Blood Plasma Proteins Modulate the Broncholytic Effects of a Muscarinic Receptor Antagonist

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Blood plasma proteins modulated the effects of muscarinic receptor antagonist methacin. Administration of methacin in combination with albumin or C-reactive protein (but not with IgG) abolished the broncholytic effect of methacin. It was probably associated with allosteric antagonism to M_2 cholinergic receptors on non-nervous cells, which increased antibody production and mediator response. The concentration of serotonin in mesenteric lymph nodes was high during shock.

Key Words: *anaphylactic shock; albumin; C-reactive protein; serotonin; muscarinic antagonist*

Dysfunction of M_2 cholinergic receptors leads to enhanced release of acetylcholine (ACh) from the vagus nerve; the action of ACh is associated with bronchoconstriction upon contact with the allergen [13]. The broncholytic effect of muscarinic antagonists (methacin, atropine, atrovent, *etc*.) during anaphylaxis is associated with blockade of M_2 cholinergic receptors in the lung tissue [1]. Studying the ACh-dependent mechanism of arresting anaphylactic shock does not take into account the fact that muscarinic antagonists not only interact with blood plasma proteins, but also modulate the effect of vasoactive mediators (*e.g.*, serotonin). Among blood plasma proteins, albumin is characterized by weak cholinesterase activity [9], while C-reactive protein (CRP) can nonspecifically bind ACh [3]. Previous studies showed that serotonin is an allosteric modulator of nicotinic cholinergic receptors, which modifies the effects of ACh [6]. It can be hypothesized that endogenous substances modulate the broncholytic effect of ACh-inducing agents.

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Here we studied the effects of albumin, CRP, and IgG on activity of muscarinic antagonist and concentration of serotonin in lymphoid organs of guinea pigs during anaphylactic shock.

MATERIALS AND METHODS

Experiments were performed on male guinea pigs (n=70) weighing 350 g and obtained from the Rappolovo nursery (Russian Academy of Medical Sciences). The animals were maintained in a vivarium at 23°C and 12:12-h light/dark conditions and had free access to food and water.

The animals were sensitized by subcutaneous injection of 0.1 ml normal equine serum. After 14 days the serum in a challenge dose of 0.3-0.5 ml was administered intracardially to cause anaphylactic shock. The severity of shock was estimated from the Weigle anaphylactic index (AI, four-plus system) [5].

Muscarinic receptor antagonist methacin (2 mg/kg) [2] and purified plasma proteins albumin (500 mg/kg), IgG, and CRP (1 mg/kg, ICN) were injected intraperitoneally 30 min before shock. Methacin in combination with proteins was injected 40 min before shock. The control group consisted of sensitized guinea pigs receiving physiological sa-

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line. Nonresponsiveness of guinea pigs to repeated treatment with equine serum was evaluated 14 days after shock.

Antibody-producing cells were counted 24 h after shock (per 10^6 splenocytes). The animals were immunized with sheep erythrocytes (10^9 cells) 4 days before shock (0.3 ml equine serum intracardially). Sensitized and intact guinea pigs served as the control.

Serotonin concentration in the thymus, spleen, and mesenteric lymph nodes was measured by high-performance liquid chromatography on a Beckman System Gold device using deproteinized tissue samples [10]. Experiments were performed on a LC-4C detector, SphereClone 5 m ODS 2 column (250× 4.60 mm), and SecurityGuard precolumn (ODS 4 mm L×3.0 mm ID, Phenomenex). The mobile phase consisted of 0.1 M citrate-phosphate buffer with 0.3 mM sodium octyl sulfate, 0.1 mM sodium ethylenediaminetetraacetate, and 8% acetonitrile (pH 3.2, Sigma). The sample was analyzed in a column for 20 min (isocratic flow rate 1 ml/min). Identification and quantitative study of the mediator were performed relative to the external standard.

The results were analyzed by Student's *t* test.

