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Advances in Histamine Research

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ADVANCES IN HISTAMINE RESEARCH

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Welcome Address

Welcome Address

K. Tasaka

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Ladies and gentlemen!
It is a great pleasure and honor for me to express my hearty welcome to all of you. Further, I would like to extend my great gratitude to the invited speakers who have traveled far distance in order to participate to this symposium.

Since early works on histamine have been brought about by extremely capable people in Europe at the first decade of this century, much progress in histamine research have been made in many fields. Nowadays, a number of papers on histamine research are reported not only by the pharmacologists but also from almost all fields of the basic sciences in globar scale. If my knowledge is correct, the first International Histamine Symposium was held in 1955 at Ciba Foundation and all papers presented in this symposium were published as one of the books in Ciba Foundation series under the title of Histamine. This book stimulates young students tremendously. Since then, international symposia some way related to histamine were opened several times and some of them are held in splendid circumstances. In this histamine symposium, simultaneous translation fascilities are not available. However, I do not think this does not hinder mutual communication at all. At the opening ceremony of 8th International Congress of Pharmacology, Prof. Ebashi told us that there are three kinds of English. The first two are the Queen's English and American English. That is no question about it. But the third one is just new for me and it was called as International English. I understand what he mentioned is if we can communicate anyhow through English, that will be all right. As far as English is concerned, I think International English is perfectly acceptable in Histamine Symposium. The most important thing is to speak what you found and to discuss about that. Furthermore, this kind of meeting provides good chance to meet each other. I know that most of invited speakers are already intimate friends each other. However, for domestic participants, this must be very good chance to meet so many of distinguished people at one time.

I sincerely hope that each participant can pick up something in this symposium and you can leave here with renewed hope or even greater enthusiasm at the end of this symposium. In the last, let me speak my deep gratitude to all the participants to this symposium, again.

Thank you.

Histamine Release

Antigen-antibody Reaction on the Black Lipid Membrane and Histamine Release from Antigen-coated Liposome in Immune Response

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ABSTRACT

When antigen (egg albumin) was added into the one side of aqueous phases separated by the black lipid membrane coated with IgE rich antiserum, a marked decrease of electrical resistance was elicited. Dose-dependent decrease of electrical resistance of the black lipid membrane was also observed after the addition of compound 48/80 at concentrations higher than 3×10^{-6} g/ml. The capacitance of black lipid membrane was 0.3 $\mu F/cm^2$ and electrical resistance was $10^6 \Omega \cdot cm^2$. When antigen-coated, histamine (Hi) containing unilamellar liposomes were exposed to IgE rich antiserum, Hi release takes place. When the liposomes prepared with the lipid mixture extracted from rat mast cells were used, a greater extent of Hi release was induced than those made from a mixture of phosphatidylcholine and cholesterol. When compound 48/80 was added into liposomal suspension at concentrations higher than 10^{-6} g/ml, Hi release was induced in a dose-dependent fashion. The order parameter which is reciprocal of the membrane fluidity was diminished by compound 48/80 at concentrations higher than 3×10^{-6} g/ml dose-dependently. These findings indicate that an increase of membrane permeability which is exhibited by the decreases of electrical resistance and order parameter of lipid membrane may be the reason for Hi release in IgE-mediated antigen-antibody reaction elicited on the surface of liposome.

KEYWORDS

Black lipid membrane; liposome; IgE; antigen-antibody reaction; histamine release; membrane fluidity; membrane conductance; membrane permeability.

INTRODUCTION

Within a few years, the knowledge of Hi release from rat mast cell advanced very rapidly not only in morphological but also in biochemical aspects. Recently, Ishizaka and her colleagues (1978) submit a working hypothesis of IgE-mediated Hi release; the aggregation of IgE receptors may take place in antigen-antibody reaction and this may be a key event for Hi release from rat mast cell. As a conceivable reason for aggregation of IgE receptors, an increase of the membrane fluidity may precede the aggregation. Hirata and Axelrod (1978) pointed out monomethylation of phosphatidylethanolamine is a major intermediate to increase the

