Histamine Receptors of the Microvascular Endothelium Revealed In Situ with a Histamine-Ferritin Conjugate: Characteristic High-affinity Binding Sites in Venules

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ABSTRACT Histamine covalently bound to glutaraldehyde-activated ferritin was prepared as either monomers or as small aggregates of ~0.05 to 0.15 μm Diam, suitable for electron microscopic detection of histamine cellular binding sites. The histamine-ferritin conjugates (MF) maintain the histamine capability to induce the opening of endothelial junctions in venules. To investigate the distribution of histamine receptors in the vascular endothelium, monomers or aggregates of MF were perfused in situ (mice), and various vascular beds, particularly that of the diaphragm, were fixed and processed for electron microscopy. The conjugate was preferentially bound on restricted areas of luminal endothelial cell plasmalemma especially in regions rich in filaments, and near the junctions between endothelial cells. The density of histamine binding sites was characteristically high in venules; it occurred to a much lesser extent in arterioles, veins, and muscular arteries whereas capillaries and aorta showed the lowest values. A similar distribution was obtained after perfusion of H₁ or H₂ receptor agonists coupled to ferritin (2-pyridylethylamine-ferritin [PF], or 4-methylhistamine-ferritin [MF], respectively). The binding specificity was assessed through control experiments with either native or activated ferritin or by competition with histamine. The findings suggest that histamine receptors are largely represented in the cell membrane of the vascular endothelium, particularly in venules. Experiments using specific H₁ and H₂ receptor agonists (PF and MF) and antagonists (mepyramine and cimetidine) indicate that the venular endothelium contains mainly H₂ receptors.

Histamine participates in a number of physiological processes particularly those of the cardiovascular system (1, 5, 6, 9, 32, 34) and is largely involved in inflammatory and anaphylactic reaction (3, 4). As identified on a physiological and pharmacological basis, its effects are mediated by two types of receptors: H₁ receptors, specifically inhibited by classical antihistamines such as mepyramine, and H₂ receptors, blocked by antagonists such as cimetidine (8, 19, 35). The distribution and properties of histamine receptors (Hr) as defined for several cells, tissues, and organs, including blood vessels, is based primarily on the target responses to histamine either alone or associated with its agonists or antagonists (7, 11–15, 18, 20, 33, 42, 45).

Histamine receptors were demonstrated to be located on the plasma membrane of several mammalian cells (10, 21, 22, 28, 29, 43). So far, their presence has been detected by the binding of various cells to histamine which was either insolubilized on albumin-treated agarose beads (28, 46, 47), or coupled to bovine serum albumin-fluorescein isothiocyanate (BSA-FITC) (12) or o-phtaldialdehyde (30). An indirect detection of Hr was achieved by determining the [³H]mepyramine binding (41).

The aim of the present study was (a) to produce an electron-opaque, biologically active histamine conjugate, and (b) to localize at the cellular level, the histamine receptors of the microvascular endothelium.

The biologically active marker obtained was a histamine-ferritin conjugate (HF) which could be produced as either monomers or small aggregates. Results reported here show that, as detected by HF binding, Hr are present on the plasma membrane of the endothelial cells particularly in venules where a remarkable number of high-affinity binding sites render the venular endothelium a characteristically differentiated domain. This can explain the special involvement of venules in inflammation.
MATERIALS AND METHODS

Materials

Reagents: Chemicals were purchased as follows: histamine diphosphate from Sigma Chemical Co., St. Louis, MO; ferritin, 6x crystallized, cadmium (removed from horse spleen) from Miles Laboratories Inc., Elkhart IN; glutaraldehyde, as a 25% solution purified according to P. J. Anderson from E. Merck, Darmstadt, Germany; [3H]histamine from New England Nuclear, Boston, MA; Sephadex G25 and Sepharose 6B from Pharmacia Fine Chemicals, Uppsala, Sweden. 2-Pyridylethylamine dihydrochloride, 4-methylhistamine dihydrochloride, mepyramine maleate, and cimetidine were a generous gift of Dr. C. Robin Ganellin from Smith, Kline, and French Laboratories LTD., Welwyn Garden City, England.

Animals: 164 RAP mice, weighing 20 g each, were kept under similar standardized conditions of feeding and housing for 10 d before being used for these experiments.

