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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, γ -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.

Key words: 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 (A χ hR) 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 et al., 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., 2001) and weak esterase-like activity (Liederer and Borchardt, 2006). C-reactive protein (CRP) can unspecifically interact with the various ligands, including 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 Fc ϵ RI 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.

Immunizations and AFC assay

Guinea pigs were immunized with sheep red blood cells (SRBC; 10^9 cells/animal) 4 days prior to injection of a challenging dose (0.3 ml) of HS. Quantity of antibody-forming cells (AFC/ 10^6 of splenocytes) in the spleen was determined 24 hours after administration of a challenging dose of HS (Jerne et al., 1974). Positive and negative control groups consisted of sensitized and intact guinea pigs, accordingly.

Determination of serotonin

The content of serotonin (5-HT, ng/g) in the thymus, spleen, mesenteric lymph nodes was determined by HPLC method in deproteinized tissue extracts at the pathochemical stage of shock (Rogausch et al., 2003).

Statistical analysis

All values are presented as the mean $\pm$ SEM. Statistical processing of results was carried out using Student's t-test.

Results

Effects of cholinergic drugs and plasma proteins on the anaphylactic shock

Administration of methacine as well as neostigmine simultaneously with a sensitizing dose of HS has a little influence on the shock induction (intracardiac injection of a challenging dose (0.5 ml) of HS) in comparison with animals which received saline (control) (Table). Application of methacine 40 min and neostigmine 15 min prior to injection of a sensitizing dose of HS does not

affect anaphylactic reaction outcome, but is characterized by prolongation of the agonal period of shock (15.0 ± 0.1 min in experimental group; 5.0 ± 0.1 min in control; $p < 0.001$, $n = 10$). Methacine in combination with IgG or albumin, but not with CRP, significantly decreases anaphylactic reaction as compared with control (Table).

Administration of IgG or CRP (not albumin) simultaneously with a sensitizing dose of HS leads to reduction of anaphylactic reaction in comparison with control (animals which received saline). Antianaphylactic action of IgG is more marked in comparison with such effect of CRP (Table).

Administration of methacine or neostigmine 30 min prior to shock (pathochemical stage) reduces anaphylactic reaction as compared with control (animals which received saline). Conversely, administration of hexamethonium or aceclidine does not reduce anaphylactic reaction index, but increases shock severity as is shown by the measurement of agonal period length: the agonal period is 2.0 ± 0.1 min in experimental group and 5.0 ± 0.1 min in control group ($p < 0.001$, $n = 5$).

Administration of methacine with neostigmine, both 15 min prior to shock induction, decreases anaphylactic reaction up to 1.8 ± 0.3 arbitrary units, and administration of methacine 30 min with neostigmine 15 min prior to shock induction reduces it up to 0.8 ± 0.1 arbitrary units as compared with control (4.0 ± 0.0 arbitrary units) ($p < 0.001$, $n = 5$). Application of methacine 40 min with neostigmine 15 min prior to shock induction results in most effective reduction of anaphylactic reaction – 0.4 ± 0.02 arbitrary units, and this effect is comparable with the effect of eufilline (reference drug), which is used for the treatment of immediate hypersensitivity.

Administration of methacine with IgG (both 40 min prior to shock) leads to the antianaphylactic effect, comparable with the effect of methacine in combination with neostigmine or with the effect of eufilline, while introduction of methacine with albumin or CRP revokes methacine action (Table).

Administration of albumin or IgG (not CRP) 30 min prior to shock prevents edema formation ($p < 0.001$, $n = 10$) and leads to the refractory status: decrease of anaphylactic response on the repeated injection (in two weeks) of a challenging dose of HS. At the same time this refractory status does not abolish anaphylactic reaction upon antigen administration.

AFC response and 5-HT level in the lymphoid organs

Guinea pigs which survived anaphylactic shock (intracardiac injection of a challenging dose of 0.3 ml HS; anaphylactic index is 3.0 ± 0.4 arbitrary units) have a high level of antibody response (the number of AFC in positive control is $1100 \pm 30/10^6$ of splenocytes, in negative control is $200 \pm 10/10^6$ of splenocytes; $p < 0.001$, $n = 5$). 5-HT concentration decreases in the thymus and spleen, but not in the lymph nodes, in comparison with its basal concentration (Fig.). Administration of methacine with neostigmine (40 and 15 min prior to shock, accordingly) or methacine with IgG (both 40 min prior to shock) results in effectively prevention of the pathochemical stage of shock: anaphylactic index is 0.4 ± 0.02 and 0.5 ± 0.1 arbitrary units, accordingly. These combinations lead to the normalization of B cell activity (the number of AFC is $200 \pm 10/10^6$ of splenocytes) and 5-HT level in the lymphoid organs (Fig.). When using methacine with CRP or methacine with albumin at the pathochemical stage of shock, the index of anaphylactic reaction is 2.7 ± 0.1 and 2.9 ± 0.3 arbitrary units, accordingly. Guinea pigs which survived shock have a great number of AFC in the spleen ($1200 \pm 40/10^6$ of splenocytes in experimental group and $200 \pm 10/10^6$ of splenocytes in control; $p < 0.001$, $n = 5$) and high concentration of 5-HT in the lymphoid organs as compared with the basal parameters (Fig.).

