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# GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

# Role of β2-Adrenoreceptors in Adrenergic Anti-Inflammatory Mechanism in Sepsis

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Experiments on random-bred albino mice showed that of β2-adrenoreceptor agonist hexaprenaline sulfate significantly reduced mortality of mice from experimental sepsis (intraperitoneal administration of *E. coli*) in 4 and 24 h after modeling by reducing blood levels of proinflammatory cytokines TNFα, IL-1β, and IL-6. The antagonist of β2AR ICI-118,551 eliminated this effect.

**Key Words:** *adrenergic anti-inflammatory mechanism; pro-inflammatory cytokines; sepsis; β2-adrenoceptors*

Mortality from sepsis constitute 12 to 60% lethal outcomes associated with diseases and their complications [8] and this parameter continues to increase [3,10]. In 1987, a cholinergic anti-inflammatory mechanism was discovered [1] that after the study of its realization at the organism, cellular, and subcellular levels [2,4,6] in 2002 was called the "cholinergic anti-inflammatory pathway" [4]. It should be noted that in 1995 the possibility of administration of cholinergic agonists for immediate activation of antimicrobial resistance during sepsis was proved [2]. A number of works were devoted to the analysis of the cholinergic anti-inflammatory pathway related to the action of acetylcholine on α7n-acetylcholine receptors (α7nAChRs) on the monocyte-macrophage system cells with subsequent inhibition of the production of pro-inflammatory cytokines TNFα, IL-1β, and IL-6 by these cells and a decrease in mortality from sepsis [3-5,7, 11.15]. The decrease in TNFα, IL-1β, and IL-6 production (onset of anti-inflammatory effect) at the cholinergic anti-inflammatory pathway (mechanism) is provided by kinase JAK2, transcription factor STAT3, transcription factor NF-κB [3,6]. We can hypothesize that along with cholinergic anti-inflammatory mechanism, an adrenergic anti-inflammatory mechanism associated with activation of nicotinic receptors of the adrenal medulla and sympathetic ganglia, is activated in sepsis, inflammatory bowel diseases, and other infectious processes. Activation of nicotinic receptors induces production of epinephrine and norepinephrine that probably stimulate adrenoreceptors of the monocyte-macrophage system cells (direct effect) [12] and β2-adrenoreceptors (β2AR) of T cells in the spleen (indirect effect) [5] produce the same effect as activation of α7nAChRs that leads to a reduction in the synthesis of proinflammatory cytokines by cells of the monocyte-macrophage system [3,4,7].

We studied the role of β2-adrenoreceptors in the realization of the adrenergic anti-inflammatory mechanism in sepsis.

## MATERIALS AND METHODS

Experiments were conducted on random-bred white mice of both sexes weighing 18-22 g. Group 1 mice (control 1, *n*=8) received intraperitoneal injection of 2 ml isotonic NaCl (physiological saline, PS) 2 h af-

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ter its subcutaneous injection (0.5 ml). Group 2 mice (control 2, $n$=55) received single subcutaneous injection of 0.5 ml PS followed in 2 h by intraperitoneal injection of 24-h culture of *E. coli* ($2.5\times10^9$ microbial bodies in 2 ml PS; sepsis modeling) [1-3]. In group 3 ($n$=32), a selective β2AR agonist hexaprenaline sulfate (Nycomed) was administered subcutaneously in a single dose of 1.5 μg/kg in 0.5 ml PS (group 3, $n$=32). Group 4 mice ($n$=30) received single subcutaneous injection of β2AR selective antagonist ICI-118,551 (Sigma-Aldrich) in a dose of 3 mg/kg in 0.5 ml PS. β2AR agonist hexaprenaline sulfate in combination with selective antagonist β2AR ICI-118,551 was administered to mice of the group 5 ($n$=32). β2AR antagonist was administered subcutaneously in 10-20 min before administration of β2AR agonist hexaprenaline sulfate. In groups 3 and 5, sepsis was modeled 2 h after the administration of the drugs. To assess the role of β2AR in the realization of an adrenergic anti-inflammatory mechanism, mortality of mice was recorded in groups 2-4 at 4 and 24 h after sepsis modeling. In all groups, the blood concentration of TNFα, IL-1β, and IL-6 was studied by ELISA using ELISA Kits (MyBioSoure) according to manufacturer's instructions. Monoclonal antibodies MBS494184, MBS494492, MBS335516 to TNFα, IL-1β, and IL-6 (MyBioSoure) were used to determine the concentration of proinflammatory cytokines. The blood for the study was taken from the retroorbital sinus.

