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Original Article

# Permeability of rat IgE across rat aortic endothelial cell is enhanced by histamine

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## Abstract

**Background:** It is a well-known fact that IgE is a key substance that induces an allergic reaction in extravascular tissue. However, it remains to be elucidated how IgE in the circulating blood transfers to the site of the allergic reaction in the extravascular tissue. In the present paper, rat IgE passage through cultured rat aortic endothelial cells (RAEC) was first examined using a dual-chamber system. Second, we examined the effects of histamine, which is thought to affect endothelial permeability, on IgE passage through the RAEC in comparison with the effects of albumin and IgG2a.

**Methods:** The permeability constant (PC) was used to evaluate the degree of IgE passage through the RAEC.

**Results:** The value of the PC for rat IgE ($0.58 \pm 0.11 \times 10^{-5}$ cm/s) was lower than that for IgG2a and albumin ($0.88 \pm 0.28 \times 10^{-5}$ and $0.93 \pm 0.26 \times 10^{-5}$ cm/s, respectively) under conditions of non-exposure to histamine. In contrast, the PC of rat IgE was significantly increased by exposure to histamine ($10^{-10}$ mol/L) at 12 h after exposure. However, the PC for IgG2a and albumin were not significantly increased following exposure to histamine. The enhancement by histamine of IgE passage through the RAEC was not inhibited by diphenhydramine, a histamine $H_1$ receptor antagonist, but were inhibited by cimetidine, a histamine $H_2$ receptor antagonist.

**Conclusions:** On the basis of results from the present study, histamine, acting via $H_2$ receptors, enhances the permeability of rat IgE across the RAEC monolayer. The increased permeability of endothelial cells induced by histamine may contribute greatly to the transfer of IgE from circulating blood to extravascular tissue.

**Key words:** IgE, endothelial cells, histamine, permeability.

## Introduction

The allergic reaction in tissues is known to be related to several factors, including allergen-specific IgE, IgE target cells, allergen and inflammatory cells. Inflammatory cells, such as eosinophils, in the circulation are also known to accumulate at the site of allergic inflammation in extravascular tissue. It has been thought that IgE, a key substance in the allergic reaction in extravascular tissues, is produced in the tissue and/or distributed from the circulating blood to the tissue. However, it remains to be elucidated how IgE in the circulating blood transfers to the extravascular tissue and acts in the process of an allergic reaction. On the basis of clinical data, elevated IgE in the circulating blood of patients with allergic diseases cannot be regarded as a pathophysiological sign of allergy[1] and it is thought that the IgE level in the circulating blood does not always reflect the amount of IgE in the tissue. Therefore, we need to determine the regulatory mechanism by which IgE passes through the vascular wall

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to the tissue. In addition, there is one report that mouse or human mast cells can secrete vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) and that this secretion is enhanced via activation of IgE receptors.[2] It is suggested from this report that the passage of IgE through the vascular wall to the tissue may be regulated by biological substances from the extravascular cells, such as mast cells. In the present study, we investigated both IgE passage through cultured endothelial cells and the effect of histamine on IgE passage in comparison with the passage of albumin and IgG2a.

## METHODS

### Animals

Wistar-strain male rats, weighing 250–300 g, were used. Rats were purchased from Kyudo (Kumamoto, Japan).

### Animal care and management

We followed the Standards Relating to the Care and Management of Experimental Animals (Notification No. 6, 27 March 1980, from the Prime Minister's Office, Tokyo, Japan) for the care and use of animals, together with the Guide for Animal Experiments issued by the University of the Ryukyus. All animal studies were reviewed and approved by the Animal Care Committee at the University of the Ryukyus.