Discussion

Administration of methacine with neostigmine (40 min and 15 min prior to a sensitizing dose of HS injection, accordingly) at the immunological stage does not abolish sensitization, but results in retardation of agonal period up to 15 min vs. 5 min in control (sensitized guinea pigs which

received saline). Methacine in combination with neostigmine and, to a lesser degree, methacine in combination with IgG or CRP, when using upon sensitization, reduces the index of anaphylactic reaction. Increase in IgG or CRP (not albumin) concentration at the stage of sensitization leads to decrease of the anaphylactic reaction index. We have established that introduction of methacine 40 min and neostigmine 15 min prior to administration of a challenging dose of HS (pathochemical stage) effectively arrests shock. Administration of methacine in combination with plasma proteins at the pathochemical stage revealed that the combination of methacine with IgG has the most effective antianaphylactic action. Methacine in combination with albumin or CRP does not affect anaphylactic shock. Increase in albumin concentration 30 min before shock induction results in significant prevention of anaphylactic reaction. Administration of IgG or CRP before shock induction decreases anaphylactic reaction too, but to a lesser degree.

Under anaphylactic shock horse serum increases antibody-producing B cell activity and has unlike influence on 5-HT level in the lymphoid organs: decreases 5-HT concentration in the thymus and spleen, but increases it in the lymph nodes as compared with the basal concentration of 5-HT.

Ineffectiveness of the combination methacine and neostigmine in anaphylactic shock prevention at the immunological stage (stage of sensitization) appears to be connected with the immunotropic activity of methacine. Using the model of primary immune response we have shown that methacine leads to maximum increase of AFC response at the productive phase of immune response (14th day), and neostigmine possesses a low immunotropic activity (unpublished data). The higher antianaphylactic activity of the combination of methacine and albumin as compared with the combination of methacine and CRP or IgG at the immunological stage needs further investigation. It is known that IgG can decrease IgE production (Strait et al., 2006). It is possible that IgG and CRP with HS are able to form inactive complexes like [HS-IgG] that prevent potentiating action of HS components on the reagin-forming B cell activity. This assumption is confirmed with indirect clinical evidences: in patients with acute allergic reactions high plasma CRP level is accompanied by low plasma histamine concentration (Lin et al., 2001).

Previously, when studying the pathochemical stage of anaphylactic shock, we have shown that administration of methacine 15, 30 and 40 min prior to shock results in its reduction up to $2.3-2.5 \pm 0.2$ arbitrary units. Administration of neostigmine 15 and 30 min prior to shock induction decreases anaphylactic reaction up to $2.5-2.8 \pm 0.2$ arbitrary units. Administration of neostigmine 40 min prior to shock induction increases anaphylactic reaction up to 3.5 ± 0.3 arbitrary units vs. 4 ± 0.0 arbitrary units in control (animals which received saline) (Nezhinskaya et al., 2004). We suppose that administration of the combination of methacine with neostigmine (40 and 15 min prior to shock induction, accordingly) is beneficial because by blocking cholinesterase endogenous ACh is increased and methacine blocks muscarinic receptors and therewith unwanted side effects in the airways (bronchoconstriction) and heart (bradycardia). Aceclidine (agonist at m-AChR) increases anaphylactic shock reaction that evidences the importance of m-AChR block. Application of ganglionic blocker hexamethonium leads to intensification of anaphylactic reaction that may be connected with the block of n-AChR of sympathetic ganglion (Mashkovskiy, 2001) and the block of n-AChR on B cells, while it is known that their stimulation results in B cell functional activity impairment (Sato et al., 1999). Antianaphylactic effect of methacine with IgG at the pathochemical stage (0.5 ± 0.1 arbitrary units) appears to be connected with the blockade of respiratory tract m-AChR by methacine and protective action of IgG. It is known that IgG are able to form the complexes with Fc γ -receptors on mast cells and basophiles (Strait et al., 2006). Combination of methacine with albumin or CRP does not affect anaphylactic shock that is possibly due to unspecific binding of methacine by proteins. Methacine itself or plasma proteins (albumin, CRP, IgG), when using before shock induction, reduce anaphylactic reaction. These suppositions need further investigation.

Lymph node B cells are known to be involved in inflammation (Mizoguchi and Bhan, 2006). B cells are affected with 5-HT_{1A} (Iken et al., 1995). It is established that CD20⁺ B cells are also the source of 5-HT_{1A} (Yang and Lackner, 2004). Effective combination of methacine with neostigmine (40 and 15 min prior to shock, accordingly) or methacine with IgG (both 40 min prior to shock) normalize antibody response of the spleen and 5-HT concentration in the lymphoid organs. It is not inconceivable that methacine due to the block of m-AChR on B cells reduces the antibody response and abundance of ACh, resulting in acetylcholinesterase inhibition, modifies 5-HT level in lymphocytes. These suppositions need further investigations.