membrane fluidity of erythrocyte ghost. Also, in rat reticulocyte, isoproterenol stimulates mono-methylation of phosphatidylethanolamine and in the following, mono-methylated phosphatidylethanolamine increases the membrane fluidity so as to fascilitate the coupling of β-adrenergic receptor and adenylate cyclase (Hirata et al., 1979). Ishizaka et al. (1980) have shown that the bridging of IgE receptor molecules initially triggers methylation of rat mast cell phospholipids, and then the increase of ^{45}Ca influx and Hi release follow sequentially. Since the bridging of IgE receptors stimulates phospholipid methylation, they suggested that IgE receptors are in close association with membrane methyltransferase. Also, as the preceding phospholipid methylation is required for Ca influx, they understand that methyltransferase may play an important role for Ca influx. Although they have not described about the morphological changes of rat mast cells exposed to antigen-antibody reaction and Hi releasers, morphology of membrane events in rat mast cell associated with Hi release were investigated extensively. Most remarkable morphological change in IgE-mediated antigen-antibody reaction in rat mast cell is exocytosis (Mota and Da Silva, 1960; Chakravarty et al., 1967; Uvnäs, 1974). As a working hypothesis which explains the sequential relation between Hi release and exocytosis, Thon and Uvnäs (1967) advocated the two stage theory; extrusion of the granules takes place in the first step and in the second step ion-exchange between Hi in the extruded granule and Na^+ in the medium will be followed. Later on, more detailed observation of degranulation was reported by Röhlich et al. (1971). As the initial change in sequential exocytosis, they found "pore formation" which was brought about in the region where the perigranular membrane and the cell membrane fused together and as a consequence of this, structureless diaphragm bridging the edges of the fused region, "pore" was formed. The idea of the membrane fusion between the perigranular membrane and the cell membrane prior to the exocytosis and Hi release greatly influenced many other investigators in various field. As the mechanism of the membrane fusion has been investigated more and more not only morphologically (Lagunoff, 1973; Chi et al., 1976; Lawson et al., 1977) but also biochemically (Strandberg et al., 1975; Strandberg and Westerberg, 1976; Kennerly et al., 1979), the importance of protein-lipid interaction of bilayer membrane became apparent (Chi et al., 1976). Protein-lipid interactions play a key role in the structural and functional properties of membranes. Recently, the properties of lipids and the interaction with protein have been studied in a variety of model systems; mostly in 1) single bilayers separating two aqueous phases (black lipid membrane) and 2) lamellar single-bilayer vesicles. In 1966, Del Castillo et al. found that electrical impedance of the black lipid film decreases markedly in antigen-antibody reaction and in 1968, Haxby et al. prepared the liposomes for the purpose to analyze the complement-mediated antibody reaction. It became apparent that these liposomes were undergoing immune damage akin to the action of antibody and complement on natural cell membranes. From those findings, it was assumed that IgE-mediated antigen-antibody reaction also can be mimicked on the model membrane. In the present experiment, it was intended to clarify the most basic mechanism of the Hi release in IgE-mediated antigen-antibody reaction by using the model membranes.

METHODS

1. Preparation of IgE rich anti-egg albumin mouse serum

IgE rich anti-egg albumin mouse serum was prepared according to a modification of Levine and Vaz (1970). 10 μg of egg albumin were suspended in 1.0 ml of 4 % alumina gel and the suspension was injected (i.p.) to balb/c mice 0.1 ml each. 4 weeks later the first booster was carried out (i.p.) with one-half of the dose. Another 2 weeks later, the second booster was injected (i.p.) with one-fourth of the original dose. A week after the last injection, mice was exsanguinated and arterial blood was collected, and the serum was separated. Further purification

of IgE rich serum was carried out according to the method of Frick and Ishizaka (1969).

2. Measurements of electrical resistance and capacitance of black lipid membrane

A small window was punched in the wall of teflon cup and this was clamped within a larger glass beaker (Fig. 1A). A diameter of the small hole is 1.5 mm.