### Reagents

2,4-Dinitrobenzene sulfonic acid sodium salt was purchased from Tokyo Kasei (Tokyo, Japan). Nutrient mixture F-12 HAM, Kaighn's modification (HAM's F-12K), collagenase type XI (cell culture grade) and 2,2'-azino-di-[3-ethyl-benzthiazoline-6-sulfonic acid] (ABTS) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Tween-20 and gelatin fine powder were obtained from Nacalai Tesque (Kyoto, Japan) and hydrogen peroxide was obtained from Santoku Chemical (Miyagi, Japan). Aminohexanoyl-biotin-*N*-hydroxysuccinimide, peroxidase-conjugated streptavidin, rat myeloma IgG2a and monoclonal mouse antirat IgG2a were obtained from Zymed Laboratories (San Francisco, CA, USA). A Falcon cell culture insert (diameter 6 mm; pore size 3 μm; pore density $8.0 \times 10^5$ /cm$^2$) and its companion plate were purchased from Becton Dickinson (Franklin Lakes, NJ, USA). Fetal bovine serum (FBS) was obtained from Sanko Junyaku (Fukuoka, Japan). Peroxidase (POD)-conjugated goat antirabbit IgG was purchased from BioRad Laboratories (Richmond, CA, USA). Rabbit antirat IgG rabbit antirat albumin was purchased from Inter-cell Technologies (New Jersey, NJ, USA). Type I collagen from the rat tail was obtained from Collaborative Biomedical Products (Bedford, MA, USA). The POD-conjugated sheep antirat albumin was purchased from Cappel Products, (ICN Pharmaceuticals, West Chester, PA, USA). Endothelial cell growth supplement (ECGS) and DiI-labeled acetylated low-density lipoprotein (DiI-Ac-LDL) were obtained from Harbor Bio-Products (Norwood, MA, USA). Unless otherwise stated, all other chemicals were of reagent grade.

### Isolation of rat aortic endothelial cells using a primary explant technique

Isolation of rat aortic endothelial cells was performed according to the method of MacGuire and Orkin.[3] The full length of the thoracic aorta was aseptically removed from the thoracic cavity of a Wistar rat and rinsed in HAM's F-12K. The vessel was gently cleaned to exclude periadventitial fat and connective tissue and was then cut into flat segments measuring approximately 5 × 5 mm. These flat aortic segments were placed endothelial-side down on a dish coated with type I collagen and 1 mL culture medium (HAM's F-12K containing 10% FBS, 50 μg/mL crude endothelial cell growth factor, 100 μg/mL penicillin and 100 μg/mL streptomycin) was added. Explants were incubated overnight in a humidified incubator (Astec, Fukuoka, Japan) at 37°C in a 95% air/5% $CO_2$ atmosphere and, 24 h later, 1 mL culture medium was added to the dish. Aortic explants were removed after 6–7 days according to their degree of outgrowth. Passaged cells from the tissue culture dish exhibited a 'cobblestone' morphology. A solution of 0.25% collagenase in serum-free medium was used to abrade the cells from the collagen-coated dish. These cells were then routinely subcultured for up to 4 passages with 0.25% collagenase. The cells obtained were identified as endothelial cells from their uptake of DiI-Acl-LDL. They were subsequently incubated in the presence of 10 μg/mL DiI-Ac-LDL for 4 h at 37°C. Finally, the medium was removed and cells were washed three times with phosphate-buffered saline (PBS). Low-density lipoprotein particles were visualized by fluorescence microscopy (Nikon, Tokyo, Japan) with filters adjusted for rhodamine fluorescence.

## Chamber preparation

Polyethylene terephthalate track-etched membranes of the Falcon cell culture insert (upper chamber) were coated with type I collagen. These upper chambers were placed into each well of a 24-well plate (lower chamber). Cells were removed from the tissue flask by treatment with 0.25% collagenase and centrifuged at 430 *g* for 5 min. The cell pellet was resuspended in complete culture medium and 0.43 mL, containing $1 \times 10^5$ cells, was seeded onto each collagen-coated membrane. Experiments were performed 3 days after seeding. Monolayer confluence on the membranes was evaluated by light microscopy.