RESULTS

Injection of muscarinic receptor antagonist methacin to guinea pigs (n=4) 30 min before induction of anaphylactic shock was followed by a decrease in AI (Table 1). It can be suggested that blockade of muscarinic receptors with methacin prevents the undesirable side effect on the lung tissue. Moreover, ACh directly stimulates nicotinic cholinergic receptors on B lymphocytes and decreases functional activity of cells [11]. As differentiated from CRP, increasing the concentration of albumin or IgG 30 min before shock significantly inhibited the development of edema (n=4) and caused adiaphoria. We revealed a decrease in the anaphylactic response to repeated administration of equine serum in the challenge dose (after 14 days). However, the development of adiaphoria did not prevent anaphylaxis in response to repeated treatment with the challenge dose of serum. Combined administration of methacin and IgG 40 min before shock (n=5) produced an antianaphylactic effect. However, the antianaphylactic effect of methacin and proteins was abolished after administration of methacin in combination with albumin or CRP (Table 1). Our previous studies showed that AI corresponds to 0.40±0.02 points after combined administration of methacin and neostigmine, which is comparable with the spasmolytic effect of euphyllin [4]. Administration of methacin in combination with IgG or neostigmine had a similar effect. However, the effect of combined treatment with these compounds is mediated by various mechanisms. The influence of IgG during anaphylaxis is probably related to receptor antagonism between this agent and allergen-specific IgE or IgG1, which contributes to a decrease in the sensitivity of effector cells to an allergen [12]. It cannot be excluded that nonspecific ACh ligands albumin and CRP [3,7,8] decrease the vasodilator effect of ACh on endothelial cells. The anaphylactic response decreased with increasing the concentration of albumin or CRP 30 min before shock induction (Table 1), which was

TABLE 1. Effect of Cholinergic Agents and Blood Plasma Proteins on Anaphylactic Shock and Antibody Production in Guinea Pigs

Agent	AI after administration of agents in the pathochemical stage[1]	Number of antibody-producing cells per 10^6 splenocytes[2]
Sheep erythrocytes	0	100±10
Control (equine serum, ml)	4.0±0.0	880±12^{+++}
Methacin, 2 mg/kg	2.2±0.1**	188±20^{+}
Albumin, 500 mg/kg	1.00±0.01**	240±10^{+++}
CRP, 1 mg/kg	2.5±0.3*	217±13^{++}
IgG, 1 mg/kg	2.3±0.2**	184±45
Methacin (2 mg/kg)+albumin (500 mg/kg)	4.0±0.5	900±40^{+++}
Methacin (2 mg/kg)+CRP (1 mg/kg)	3.8±0.1	980±43^{+++}
Methacin (2 mg/kg)+IgG (1 mg/kg)	0.5±0.1**	130±14

Note. [1]Sensitized guinea pigs after intracardiac administration of 0.5 ml equine serum; [2]sensitized guinea pigs after administration of 0.3 ml equine serum. *p<0.01 and **p<0.001 compared to the control; ^{+}p<0.05, ^{++}p<0.01, and ^{+++}p<0.001 compared to the animals immunized with sheep erythrocytes.

TABLE 2. Serotonin Concentration in Lymphoid Organs of Guinea Pigs after Anaphylactic Shock (ng/mg protein)

Experimental conditions	Thymus	Spleen	Lymph nodes
Basal concentration	1.081±0.004	1.532±0.004	0.981±0.004
No treatment	0.541±0.004	0.443±0.005	2.104±0.004
Methacin, 2 mg/kg	1.256±0.004	1.452±0.004	1.570±0.005
Albumin, 500 mg/kg	1.182±0.004	1.234±0.005	0.826±0.006
CRP, 1 mg/kg	1.380±0.005	1.422±0.004	1.631±0.005
IgG, 1 mg/kg	1.272±0.004	1.410±0.004	1.508±0.004
Methacin (2 mg/kg)+albumin (500 mg/kg)	1.640±0.004	1.970±0.004	2.404±0.005
Methacin (2 mg/kg)+CRP (1 mg/kg)	1.570±0.006	1.824±0.004	2.322±0.004
Methacin (2 mg/kg)+IgG (1 mg/kg)	1.168±0.006	1.091±0.004	0.670±0.005

probably related to the interaction of proteins with equine serum-induced ACh.

Combined administration of methacin and IgG suppressed the pathochemical stage of shock, which normalized B lymphocyte activity (Table 1, n=4) and serotonin concentration in lymphoid organs (Table 2). It should be emphasized that antibody production and serotonin concentration were high in lymph nodes of control animals. Published data show that B lymphocytes of lymph nodes are involved in inflammation [9] and may serve as the source of serotonin [14]. It cannot be excluded that blockade of muscarinic cholinergic receptors on lymphocytes by methacin decreases antibody production. Moreover, excess ACh probably produces a modulatory effect on serotonin concentration in lymphocytes. This hypothesis requires further investigations.

Our results indicate that blood plasma proteins modify the effectiveness of methacin. IgG potentiates, while albumin and CRP abolish the antianaphylactic effect of methacin. Blood plasma proteins can simulate the anaphylactic response. They protect other antiinflammatory mechanisms (IgG) and serve as allosteric modulators that cause dysfunction of muscarinic cholinergic receptors or ACh ligands (albumin and CRP). Desensitization has a normalizing effect on activity of B lymphocytes and serotonin concentration in lymphoid organs.

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