The data were statistically processed using Student's $t$ test.

## RESULTS

Administration of β2AR agonist hexaprenaline sulfate reduced animal mortality by 2.33 times in comparison with group 2 (*i.e*. by 20.8%, $p$<0.05) in 4 h after sepsis modeling and by 1.8 times (by 49.9%; $p$<0.05) in 24 h. Administration of β2AR antagonist ICI-118,551 separately and in combination with β2AR agonist did not significantly affect mortality of mice in comparison with control group 2 (Table 1).

The results indicate that β2AR agonist significantly reduced mortality in sepsis, while its administration in combination with β2AR antagonist eliminates this effect. The effect of β2AR agonist of hexaprenaline sulfate is mediated by activation of β2AR in monocyte-macrophage system cells due to activation of the sympathetic nervous system ganglia [3,5]. It is possible that β2AR agonist acts on β2AR of T cells in the spleen producing acetylcholine and block the synthesis of proinflammatory cytokines via activation of α7nAChRs cells of monocytic-macrophage system [5,6,15]. It should be noted that this effect also belongs to the adrenergic anti-inflammatory mechanism, but, apparently, is not the main and decisive in the realization of the anti-inflammatory action of the β2AR agonist.

In control group 2, the concentrations of cytokines TNFα, IL-1β, and IL-6 in mouse blood in 4 h after sepsis modeling increased in comparison with those in the control group 1 by 17.8, 19.5, and 57.7 times ($p$<0.05), respectively; in 24 h, the concentrations of IL-1β and IL-6 decreased in comparison with those in 4 h and exceeded the parameters in control group 1 by 4.5 and 8.2 times ($p$<0.05), respectively (Table 2). The concentrations of TNFα in both control groups did not differ significantly.

β2AR agonist hexaprenaline sulfate reduced blood concentrations of TNFα, IL-1β, and IL-6 by 3.8, 3.3, and 11.2 times in comparison with the control group 2, respectively ($p$<0.05) in 4 h after sepsis modeling (group 3). At the same time, the content of proinflammatory cytokines in the blood significantly ($p$<0.05) exceeded the corresponding parameters of the control group 1. In 24 h after sepsis modeling, the concentrations of proinflammatory cytokines significantly decreased in comparison with the previous term. In this case, the concentration of TNFα did not differ from that in control group 2, and IL-1β and IL-6 remained reduced by 2.6 and 3.0 times ($p$<0.05), respectively.

**TABLE 1**. Effect of β2AR Agonist (Hexaprenaline Sulphate, 1.5 μg/kg), β2AR Antagonist (ICI-118,551, 3 mg/kg) and Their Combination on Mortality of Mice from Sepsis (%; $M\pm m$)

| Group | Time after administration of *E. coli* | |
|---|---|---|
| | 4 h | 24 h |
| Control 2 (sepsis, $n$=55) | 36.4±6.5 | 90.9±3.9 |
| Group 3 (β2AR agonist hexaprenaline sulfate; $n$=32) | 15.6±6.6* | 50.0±9.0* |
| Group 4 (β2AR antagonist ICI-118,551; $n$=30) | 50.0±9.3 | 83.3±7.0 |
| Group 5 (β2AR agonist+β2AR antagonist; $n$=32) | 40.6±8.8 | 78.1±7.4 |