## Preparation of monoclonal dinitrophenyl-specific rat IgE and rat albumin

The procedure used for the preparation of monoclonal dinitrophenyl (DNP)-specific rat IgE was as described previously.[4] Rat albumin was purified from the serum of normal rats as follows. Normal rat serum was precipitated with ammonium sulfate at a 50% saturation at 4°C, dissolved in PBS and extensively dialyzed against 5 mmol/L phosphate buffer (PB), pH 7.0. Rat albumin was further purified by means of ion-exchange chromatography using DEAE-Sephacel (Amersham Pharmacia Biotech, Uppsala, Sweden). The adsorbed proteins were eluted with 0.1 mol/L PB, pH 5.8. Two protein peaks were observed in the ion-exchange chromatography profile. The second protein peak was a 67 kDa band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and it reacted with rabbit antirat albumin on western blot analysis.

## IgE-capture ELISA to estimate monoclonal DNP-specific rat IgE

The IgE-capture ELISA to estimate rat IgE antibodies to DNP–Ascaris (As) was performed as described previously.[4] The ELISA plate was coated with 1 μg/mL rabbit antibodies to monoclonal DNP-specific rat IgE. After blocking with Dulbecco's PBS containing 2% gelatin for 1 h at room temperature, the wells were incubated with monoclonal DNP-specific rat IgE or samples collected from the upper and lower chambers. Following washing, the wells were incubated with 1 μg/mL biotinylated DNP-As in 2% gelatin–PBS containing 0.05% Tween 20 (PBST) for 30 min at room temperature. After further washing, the wells were incubated with a 1/3000 dilution of POD-conjugated streptavidin in 2% gelatin-PBST for 30 min at room temperature. Substrate solution (ABTS) was then added to each well, and spectrophotometric readings were made using $\lambda_1 = 415$ nm and $\lambda_2 = 492$ nm wavelength filters of a dual wavelength microplate photometer (MTP-22; Corona Electric, Ibaraki, Japan).

## ELISA to estimate rat albumin

The ELISA plate was coated with 1 μg/mL rabbit antirat albumin. After blocking with Dulbecco's PBS containing 1% skimmed milk for 1 h at room temperature, the wells were incubated with rat albumin or samples collected from the upper and lower chambers. Following washing, the wells were incubated with a 1/3000 dilution of POD-conjugated sheep antirat albumin in PBST for 1 h at room temperature. Other details of the ELISA procedure were as described for the IgE-capture ELISA to estimate monoclonal DNP-specific rat IgE.

## ELISA to estimate rat IgG2a

The ELISA plate was coated with 1 μg/mL monoclonal mouse antirat IgG2a. After blocking with Dulbecco's PBS containing 1% skimmed milk for 1 h at room temperature, the wells were incubated with rat myeloma IgG2a or samples collected from the upper and lower chambers. Following washing, the wells were incubated with a 1/1000 dilution of polyclonal rabbit antirat IgG in PBST for 1 h at room temperature. After further washing, the wells were incubated with a 1/3000 dilution of POD-conjugated goat antirabbit IgG in PBST for 1 h at room temperature. Other details of the ELISA procedure were as described for IgE-capture ELISA to estimate monoclonal DNP-specific rat IgE.

## Experimental protocol for IgE passage through the endothelial cell monolayer

A 0.43 mL aliquot of serum-free medium containing monoclonal DNP-specific rat IgE, rat serum albumin or rat myeloma IgG2a was added to the upper chamber and 1 mL serum-free medium was added to the lower chamber. Samples (30 μL) from the upper chamber were taken out and an equal volume of medium was added at different time points. Samples (1 mL) from the lower chamber were taken out and an equal volume of medium was added to the lower chamber at different time points. The IgE in the medium of the upper and lower chambers was determined by IgE-capture ELISA and albumin and IgG2a were estimated by ELISA.

### Effect of histamine on IgE passage through endothelial cells

Serum-free medium containing different concentrations of histamine ($10^{-13}$ to $10^{-9}$ mol/L) was added to the upper chamber, which contained a rat endothelial cell monolayer. Unless otherwise stated, the reaction time was 24 h. The degree of IgE passage through the endothelial cells was calculated after the endothelial cells were exposed to histamine.

