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Cholinergic modulation of anaphylactic shock: plasma proteins influence

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Abstract

Cholinergic drugs can modulate anaphylactic shock and change lymphocyte functions. Plasma proteins modulate effects of muscarinic antagonists during anaphylactic shock. The present investigation was carried out to study the antianaphylactic activity of methacine (antagonist at muscarinic receptors) in combination with neostigmine (anticholinesterase drug). However, it is not known whether plasma proteins-albumin, C-reactive protein (CRP) and immunoglobulin G (IgG) – modify the effects of cholinergic drugs like methacine, serotonin (5-HT) level in the lymphoid organs and quantity of antibody-forming cells (AFC) in the spleen of guinea pigs during experimental anaphylactic shock. It was shown that administration of methacine with neostigmine (40 min and 15 min prior to shock induction, accordingly) at the pathochemical stage revokes shock development. By blocking cholinesterase endogenous acetylcholine is increased and methacine blocks muscarinic receptors and therewith unwanted side effects in the airways (bronchoconstriction) and heart (bradycardia). Administration of the combination of methacine with neostigmine at the immunological stage (guinea pig sensitization) does not affect the course of anaphylactic shock. Administration of methacine with IgG at the pathochemical stage of shock significantly decreases shock intensity, while administration of methacine with CRP or albumin has no influence on the shock. Administration of IgG or CRP (not albumin) at the immunological stage of shock and albumin or IgG (not CRP) at the pathochemical stage leads to reduction of the anaphylactic reaction. Application of methacine with neostigmine or IgG (effective combinations of drugs) results in normalization of antibody response in the spleen and 5-HT level in the lymphoid organs. Administration of methacine with CRP or albumin (ineffective combinations of drugs) leads to increase of antibody response in the spleen and 5-HT level in the lymphoid organs. Administration of hexamethonium or aceclidine aggravated anaphylactic shock reaction. Thus, the combination of methacine with neostigmine can regulate the pathochemical stage of shock and the 5-HT release. At the pathochemical stage of shock IgG increases the antianaphylactic activity of methacine, but albumin and CRP abolish it.

Keywords: B cell; C-reactive protein; Immunoglobulin G; Serotonin; Anaphylactic shock; Cholinergic drugs

Introduction

The cholinergic system plays a crucial part in the pathogenesis of anaphylactic shock. The broncholytic effect of muscarinic antagonists during anaphylactic reaction is connected with the block of muscarinic cholinoreceptors (AChR) in the smooth muscle cells and mast cells of the respiratory tract, therefore the sensitivity of effector-cells to IgE or IgG1 complexes with allergen is reduced (Gushhin, 1994). It is known that neostigmine (anticholinesterase drug) increases the protective action of methacine (antagonist at muscarinic receptors) (Losev, 1985). By blocking cholinesterase endogenous acetylcholine (ACh) is increased and methacine blocks muscarinic receptors and therewith unwanted side effects in the organism. An immune and neuromediator response, particularly the serotonin (5-HT) response of the lymphoid organs, induced by the cholinergic drugs is not investigated during anaphylactic shock. At the same time it is known that muscarinic antagonists affect B cell activity (Kawashima and Fujii, 2003). It is also known that 5-HT can play a role of n-AChR endogenous allosteric activator (Schrattenholz et al., 1996; Garcia-Colunga and Miledi, 1999) that confirms a functional relationship between the cholinergic and serotonergic systems.

It is found that albumin is the protein with unspecific binding property (Hansen et al., 2002) and weak esterase-like activity (Liederer and Borchardt, 2006). C-reactive protein (CRP) can unspecifically interact with the various ligands, including

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biologically active substances (Nazarov, 2001); in particular it binds ACh (Nazarov et al., 2006). Effect of immunoglobulin G (IgG) under anaphylactic shock is connected with its protective function (Strait et al., 2006). The influence of plasma proteins on the effects of muscarinic antagonist methacine has not yet been ascertained during anaphylactic shock.

The goal of the present work was to investigate the possibility of simultaneous stimulation of n-AChR and block of m-AChR, role of plasma proteins (albumin, CRP, IgG) in the broncholytic effect of methacine and 5-HT level in the lymphoid organs during anaphylactic shock in guinea pigs.

Materials and methods

Animal model of anaphylactic shock

The study was carried out on male guinea pigs ($n=145$) weighing 350 g with the guidelines for animal experimentation set by Institute of Experimental Medicine, the Russian Academy of Medical Sciences. Guinea pigs were allowed access to food and water ad libitum and maintained at 23 °C under a cycle of 12-h light/12-h darkness.

Sensitization of guinea pigs (immunological stage) was performed with a subcutaneous injection of 0.1 ml of normal horse serum (HS) (Stavropol, Russia).

It is known that the immunological stage of anaphylactic shock is connected with the production of IgE or IgG1 by B cells and sensitization of mast cells and basophiles (Brown, 1995).

Anaphylactic shock (pathochemical stage) was induced 14 days after injection of a sensitizing dose of HS. Animals received a challenging dose of HS (0.5 ml) with the intracardiac injection.

