intraperitoneal injection of LPS *Salmonella Typhi* strain ty-4441 (St. Petersburg Institute of Vaccines and Sera) in a sublethal dose (20 mg/kg dissolved in 0.5 physiological saline) [13]. The spleen was denervated by excision of the anterior and posterior branches of the vagus nerve (3-5 mm) [5]. Sham-operated rats served as the control.

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The preparations of atropine sulfate (0.09 mg/kg), galantamine hydrobromide (0.45 mg/kg), choline alfoscerate (90 mg/kg), and ipratropium bromide (7.2 μg/kg) were dissolved in physiological saline and injected intraperitoneally 30 min before LPS. The effects of these agents were assessed 2 h postinjection according to HR changes [15]. HR was determined by the mean value of *R-R* interval using the formula HR (bpm)=60/(duration of *R-R* interval in seconds). The control HR values were measured before injection of the preparations in the experimental rats and in the rats with shock "treated" with physiological saline.

The number of antibody-producing cells (APC) per 10^6 splenocytes was determined according to [7] in rats immunized with sheep erythrocytes in a dose of 10^8 per rat performed 2 h after the surgery. The cholinotropic drugs were injected on postimmunization day 4 and 30 min before LPS. The content of APC was determined after 24 h. The controls were splenectomized rats not treated with the drugs and sham-operated rats.

Serotonin level (ng/mg protein) in deproteinized spleen specimens [10] was measured with HPLC (Beckman System Gold) employing LC-4C detector, SphereClone 5 μ ODS 2 (250×4.60 mm) column, and SecurityGuard precolumn (ODS 4 mm L×3.0 mm ID; Phenomenex).

The data are presented as parameter value±*SEM*. The results were analyzed statistically using Statistica 6.0 software and Student's *t* test.

RESULTS

Application of cholinotropic agents to sham-operated rats subjected to endotoxic shock induced changes in HR: muscarinic antagonist atropine or anticholinesterase agent galantamine decreased HR; muscarinic and nicotinic receptor agonist choline alfoscerate also produced a negative chronotropic effect in comparison with the control sham-operated rats (Table 1). The effects of atropine can be explained by the fact that it blocks presynaptic muscarinic receptors in central cholinergic synapses, thereby increasing secretion of acetylcholine and potentiating neurotransmission with the involvement of postsynaptic nicotinic cholinoreceptors, which leads to moderation of LPS-caused tachycardia [2]. This hypothesis is indirectly corroborated by inefficiency of ipratropium, which probably blocks the peripheral postsynaptic muscarinic cholinoreceptors. Bradycardia caused by intraperitoneal injection of choline alfoscerate (choline ester subjected to metabolism) results from up-regulation of acetylcholine synthesis in the atrium by choline followed by potentiation of the cholinergic transmission in parasympathetic nerve fibers [11]. In addition, choline exerts an agonistic effect on M_2 muscarinic cholinoreceptors of cardiac pacemakers [6]. It cannot be excluded that the effects of galantamine are related to elevation of acetylcholine concentration. In splenectomized rats, the employed preparations of atropine, ipratropium, galantamine, or choline alfoscerate moderated HR to the end of a 2-h observation period in comparison with experimental control rats with denervated spleen. Probably, disturbance of vagus nerve integrity can modify the effect of some cardiac chronotropic agents.

The parasympathetic denervation of the spleen did not change the intensity of antibody genesis in response to administration of sheep erythrocytes in comparison to the animals with intact innervations of the spleen. However, the effects of cholinergic preparations were different in intact and splenectomized rats (Table 2). Injection of atropine decreased the content of APC, while choline alfoscerate or ipratropium increased it. These data corroborate the view on the control of the immune function of the spleen during

TABLE 1. Effect of Splenic Denervation on HR in Rats with Endotoxic Shock Treated with Cholinergic Preparations (*M*±*SEM*)

Preparation	Initial HR before LPS injection		2 h after injection of the preparations*	
	sham-operated rats	splenectomized rats	sham-operated rats	splenectomized rats
Control	428±12	469±17	+76±4	+114±8^{xxx}
Atropine, 0.09 mg/kg	389±10	383±14	+34±3***	+26±7***
Galantamine, 0.45 mg/kg	363±9	377±12	+9±2***	+26±8***^{xxx}
Choline alfoscerate, 90 mg/kg	339±10	374±14	-16±3***	+19±6***^{xxx}
Ipratropium, 7.2 μg/kg	423±10	406±13	+66±7	+54±8**

Note. *Difference in HR in rats treated with the corresponding agent and HR before its injection. **p<0.01, ***p<0.001 compared to experimental control rats subjected to endotoxic shock treated preventively with physiological saline; ^{xxx}p<0.001 compared to sham-operated rats.