### Effect of histamine antagonists on IgE passage through endothelial cells exposed to histamine

Serum-free medium containing histamine and a histamine antagonist or anti-allergic drugs at different concentrations was added to the upper chamber, which contained a rat endothelial cell monolayer. The reaction time was 24 h. The degree of IgE passage through endothelial cells was calculated after washing the endothelial cell monolayer on the membrane with serum-free medium.

### Data analysis

The degree of IgE passage through endothelial cells was expressed as a permeability constant (PC) according to the method of Cooper *et al*.[5] The clearance volume of IgE at different time points ($V_{IgEt}$), which indicates the volume of the upper chamber cleared of IgE through the endothelial cell monolayer, was calculated by summing the incremental clearance volumes up to that time point:

$$V_{IgEt} = \Sigma(V_{Li} \cdot \Delta[L]_i/[U]_i)$$

where $V_{Li}$ is the volume of the lower chamber at different time points, $\Delta[L]_i$ is the increase in IgE concentration between time points and $[U]_i$ is the concentration of IgE in the upper chamber at different time points. The change in $V_{IgE}$ over time ($dV_{IgE}/dt$), equal to the clearance rate in μL/min, was determined by weighted least-squares non-linear regression for the reaction time. Furthermore, if:

$$(PS \cdot (V_U + V_L)/(V_U \cdot V_L)) \cdot t < 0.1$$

then, with an error of $< 5\%$, $dV_{IgE}/dt = PS$, where P is the PC, S is the surface area, $V_U$ is the volume of the upper chamber and $V_L$ is the volume of the lower chamber.

### Statistical analysis

Statistical analysis was performed by unpaired Student's *t*-test for between-group comparisons. Data are expressed as the mean±SD. When $P < 0.05$ was obtained, the means were considered to be significantly different.

## RESULTS

### Correlation of clearance volume and reaction time of IgE, albumin and IgG2a

Three continuous lines formed the weighted least-squares regression curves for the experimental period in the case of IgE. The slope of each line indicated the clearance rate for the experimental period. Therefore, the PC of IgE across the endothelial cell monolayer was calculated on the basis of its clearance rate. In contrast, in the case of 5 μg/mL IgE, which was added to the upper chamber, the continuous line did not pass over the origin and the value of the clearance rate was lower than in other cases. However, in the case of the addition of 10 and 20 μg/mL IgE to the upper chamber, both lines passed over near the point of origin. The value of the clearance rate in the case of 10 μg/mL IgE added to the upper chamber was larger than that in the case of 20 μg/mL IgE. From these results, 10 μg/mL was selected as the proper concentration of IgE in the medium to be added to the upper chamber in the present study (Fig. 1a). The PC of IgE across the endothelial cell monolayer was approximately $0.58 \times 10^{-5}$ cm/s under these conditions. In the case of the addition of 5, 10 and 20 μg/mL IgG2a or albumin to the upper chamber, each line passed over near the point of origin and the values for the clearance rate were almost the same at different concentrations (Fig. 1b,c). Consequently, 10 μg/mL IgG2a and albumin was used in the present study.

### Permeability constant of rat IgE, IgG2a and albumin across the endothelial cell monolayer

The PC of rat IgE was smaller than that of IgG2a or albumin ($P < 0.05$). In contrast, no significant differences in PC were noted between rat IgG2a and albumin (Fig. 2).

### Effect of histamine on the PC of IgE, IgG2a and albumin across endothelial cells

The PC of IgE across the endothelial cell monolayer exposed to histamine (above $10^{-11}$ mol/L) was signifi-

cantly increased compared with the PC of IgE across the endothelial cell monolayer not exposed to histamine (Fig. 3a). At $10^{-10}$ mol/L, histamine produced the greatest enhancement of the PC of rat IgE. The kinetics of the enhancing effect of histamine ($10^{-10}$ mol/L) on the PC of rat IgE were examined over the time range 3–24 h. A significant increase in PC following exposure to histamine appeared after 12 h compared with cells not exposed to histamine and reached to its peak (approximately $1.5 \times 10^{-5}$ cm/s) after 24 h (Fig. 3b). In contrast, no significant differences were observed between PC of rat IgG2a and albumin in cell monolayers exposed or not exposed to histamine (Fig. 4).

