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Influence of Mepacrine on the Reaction of Adoptive Cutaneous Anaphylaxis

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Abstract

In a test of adoptive cutaneous anaphylaxis, the influence of the phospholipase A₂ inhibitor mepacrine, on the intensity of the local anaphylactic reaction was investigated in the skin of recipients following intracutaneous injection of syngenic immune splenocytes. Injection of the mepacrine solution with **preincubated** sensitized splenocytes inhibits the cutaneous anaphylactic reaction after a single intravenous administration of allergen to recipients. The inoculation of immune splenocytes, preincubated in mepacrine but without the phospholipase A₂ inhibitor, to the skin of syngenic recipients is accompanied by less suppression of the local skin anaphylactic reaction than with a common injection of mepacrine with immune splenocytes.

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Introduction

The present knowledge of the molecular bases of allergy development is founded on the extremely important role of the phospholipase A₂ system in the formation of immunological, pathochemical and patho-physiological hypersensitivity mechanisms. The permissive function of phospholipase A₂ in the induction of the synthesis by T cells of IgE-binding factors in the presence of bradykinin or the factor intensifying the glycosylation is known [1]. Evidence of the role of membrane phospholipids in the expression of surface antigens [2], as well as data on the amphipathic nature of FcR [3], whose affinity depends on phospholipid contamination, show the importance of the membrane phospholipid system not only in the transduction of a membrane signal but also in the mechanism of ligand-receptor interactions. Information on the phospholipase A₂ role in the isotype regulation mechanism of the immune response acquires special importance in connection with reports on the availability of a structural interaction between FcR and phospholipase A₂ in membranes of lymphocytes [4] and macrophages [5]. The aim of the present investigation was to study the influence of the phospholipase inhibitor, mepacrine, on the reaction of adoptive cutaneous anaphylaxis, the expression of which is determined by the relation of the most important pathogenetic mechanisms of allergy: IgE secretion by transplanted lymphocytes, mediator formation by mast cells and macrophages of recipient tissues, as well as by vascular reactivity of the transplantation locus to the action of endogenous mediators [6].

Materials and Methods

The experiments were performed on male Fischer rats. Twenty donor rats were immunized by a single intraperitoneal administration of 0.1 ml of ambrosia pollen allergen (20,000 PNU/ml, USSR). 12 days after the allergen injection, a splenocyte suspension was prepared from the dispersed spleen tissue freed of erythrocytes by the osmogenic shock. The reaction of

adoptive cutaneous anaphylaxis [7] was implemented by intracutaneous injection of a $0.1 \text{ ml/l} \times 10^6$ splenocyte suspension in a 0.15 M NaCl solution into three equally spaced points on the body of the rat recipients. For the reaction 7 donor splenocyte suspensions with a vital capacity of up to 95% were selected. Each recipient received splenocytes from only 1 donor. Splenocyte preincubation was performed for 30 min in 0.02, 0.2, and 0.4 mM mepacrine solutions (Avion, England) dissolved in 0.15 M NaCl. Control samples of splenocytes, preincubated with 0.15 M NaCl, were intracutaneously injected contralaterally. 24 h after injection of normal and immune suspensions of lymphocytes, 0.4 ml of ambrosia pollen allergen (20,000 PNU/ml) and 0.6 ml of Evans blue were intravenously injected into the recipients.

During a special series of the experiment, 7 animals received intracutaneous injections of donor splenocyte suspension preincubated in a 0.02- mM mepacrine solution after which phospholipase A2 inhibitor had been washed out by double centrifugation in 0.15 M NaCl. The results were recorded 30–50 min later and expressed as a product of the maximum and minimum diameters of the dye skin extravasate. Statistics processing was done using the Student method.

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Results

The injection of splenocytes preincubated with mepacrine solutions was accompanied by suppression of the local anaphylactic reaction in the skin dependent on the concentration of phospholipase A2 inhibitor (table 1). Because the size of the dye skin extra-vasate in the reaction of adoptive cutaneous anaphylaxis is the integral indicator dependent on the activity of reagin-secreting cells and the reactivity of the recipient tissues to the mediator action, the results obtained do not explain the mechanism of the established anaphylactic action of mepacrine.

When performing the reaction of adoptive cutaneous anaphylaxis with splenocytes preincubated in a 0.02 mM solution of mepacrine, but washed of phospholipase A2 inhibitor, the experiment excludes the possibility of mepacrine influencing the cell elements of the transplantation locus: a not very essential suppression of the skin anaphylactic reaction was noted in comparison with common injection of splenocytes and mepacrine. While the size of extravasate was suppressed by 79.2% when injecting a splenocyte suspension in a 0.02 mM mepacrine solution, injection of splenocytes washed from mepacrine was accompanied by extravasate suppression of only 44.1% ($p < 0.05$). In this connection the anaphylactic mepacrine action in the test of adoptive cutaneous anaphylaxis should be considered as a manifestation of an inhibiting action of phospholipase A2 blocker on both the process of reagin secretion by immune splenocytes and the mechanisms of pathochemical and vascular reaction in the transplantation locus.

Conclusion

The established dependence of the reagin secretion process by sensitized splenocytes on the activity of the phospholipase A2 fermentative system agrees with the existing knowledge on the mechanisms of isotype-specific regulation of the immune response. The pharmacological inhibition of phospholipase A2 by mepacrine, being the coupling system between the action upon the lymphocyte of the factor intensifying glycosylation and the process of formation of the IgE-binding factors, represses the IgE products similar to the lipomodulators also possessing antiphospholipase activity. Mepacrine blocking of the local vascular reaction in the test of adoptive cutaneous anaphylaxis

Table 1. Size of dye extravasates when performing reaction of adoptive cutaneous anaphylaxis with immune splenocytes preincubated and not preincubated in mepacrine

Experiment Mepracrine Size of dye p
form concentration extravasate, cm²
mM

Inoculation
of intact immune

splenocytes	-	1.11 ± 0.10	
Inoculation of 0.02	0.23 ± 0.04	< 0.05	
immune splenocytes	0.2	0.15 ± 0.04	< 0.05
with mepacrine	0.4	0.018 ± 0.001	< 0.05

Mean ± SEM from 28 determinations on 7 donors and 7 recipients, is the reliability index of difference from the control.

laxis may be associated with suppression of arachido-nate liberation in phospholipase reaction and accumulation of its metabolites in the recipient tissues.

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