pression of the myogenic tonus and disturbed regulation of microvessels in skeletal muscles, to induce a complex of circulatory symptoms characteristic for neurocirculatory dystonia.

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# MICROBIOLOGY AND IMMUNOLOGY

# Regulation of the Adenylate Cyclase System in the Lungs of Rats Treated with Plague Toxin

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It is demonstrated that the "murine" lethal toxin of *Yersinia pestis* desensitizes the β-adrenergic receptors coupled to the pulmonary adenylate cyclase system. The degree of adenylate cyclase activation in rat pulmonary membrane preparation by isoproterenol or histamine and the number of β-adrenergic receptors decrease after a 2-h incubation with the toxin.

**Key Words:** *plague toxin; adenylate cyclase; rat lungs; β-adrenergic receptors; histamine $H_1$ receptors*

Experimental intoxication with the bacteria *Yersinia pestis* and their toxins leads to the development of respiratory insufficiency, inflammation, and edema with a high occurrence of lung tissue necrosis [3,5]. The mechanisms underlying these processes have been insufficiently investigated. It is known that hormones and biologically active substances (catecholamines, acetylcholine, serotonin, and histamine) play an important role in lung physiology. They regulate the airway and vascular tone, vascular permeability for electrolytes and water, and the synthesis and secretion of mucus and surfactant via the corresponding receptors coupled to the adenylate cyclase (AC) and Ca-mobilizing systems [9].

In this work we studied the regulatory properties of pulmonary AC and the number of β-adrenergic and histamine $H_1$ receptors in rats treated with the *Yersinia pestis* toxin.

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TABLE 1. Content of $H_1$ Histamine and β-Adrenergic Receptors in the Lungs of Rats Treated with Plague Toxin ($M \pm m$)

| Group of animals | $H_1$ Histamine receptors | | $\beta_2$-Adrenergic receptors | |
|---|---|---|---|---|
| | Bmax, fmol/mg | Kd, nmol | Bmax, fmol/mg | Kd, nmol |
| Control group ($n=10$) | 143±21 | 1.45±0.21 | 288±30 | 1.4±0.18 |
| 1 h of intoxication ($n=10$) | 119±15 | 1.8±0.26 | 270±26 | 1.6±0.21 |
| 2 h of intoxication ($n=10$) | 96.6±10 | 2.40±0.28* | 220±23* | 2.1±0.26* |
| 5 h of intoxication ($n=8$) | 90.8±9.5* | 2.60±0.23* | 212±20* | 2.9±0.32* |

Note. Here and in Table 2 an asterisk indicates significance of differences between control and experimental group at $p<0.05$.

## MATERIALS AND METHODS

Experiments were performed on Wistar rats weighing 150-200 g. Plague toxin (Bekker's fraction II) was injected into the caudal vein (1 mg in 0.5 ml of normal saline, which corresponds to $LD_{100}$). Control animals were injected with normal saline. All procedures were performed under light ether anesthesia. The lungs were excised 1, 2 and 5 h after toxin injection, washed with normal saline, and frozen in liquid nitrogen.

Lung plasma membrane preparations were obtained as described [1]. Binding of [-]-$^3$H-dihydroalprenolol (DHA) was carried out by a method reported elsewhere [10]. Binding of $^3$H-pyrilamine was measured as described [13]. The binding reaction was terminated by the addition of cold buffer (15 ml) followed by passage through GF filters (Whatman, UK). The filters were transferred to vials containing dioxane scintillator, and radioactivity was measured in a Rack Beta counter (LKB, Sweden). The number of β-receptors ($B_{max}$) and the ligand dissociation constant ($K_d$), a value inverse to the receptor affinity, were calculated on a PC using EVDA (Ligand) and IBM-PC McPherson (1984) software.

Adenylate cyclase activity in lung membranes was assayed as previously [6]. The reaction medium contained 50 mM Tris-HCl, pH 7.5 (30°C), 5 mM $MgCl_2$, 0.5 mM cAMP, 0.5 mM isobutylmethylxanthine, 0.1 mM ATP (0.5 μCi $\alpha^{32}$P-ATP], 20 mM creatine phosphate, 0.2 mg/ml creatine kinase, and 0.1 mM GTP. The reaction was initiated by the addition of 10-50 mg protein and conducted for 20 min at 30°C. Protein was measured after Peterson [12]. Statistical analysis was performed using Student's $t$ test.

## RESULTS

The binding experiments showed that 2 h after treatment of rats with plaque toxin (PT) the number of b-adrenoreceptors in pulmonary membranes and the receptor affinity for DHA decreased 1.5 and 1.6 fold, respectively, compared with intact animals (Table 1). The decrease was even greater at the terminal stage.