Conclusion

In the present paper we have shown that methacine itself or in combination with neostigmine (40 min and 15 min prior to shock induction, accordingly) or with IgG (both 40 min prior to shock induction) effectively prevents the pathochemical stage of anaphylactic shock. Methacine in combination with albumin or CRP (40 min prior to shock induction) does not affect the course of anaphylactic shock. The beneficial effect of a methacine together with neostigmine on the pathochemical stage results in normalization of B cell activity and reduction of 5-HT level up to its basal concentration in the lymphoid organs. Plasma proteins may regulate the immunological (IgG and CRP) and pathochemical (albumin and IgG) stages of anaphylactic shock.

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Table and Figure legends

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

¹ – immunological stage: administration of drugs simultaneously with a sensitizing dose of HS (0.1 ml, subcutaneous injection); ² – pathochemical stage: administration of drugs in sensitized guinea pigs just before introduction of a challenging dose of HS (0.5 ml, intracardiac injection). 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 1st ?);
- ++ – 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 $\pm$ 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.

Fig. Effects of combinations of methacine with neostigmine or plasma proteins (albumin, CRP, IgG) on the serotonin level in the lymphoid organs of guinea pigs.

1 – thymus, 2 – spleen, 3 – lymph nodes.

Methacine and neostigmine were given 40 and 15 min prior to shock, accordingly. Methacine in combination with plasma proteins (albumin, CRP, IgG) were used 40 min prior to shock. Values are presented as the mean $\pm$ SEM from 5 animals in each groups. $p < 0.05$ vs. 5-HT basal concentration.

Table

Effects of cholinergic drugs and plasma proteins on the anaphylactic shock

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

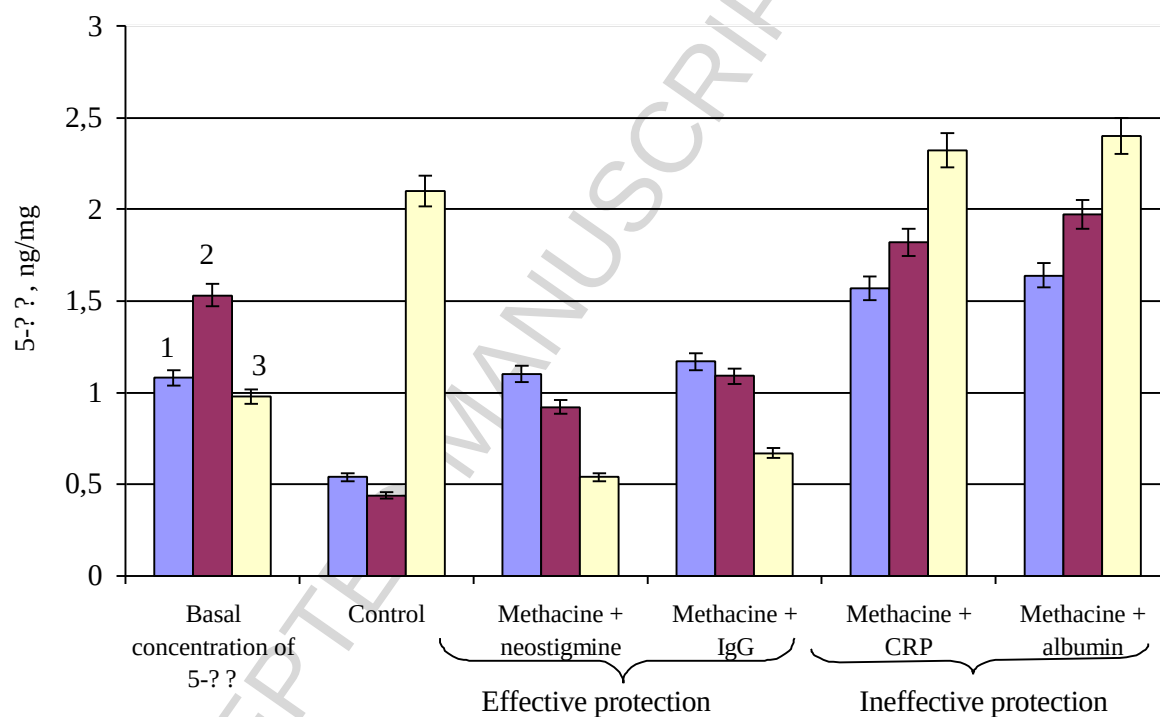


Fig. Effects of combinations of methacine with neostigmine or plasma proteins (albumin, CRP, IgG) on the serotonin level in the lymphoid organs of guinea pigs.