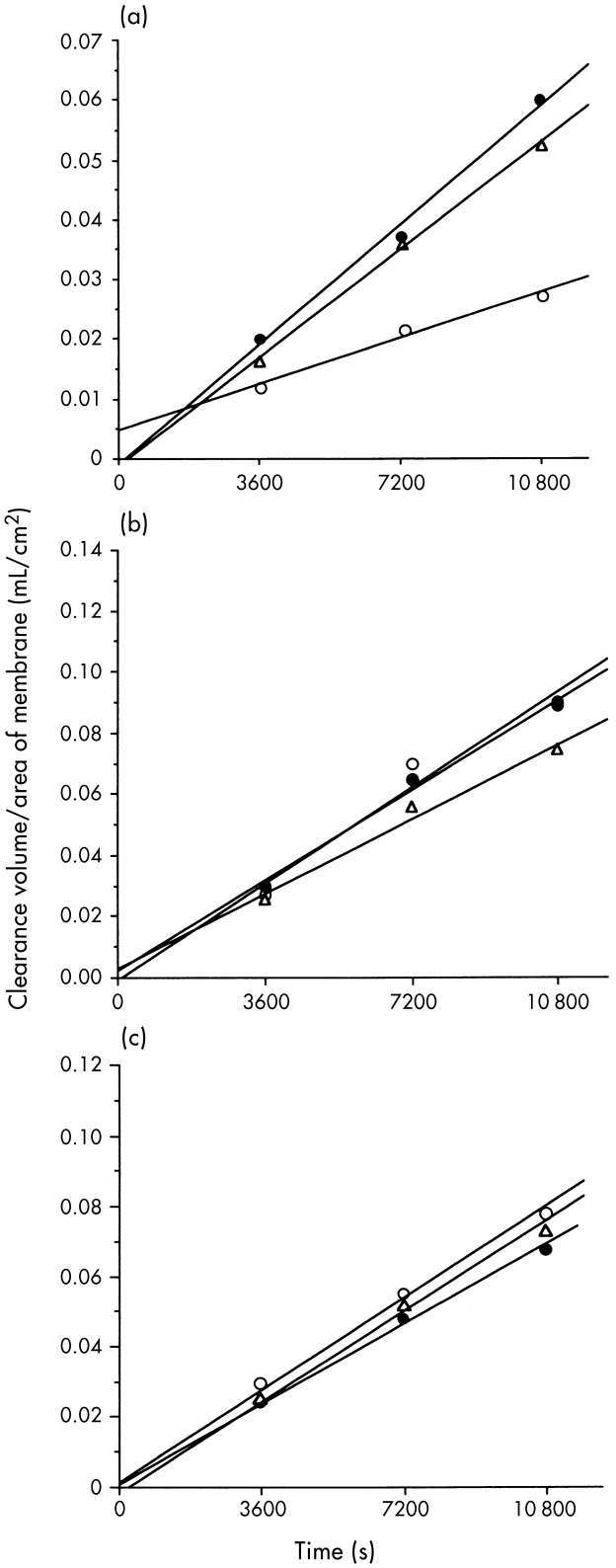


**Fig. 1** Clearance volume–reaction time curves for rat (a) IgE, (b) IgG2a and (c) albumin. A single passage assay was performed in serum-free medium (HAM's F-12K). Different concentrations of IgE, IgG2a and albumin (0.43 mL) were added to the upper chamber at the start of the assay: (○), 5 μg/mL; (●), 10 μg/mL; (△), 20 μg/mL.The concentrations of IgE, IgG2a and albumin in the upper and lower chambers were determined at different time points and the clearance volume was calculated, as described in Methods.