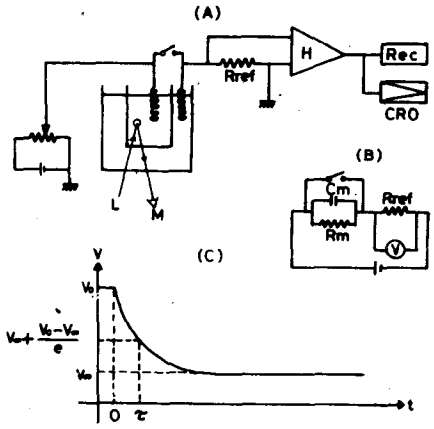

Fig. 1. Experimental arrangement for determination of black lipid membrane resistance and capacitance. A) experimental apparatus; H: high input impedance amplifier, Rec: recorder, CRO: oscilloscope, L: light, M: stereoscope. B) equivalent circuit; Cm: capacitance of black lipid membrane, Rm: electrical resistance of black lipid membrane, Rref: a fixed resistor (reference resistor), V: a voltage drop through Rref. C) schematic diagram of voltage change.

Both compartments are filled to the same level with HEPES-buffered physiological salt solution which contains diluted mouse antiserum (1:1,000) and maintained at room temperature. The composition of bathing solution was NaCl 154 mM, KCl 2.7 mM, $CaCl_2$ 0.9 mM, HEPES 5 mM (pH 7.4). 5 mg of phosphatidylcholine and 5 mg of cholesterol were dissolved in 1 ml of n-decane and this lipid solution was spread across the hole through a fine polyethylene tube which was connected to a microsyringe. When the purity of phosphatidylcholine was tested by thin layer chromatography, it was found as a single spot. As the solvent diffuses out into the bathing solution, a thin films is formed which shows interference colors at first and then the "black spots" are seen single or several at once and growing at a fairly rapid rate. Finally whole area are covered by black color (black lipid membrane). The electrical resistance of the black lipid membrane was measured by applying a dc-potential of 20 mV between two Ag/AgCl electrodes which were placed at the both sides of the membranes. When the switch is closed, voltage drop through the reference resistor was recorded as V_0 (Fig. 1C). As the switch is open, the electric current flows the film and the reference resistor, and voltage drop via reference resistor decreases exponentially and reaches steady state voltage V_∞. By measuring V_0, V_∞ and time constant (τ), the electrical resistance and capacitance of the black lipid membrane can be calculated by the following equations:

$$Rm : Rref = V_0 - V\infty : V\infty \qquad RmCm = \tau$$

$$Rm = \frac{V_0 - V\infty}{V\infty} Rref \qquad Cm = \frac{\tau}{Rm} = \frac{V\infty \cdot \tau}{(V_0 - V\infty)Rref}$$

3. Preparation of antigen-coated, Hi-trapped liposome

1 mg of phosphatidylcholine and 0.5 mg cholesterol were dissolved in 0.5 ml of n-decane in the test tube and evaporated in vacuum desiccator. Then, 20 μg of egg albumin dissolved in 0.5 ml of saline were added to the test tube and mixed with vortex mixer for 1 min at room temperature. After that, sonication (TOMY, UR-20P, 28 KHz) was carried out for 10 min in cold water and then evaporated again. In the following, 1 ml of saline containing 1 mg of Hi was added and then mixing and sonication were carried out. After that, sonicated solution was transferred into visking tube (No. 8). Hi remained in visking tube was removed by dialysis against saline for 2 hr at room temperature (Fig. 2). Saline was flown at a constant rate of 2 ml/min through the inlet, and the effluent was collected every 1 min and Hi concentration was determined fluorometrically (Shore, 1971).

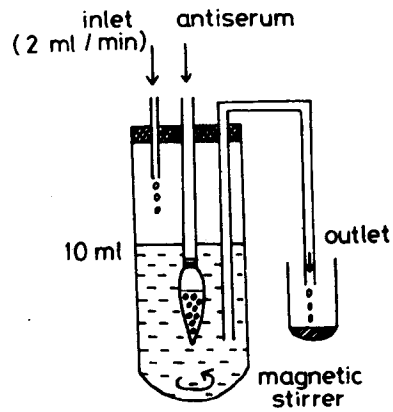

Fig. 2. Experimental arrangement for Hi release from antigen-coated liposomes.