The pathochemical stage is the clinical manifestation of anaphylaxis. On reexposure to an antigen, antigen cross-linking of antigen-specific IgE bound to mast cell FcεRI stimulates mast cell degranulation, with the rapid release of histamine and other mediators which are implicated in vascular permeability changes, flushing, angioedema, hypotension and bronchoconstriction (Hepner and Castells, 2003).

The refractory status, connected with the pathochemical stage of shock, was estimated in guinea pigs, which in 14 days after survival the anaphylactic shock were again given an intracardiac injection of a challenging dose (0.5 ml) of HS. The refractory status, connected with the immunological stage of shock, was estimated in guinea pigs, which in 30 days after survival the anaphylactic shock were again sensitized with a subcutaneous injection of 0.1 ml of HS, and in 14 days after this injection received an intracardiac injection of a challenging dose (0.5 ml) of HS.

The severity of anaphylactic shock was estimated with the Weigle index (arbitrary units) (Khaitov et al., 2000).

Treatment with cholinergic drugs and plasma proteins

At the immunological stage of shock muscarinic antagonist methacine (ICN Octjabr', Russia) (2 mg/kg), cholinesterase inhibitor neostigmine (ICN Octjabr', Russia) (0.02 mg/kg) (Mashkovskiy, 2001) were given simultaneously with HS injection. Methacine (2 mg/kg) in combination with neostigmine (0.02 mg/kg) were applied 40 min and 15 min before administration of HS, accordingly. CRP (ICN, USA), IgG (ICN Octjabr', Russia) in a dose 1 mg/kg and albumin (Budapest, Hungary) in a dose 500 mg/kg were given simultaneously with HS or with methacine and HS (intraperitoneal injection).

At the pathochemical stage of shock methacine (2 mg/kg), muscarinic agonist aceclidine (Nizhfarm, Russia) (0.35 mg/kg), nicotinic antagonist hexamethonium (Nizhfarm, Russia) (10 mg/kg) (Mashkovskiy, 2001), neostigmine (0.02 mg/kg) were introduced 30 min prior to shock with an intraperitoneal injection. Methacine in combination with neostigmine (2 and 0.02 mg/kg, accordingly) were applied: 1) 15 min prior to shock, simultaneously, 2) methacine 30 min, neostigmine 15 min prior to shock, 3) methacine 40 min, neostigmine 15 min prior to shock induction. Methacine in combination with plasma proteins were given 40 min prior to shock. Plasma proteins were introduced 40 min prior to shock.

The reference drug was eufilline (5 mg/kg) (Mashkovskiy, 2001). Control group consisted of sensitized guinea pigs which received physiological solution.

Table 1
Effects of cholinergic drugs and plasma proteins on the anaphylactic shock

Drug	Anaphylactic index (arbitrary units)	
	Immunological stage [a]	Pathochemical stage [b]
Control (salt solution)	4.0±0.0	4.0±0.0
Methacine (2 mg/kg)	3.4±0.3	2.2±0.1
Neostigmine (0.02 mg/kg)	3.8±0.1	2.3±0.2**
Albumin (500 mg/kg)	3.5±0.3	1.0±0.01***
CRP (1 mg/kg)	2.7±0.2***	2.5±0.3***
IgG (1 mg/kg)	1.5±0.1***	2.3±0.2***
Methacine (2 mg/kg)+neostigmine (0.02 mg/kg)	4.0±0.0	0.4±0.02***
Methacine (2 mg/kg)+albumin (500 mg/kg)	2.0±0.2***	4.0±0.5
Methacine (2 mg/kg)+CRP (1 mg/kg)	2.9±0.4*	3.8±0.1
Methacine (2 mg/kg)+IgG (1 mg/kg)	2.9±0.4*	0.5±0.1***
Eufilline (5 mg/kg)	4.0±0.1	0.6±0.2***

Methacine, neostigmine and purified plasma proteins (albumin, CRP, IgG) were given 30 min prior to shock. When being used in the combination, methacine and neostigmine were introduced 40 and 15 min prior to shock, accordingly, while methacine and plasma proteins were introduced 40 min prior to shock.
Anaphylactic reaction intensity is evaluated by the Weigle index (Khaitov et al., 2000):

\+ – short-term nose scratch, roughing of hair, temperature decrease (no less than 1 °C);

++ – well-defined frequent scratches, single sneezing, temperature decrease;

+++ – spastic cough, lateral position of the animal, dejection and urination;

++++ – respiratory tract spasm, convulsive jumps, cramps. Animals die at 5th min (agonal period).

All values are presented as the mean±SEM from 5 animals in each groups. *$p<0.05$ vs. control, **$p<0.01$ vs. control, ***$p<0.001$ vs. control. Control group consists of rats which received intracardiac injection of 0.5 ml HS.

[a] Immunological stage: administration of drugs simultaneously with a sensitizing dose of HS (0.1 ml, subcutaneous injection).

[b] Pathochemical stage: administration of drugs in sensitized guinea pigs just before introduction of a challenging dose of HS (0.5 ml, intracardiac injection).