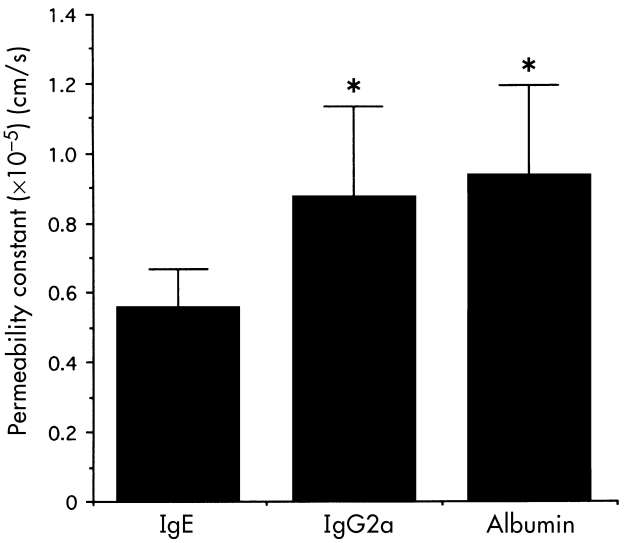


**Fig. 2** Permeability constant (PC) of rat IgE, IgG2a and albumin across rat endothelial cells.The PC of rat IgE, IgG2a and albumin across the cells was determined following incubation of cells with serum-free medium for 24 h and was calculated as described in Methods. Data are the mean±SD ($n = 10$). *$P < 0.05$ compared with IgE.

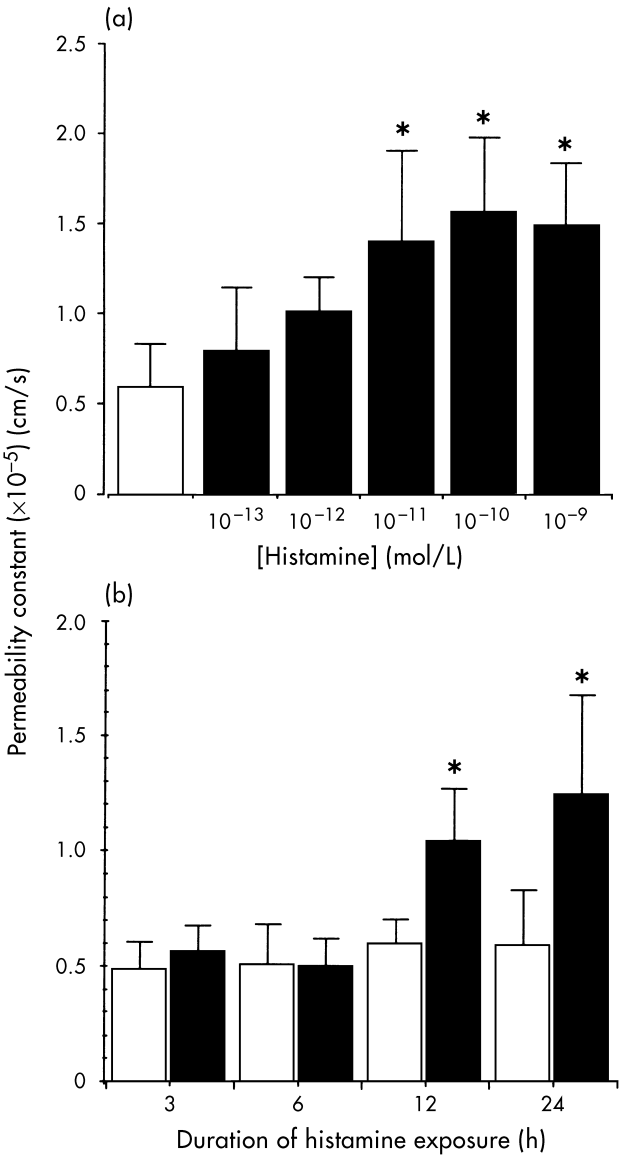


**Fig. 3** Effect of histamine on the permeability of rat IgE across rat endothelial cells. Histamine (■) was applied to the endothelial cell monolayer in the upper chamber at different concentrations, and the permeability of the monolayer of rat IgE was examined (a). The time-course of the increased permeability of IgE across the endothelial monolayer after exposed to $10^{-10}$ mol/L histamine was also examined (b). The permeability constant (PC) of IgE across the endothelial cells was calculated as described in Methods. Data are the mean±SD ($n = 3$). *$P < 0.05$ compared with serum-free medium (□).