In some experiment, antigen-coated, Hi-trapped liposome was prepared with total mast cell lipids instead of a mixture of phosphatidylcholine and cholesterol. Total lipids of rat mast cells were obtained as follows. Rats were decapitated and 10 ml of buffered physiological salt solution were injected into the abdominal cavity and after gentle massaging of the abdominal wall, mast cells were collected by repeating the gum arabic density gradient centrifugation (Kimura et al., 1960; Sugiyama, 1971). Mast cells obtained from several rats were pooled to provide a sufficient number of cells for an experiment. Mast cells were at least 95 % pure and counted in a Bürker chamber.

Total mast cell lipids were extracted according to a modification of Strandberg and Westerberg (1976). Mast cell pellet was suspended in a distilled water and sonicated, and then extracted with $CHCl_3$ + MeOH (2:1) solution. The following procedure are shown in Fig. 3. Total lipid extracted and reference phospholipids were dissolved in chloroform and applied on Silica gel plate. The chromato-plates were placed in glass jars and developed at room temperature. A series of procedures for quantitative analysis of phospholipids were also shown in Fig. 3.

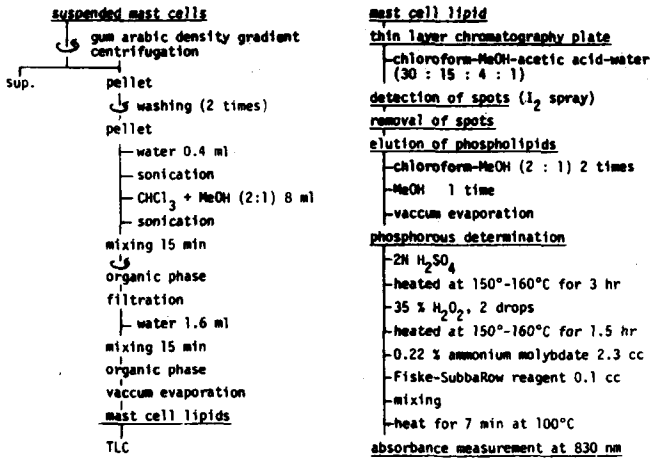

Fig. 3. Procedure for lipids extraction from isolated rat mast cell and phospholipids analysis.

Butylhydroxy toluene was used as anti-oxidant in all of these procedures. Lipid phosphorus in each fraction was determined by the method of Bartlett (1957). Determination of cholesterol in the total mast cell lipids was carried out by the method of Rudel and Morris (1973).

4. Hi release from liposomal suspension by compound 48/80

Liposomes were prepared as previously described with phosphatidylcholine and cholesterol which were dissolved in n-decane and Hi was trapped in the liposome. The extrinsic association of egg albumin with the surface of liposome was carried out in some cases. Liposomes were suspended in physiological salt solution. 2 ml of liposome suspension were placed in the test tube and compound 48/80 was added to make the final concentrations of 10^{-7}-$5x10^{-5}$g/ml and incubated at 37°C for 15 min, and then centrifuged for 30 min at 10^5x *g*.Hi concentration in the supernatant was determined fluorometrically. Total Hi content in the liposomal suspension was determined after the treatment with 1 % triton X-100 (as the final concentration) to solubilize the liposomes.

5. Electron microscopy of liposome

(1) negative staining: A drop of liposome solution was spread over a copper grid and water wiped off, and then a drop of 2 % ammonium molybdate solution (pH 7.4) was superimposed and excess solution was wiped off. The specimen was examined at 40,000 x magnification of electron microscope (Hitachi, H-500).

(2) freeze-fracture replica: A drop of liposome solution was placed on specimen support and was frozen in liquid nitrogen. Fracturing was carried out and replication was made with Pt and C coating and replica membrane was cleaned with a commercial bleach. The specimen was examined at 20,000 x magnification.

(3) ferritin conjugation with egg albumin: According to a modification of the method of Matukura et al. (1969), ferritin conjugation with egg albumin was performed. Since a marked reduction of PCA titer was noticed when antibody was used for conjugation with ferritin, egg albumin (antigen) was used instead of antiserum.