TABLE 2. Effect of Splenic Denervation on APC (per 10^6 cells) and Serotonin (ng/mg protein) in Rats with Endotoxic Shock (*M*±*SEM*)

Preparation	Sham-operated rats		Splenectomized rats	
	APC[1]	serotonin[2]	APC[1]	serotonin[2]
Control	452±13[x]	5.318±0.005[o]	345±13[x]	1.564±0.005
Without preparations	824±37***	56.588±0.006***	1676±52***	0.060±0.005***
Atropine, 0.09 mg/kg	113±16***+++	3.413±0.005***+++	416±64+++	9.338±0.004***+++
Galantamine, 0.45 mg/kg	163±24***+++	5.441±0.005***+++	511±77+++	5.743±0.004***+++
Choline alfoscerate, 90 mg/kg	165±19***+++	5.250±0.004***+++	366±61+++	5.451±0.005***+++
Ipratropium, 7.2 μg/kg	468±35+	2.307±0.006***+++	500±58+++	6.19±0.006***+++

Note. [1]In 5 days after denervation of the spleen, immunization with sheep erythrocytes, injection of cholinotropic preparations and LPS (1 day before examination); [x]the number of APC in non-operated rats was 350±82/10^6 splenocytes; [2]in 24 h after sham surgery and vagotomy followed by injection of the preparations and LPS; [o]baseline serotonin level was 5.465±0.005 ng/mg protein. ***p<0.001 compared to intact control rats; +++p<0.001 compared to rats with spleen denervation and endotoxic shock not treated with the cholinotropic agents.

sepsis by the parasympathetic system [5]. Moreover, they attest to the influence of the cholinergic drugs on the non-innervated B cells. Potentiation of activity of B cells during shock was accompanied by pronounced elevation of serotonin in spleen in comparison with its baseline level (Table 2). The release of this transmitter can be enhanced due to up-regulation of activity of splenic sympathetic and parasympathetic neurons [12]. This is indirectly supported by the fact that spleen denervation decreased serotonin concentration 90-fold, while choline alfoscerate or galantamine elevated its concentration in the spleen.

Thus, the final hemodynamic state during endotoxic shock is determined by the balance of activity of the constrictor and dilator influences onto the vascular tone and by the cardiotropic effects of autonomic nervous system. The preparations potentiating cholinergic neurotransmission (choline alfoscerate and galantamine) can normalize the antibody-producing function of splenic B cells and restore activity of splenic serotoninergic system even without the vagal supply. It cannot be excluded that modulation of the transmitter response of the spleen with cholinotropic agents can also affect the serotoninergic system of B cells, which indicates one of the alternative approaches to the regulation of their function during inflammation.

REFERENCES

1. K. M. Buller, C. V. Dayas, and T. A. Day, *Neuroscience*, **118**, No. 1, 189-203 (2003).
2. R. L. Buyukuysal, I. H. Ulus, S. Aydin, and B. K. Kiran, *Eur. J. Pharmacol.*, **281**, No. 2, 179-185 (1995).
3. M. Gallowitsch-Puerta and V. A. Pavlov, *Life Sci.*, **80**, Nos. 24-25, 2325-2329 (2007).
4. R. P. Gaykema, C. C. Chen, and L. E. Goehler, *Brain Res.*, **1130**, No. 1, 130-145 (2007).
5. J. M. Huston, M. Ochani, M. Rosas-Ballina, *et al., J. Exp. Med.*, **203**, No. 7, 1623-1628 (2006).
6. Y. O. Ilcol, M. S. Gurun, Y. Taga, and I. H. Ulus, *Eur. J. Biochem.*, **270**, No. 5, 991-999 (2003).
7. N. K. Jerne, C. Henry, A. A. Nordin, et al., *Transplant. Rev.*, **18**, 130-191 (1974).
8. V. A. Pavlov, M. Ochani, L. H. Yang, et al., *Crit. Care Med.*, **35**, No. 4, 1139-1144 (2007).
9. G. N. Pontes, S. G. Massironi, C. Arslanian, *et al., Scand. J. Immunol.*, **62**, No. 4, 353-360 (2005).
10. H. Rogausch, A. del Rey, A. Kabiersch, *et al., Am. J. Physiol.*, **268**, No. 4, Pt. 2, R902-R908 (1995).
11. V. Savci and I. H. Ulus, *J. Cardiovasc. Pharmacol.*, **30**, No. 5, 667-675 (1997).
12. R. H. Straub, *Trends Pharmacol. Sci.,* **25**, No. 12, 640-646 (2004).
13. V. Strunk, K. Hahnenkamp, M. Schneuing, *et al., Anesth. Analg.*, **92**, No. 3, 681-687 (2001).
14. K. J. Tracey, *J. Clin. Invest.*, 117, No. 2, 289-296 (2007).
15. C. C. Wu, S. J. Chen, and M. H. Yen, *Am. J. Physiol.*, **275**, No. 4, Pt. 2, H1148-H1157 (1998).