## Effect of histamine antagonists on the PC of IgE across endothelial cells exposed to histamine

At concentrations of $10^{-10}$ to $10^{-6}$ mol/L, cimetidine, a histamine $H_2$ receptor antagonist, lowered the increased PC of IgE across endothelial cells following exposure to

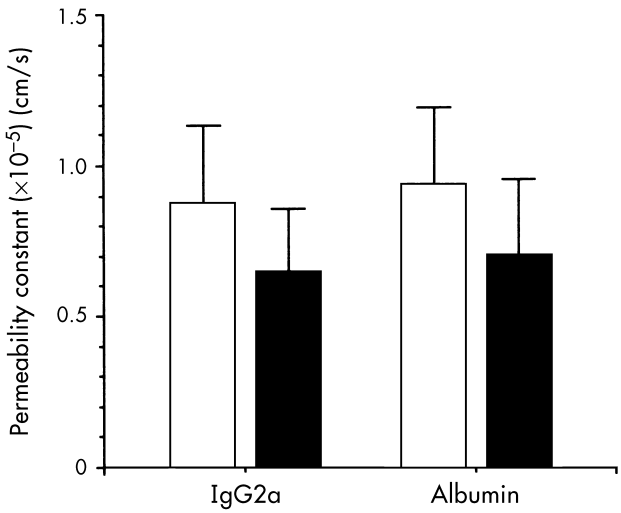


**Fig. 4** Comparison of permeability constants (PC) of rat endothelial cells exposed to histamine for rat IgG2a and albumin. Histamine ($10^{-10}$ mol/L; ■) was applied to the endothelial cell monolayer in the upper chamber for 24 h. The PC of the monolayer for rat IgG2a and albumin was calculated as described in Methods. Data are the mean±SD ($n = 5$). (□), serum-free medium

histamine ($P < 0.01$). In contrast, diphenhydramine, a histamine $H_1$ receptor antagonist, did not exert any significant effect on the PC of IgE at the indicated concentrations (Fig. 5).

## Discussion

The PC of rat IgE ($0.58 \pm 0.11 \times 10^{-5}$ cm/s) was smaller than that of IgG2a ($0.88 \pm 0.28 \times 10^{-5}$ cm/s) and albumin ($0.93 \pm 0.26 \times 10^{-5}$ cm/s). It was, however, indicated that the permeability of rat IgE across rat aortic endothelial cells (RAEC) was enhanced following the exposure of the cells to histamine. In contrast, the permeability of IgG2a and albumin was not enhanced following exposure of RAEC to histamine. Morphological changes in RAEC were not observed after exposure to histamine. On the basis of these results, we suggest that the passage of IgE through endothelial cells was more susceptible to enhancment by histamine than was the passage of either IgG2a or albumin. Histamine is a preformed mediator of mast cells and is released following degranulation of mast cells induced by stimuli such as exposure to allergen.[6] Therefore, from the results obtained in the present study, an acceptable interpretation is that the passage of IgE through the vascular wall via endothelial cells to extravascular tissue may be