The maximum number and the affinity of $H_1$ histamine receptors for $^3$H-pyrilamine after 2 h of intoxication were increased 1.5 and 1.3 fold, respectively, compared with the control animals. The decrease was still greater 5 h after toxin administration (Table 1).

Thus, under the action of plague toxin both the maximum number of cAMP-coupled β-adrenergic receptors and the number of $H_1$ histamine calcium-mobilizing receptors are decreased [7,10]. The decrease in the number of $H_1$ histamine receptors may be due to their desensitization by high doses of histamine acting on the lungs during a long time [11]. An increased histamine content, accompanied by a reduced activity of serum histaminase during intoxication with PT, was demonstrated previously [2].

The cAMP-dependent signal transducing system consists of receptor protein, GTP-binding proteins, and the catalytic subunit of AC. Damage to any of these components or derangement

TABLE 2. Regulatory Properties of Adenylate Cyclase (pmol/mg×min) in Lung Membranes of Rats Treated with Plague Toxin ($M \pm m$)

| Group of animals | Baseline activity | Isoproterenol, $10^{-4}$ M | Histamine, $10^{-7}$ M | NaF, $10^{-2}$ M | GIDP, $10^{-4}$ M |
|---|---|---|---|---|---|
| Control group ($n=10$) | 1.5±0.1 | 7.3±0.8 | 3.0±0.3 | 14.9±1.7 | 4.3±0.3 |
| 1 h of intoxication ($n=10$) | 1.7±0.2 | 7.4±0.6 | 2.9±0.3 | 14.8±1.8 | 4.3±0.4 |
| 2 h of intoxication ($n=10$) | 1.4±0.1 | 5.4±0.4* | 2.0±0.2 | 11.8±1.3 | 3.8±0.3 |
| 5 h of intoxication ($n=8$) | 1.3±0.1 | 5.0±0.4* | 1.5±0.2* | 9.3±0.6* | 2.8±0.2* |

of their coupling caused by intoxication may change the cAMP concentration, which affects pulmonary function.

It can be seen from Table 2 that the baseline AC activity (nonstimulated by hormones) does not differ significantly in plasma membranes of control and PT-treated animals.

Under the action of sodium fluoride and guanidylimidodiphosphate (GIDP) AC activity in the lung membranes of control animals increased 10 and 2.8 fold, respectively. At the terminal stage, in PT-treated rats adenylate cyclase sensitivity to these compounds decreased 38 and 35%, respectively. The reduction in the stimulatory potential of these compounds is not due to changes in $K_m$ for ATP and in pH optimum for the adenylate cyclase reaction (data not shown).

In the lungs, catecholamines and histamine realize their activatory effects via the $\beta_2$-adrenergic and $H_2$ histamine receptors coupled to the AC complex [10,14]. It can be seen from Table 2 that isoproterenol and histamine increase AC activity in lung membranes of control animals 4.8 and 2 fold, respectively. After 2 h of intoxication the degree of AC activation by isoproterenol and histamine decreased 26 and 33%, respectively, the decrease being even greater after 5 h of intoxication. The diminished stimulatory potential of isoproterenol toward AC is not accompanied by changes in the enzyme affinity for this agonist (data not shown) and may be attributed to the decreased number and lowered affinity of the β-adrenergic receptors. It is known that upon desensitization caused by long-term action of an agonist on the lung tissue *in vivo*, the number of corresponding receptors decreases and the degree of AC activation by this agonist is lowered [8]. Once the degree of desensitization increases, AC sensitivity drops first to external stimuli (histamine and catecholamines) and then to the nonspecific activators of the AC-complex (guanine nucleotides and sodium fluoride). It should be mentioned that the degree of AC activation by these compounds in the myocardium does not decrease [6]. It is likely that in the lung PT not only impairs coupling of hormone receptors to nucleotide-binding N-protein, but also affects the interaction between the regulatory and catalytic components of the AC complex.

The results obtained may indicate that "murine" PT is not a specific antagonist of β-adrenergic receptors [15], since during intoxication the number of $H_1$ histamine receptors coupled to the calcium mobilizing system also decreases. We think that desensitization of the AC system is associated not only with prolonged action of hormones, the levels of which are markedly increased both during plague intoxication and in toxic shock of other etiology [2,5], but also with the changes in the phospholipid composition of pulmonary plasma membranes induced by free-radical oxidation of lipids, which was demonstrated in our previous work [4].

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