**Note.** *$p$<0.05 in comparison with group 2.

**TABLE 2.** Effect of β2AR Agonist (Hexaprenaline Sulphate, 1.5 μg/kg), β2AR Antagonist (ICI-118,551, 3 mg/kg) and Their Combination on the Concentration of Proinflammatory Cytokines in the Blood of Mice in 4 and 24 h after Sepsis Modeling (pg/ml, *M*±*m*)

| Group | TNFα | | IL-1β | | IL-6 | |
|---|---|---|---|---|---|---|
| | 4 h | 24 h | 4 h | 24 h | 4 h | 24 h |
| Control 1 | 34±5 (*n*=8) | 38±6 (*n*=9) | 26±4 (*n*=8) | 28±5 (*n*=8) | 33±6 (*n*=8) | 25±4 (*n*=8) |
| Group2 (sepsis) | 606±84* (*n*=8) | 55±8° (*n*=5) | 507±68* (*n*=8) | 125±21*° (*n*=5) | 1905±243* (*n*=7) | 205±34*° (*n*=5) |
| Group 3 (β2AR agonist of hexaprenaline sulfate) | 160±28*+ (*n*=7) | 48±8° (*n*=7) | 155±30*+ (*n*=7) | 48±8*+° (*n*=7) | 170±29*+ (*n*=7) | 69±12*+° (*n*=5) |
| Group 4 (β2AR antagonist of ICI-118,551) | 620±107* (*n*=6) | 50±8° (*n*=5) | 510±87° (*n*=6) | 96±18*° (*n*=5) | 1611±250* (*n*=6) | 184±35*° (*n*=5) |
| Group 5 (β2AR agonist+ β2AR antagonist) | 568±99* (*n*=7) | 32±7° (*n*=7) | 445±80* (*n*=7) | 136±29*° (*n*=7) | 1405±262 (*n*=7) | 160±28*° (*n*=7) |

**Note.** Number of animals is shown in parentheses. $p<0.05$ in comparison with *control group 1, +control group 2, °with parameter in 4 h.

After administration of β2AR antagonist ICI-118,551, the concentrations of TNFα, IL-1β and IL-6 in the blood (group 4) in 4 and 24 h after sepsis modeling did not differ significantly from the parameters of the control group 2.

Similar data were obtained after combined administration of β2AR agonist hexaprenaline sulfate and β2AR antagonist ICI-118,551 (group 5), which proves reduction of mouse mortality from sepsis due to suppression of the synthesis of proinflammatory cytokines as a result of direct and indirect (though activation of β2AR of splenic T cells) activation of monocyte-macrophage system cells with β2AR agonist [3,5]. It should be noted that parameters of proinflammatory cytokines in 4 and 24 h after sepsis modeling in group 4 (β2AR antagonist) are by 1.25 times higher than in group 5 (agonist+antagonist of β2AR), which indicates that β2AR antagonist eliminates this anti-inflammatory mechanism via inhibition of β2AR of T cells in the spleen [3.5], which is associated with the subsequent attenuation of acetylcholine effect on α7nAChRs of monocytes and macrophages of the spleen and elimination of TNFα, IL-1β, and IL-6 synthesis reduction.

The blood concentrations of IL-1β and IL-6 in groups 3, 4, and 5 were significantly higher ($p<0.05$) that in the control group 1.

It is known that monocytes and macrophages have βAR and their activation usually leads to anti-inflammatory effect [12] due to inhibition of the nuclear transcription factor NF-κB [14]. The mechanisms underlying reduction of the synthesis of proinflammatory cytokines under the action of β2AR agonist have not been studied sufficiently, and the results of the studies are inconsistent [9,12,13].

Thus, β2 adrenoceptor agonists reduce mortality of mice with sepsis and decrease the content of proinflammatory cytokines TNFα, IL-1β, and IL-6 in the blood by the adrenergic anti-inflammatory mechanism.

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