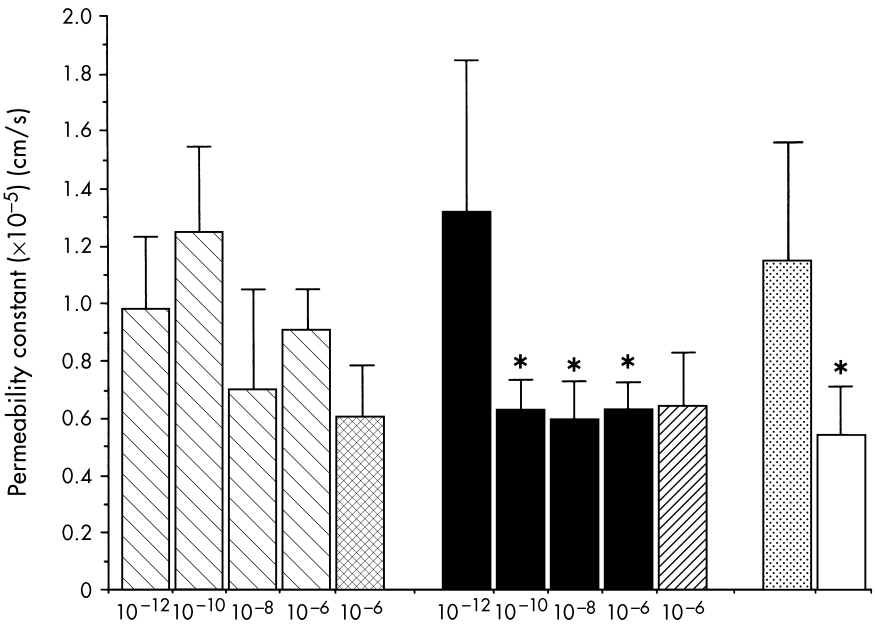


**Fig. 5** Effect of histamine antagonists on the permeability of IgE across endothelial cells exposed to histamine. Serum-free medium containing histamine ($10^{-10}$ mol/L) and the histamine antagonists diphenhydramine and cimetidine at the concentrations indicated was added to the rat endothelial monolayer in the upper chamber. The reaction time was 24 h. Data are the mean±SD ($n = 5$). *$P < 0.01$ compared with the permeability constant (PC) following exposure to histamine alone. (▨), diphenhydramine + histamine; (▩), diphenhydramine alone; (■), cimetidine + histamine; (▨), cimetidine alone; (▨), histamine alone; (□), serum-free medium.

enhanced by histamine released from mast cells at the site of the allergic reaction.

Boesiger *et al*. have reported that human and mouse mast cells secrete VPF/VEGF and that secretion of VPF/VEGF was enhanced after IgE-dependent upregulation of FcεRI expression, irrespective of exposure to allergen.[2] It has been shown that VPF/VEGF can enhance vascular permeability and induce proliferation of vascular endothelial cells.[7–10] Both tumor necrosis factor-α and interluekin-6 have an enhancing effect on endothelial permeability and are newly synthesized mediators of mast cells.[11–13] It is suggested from the results of the present study and from results of other published studies that the permeable IgE itself, due to release of chemical mediators or cytokines from mast cells, is an enhancer of IgE passage through the endothelial cells to the tissue.

The enhancement by histamine of the passage of IgE through endothelial cells was not inhibited by diphenhydramine, a $H_1$ receptor antagonist, but was blocked by cimetidine, a $H_2$ receptor antagonist. These findings suggest that the aforementioned effects of histamine on endothelial cells are mediated via activation of $H_2$ and not $H_1$ receptors.

The mechanism responsible for the effects of histamine, following stimulation of histamine receptors, on the passage of IgE through endothelial cells remains to be clarified. It is generally accepted that vascular endothelial cells represent a permselective barrier for the transport of a broad variety of molecules from the circulating blood to the interstitial fluid. Under physiological conditions, a highly controlled mechanism should be related to the barrier function of endothelial cells. With regard to the permeability of serum albumin, it has been reported that the endothelial albumin-binding protein is involved in the specific binding of albumin and is followed by transport of albumin.[14–16] Histamine was more effective in enhancing the permeability of IgE than IgG2a and albumin. Therefore, it is inferred that there is a specific mechanism for the passage of IgE through the endothelial cells via an alternative cell-surface protein with a binding affinity for IgE. The cell-surface protein galectin-3 has an affinity for IgE and is located on the membrane of endothelial cells;[17,18] the expression of galectin-3 may be associated with the activation of signal transudation in endothelial cells via histamine $H_2$ receptors.

We are studying whether any biological substances derived from mast cell other than histamine can or cannot enhance the permeability of IgE through RAEC.

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