Methods

Preparation of Histamine-Ferritin Conjugate: Ferritin was activated according to the method of Kishida et al. (23) slightly modified in that a 340-fold molar excess of glutaraldehyde per amino group of apoferritin was used: 7 mg (0.1 ml) of ferritin in 1.7 ml of 0.1 M sodium phosphate buffer, pH 7.3 was mixed with 0.4 ml of 25% glutaraldehyde solution. The reaction was carried out at 22°C for 30 min, with stirring. The small amount of precipitate formed during the reaction was removed by centrifugation at 20,000 g for 10 min. The activated ferritin was separated from the unreacted glutaraldehyde by gel chromatography on a Sepharose 6B coarse column (1.3 x 80 cm) equilibrated and eluted with 0.1 M Tris-HCl buffer, pH 7.3 at 4°C. The resulting opalescent suspension was stored at 4°C. (After extended storage, the solution should be thoroughly shaken.) Before use, the suspension was dialyzed against an excess of 0.15 M NaCl and sonicated for 10 min. Aliquots of the solution were examined by electron microscopy to determine their average diameter and size distribution.

Control of the Purity of HF Conjugate

Page: Aliquots of HF conjugate (crude HF, and HF monomers [HFm]) were electrophoresed on slab gels containing 5% acrylamide in 0.6 M barbital buffer, pH 8.5 to 8.6. Electrophoresis was carried out in the same buffer for 8 h at a field strength of 3.5 mA/cm. Gels were then fixed overnight in a mixture of 45% methanol and 45% acetic acid, subsequently stained for 1 h with 0.033 Coomassie Brilliant Blue R-250 in 24% (vol/vol) isopropanol, and 10% (vol/vol) acetic acid, followed by a final destaining wash in the same isopropanol-acetic acid solvent (40, 50) (Fig. 2).

Thin-layer Chromatography: The analysis of HF conjugates and their components was carried out on plastic plates precoated with Kieselgel 60 F254, 250 μm thick (E. Merck). The developing solvent was a mixture of 96% ethanol (80 vol) and 25% ammonium hydroxide (20 vol). For staining, 0.3% ninhydrin in n-butanol (wt/vol) solution was used (39) (Fig. 3).

Isoelectric Focusing: The method was applied to small samples of ferritin, and HF monomers with or without NaBH4. Treatment. The procedure was performed in a polyacrylamide gel containing 4.5% acrylamide and 2% ampholyte pH 3.5 to 10 (LKB Ampholine, LKB, Bromma, Sweden). The anode was 1 M H3PO4, and the catholyte 1 M NaOH. An LKB power supply was used to generate 320 V at the beginning of the experiment; voltage was increased to a maximum of 870 V (12 W constant power). The determination of the electrofocusing point was done by using a stibium surface electrode. Gel fixation and staining was carried out by the standard procedure (2, 49).

Estimation of Molar Ratio: 33 mg of histamine diophosphate containing H-histamine (final sp act: ~1 x 10^6 cpm/mg) in 1 ml of sodium phosphate buffer was coupled to 2 mg of concentrated, activated ferritin. The crude [H]HF conjugate was separated from unbound [H]histamine by gel filtration under the conditions mentioned above (Fig. 1). The fractions containing the conjugate were collected and the protein concentration of [H]HF peak was determined by the absorbance at 440 nm using an extinction coefficient (E280) of 11.6. The radioactivity in 0.5-ml aliquots from the collected fractions was determined in an L-100 C Beckman liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Aliquots from the two peaks (A and B in Fig. 1) demonstrated an equivalent quenching. The amount of histamine in the monomeric peak of conjugate (A) was calculated from the amount of radioactivity present, based on the assumption that [H]histamine reacted to the same extent as unlabeled histamine. Molar concentrations of histamine and ferritin were calculated on the basis of Mr of 111 and 440,000, respectively.

Figure 1: Chromatographic separation of histamine-ferritin conjugate from unbound histamine, tested by using [3H]histamine; Sepharose 6B column (1.3 x 80 cm), equilibrated and eluted with 0.1 M Tris-HCl buffer, pH 7.5, at 4°C. HF monomers eluted as a relatively sharp peak (A), followed by free [H]histamine (peak B).

Figure 2: Electrophoretic pattern (PAGE) of native ferritin, crude HF conjugate, and HF monomers; the latter were obtained after gel chromatography of the crude HF conjugate on a Sepharose 6B column. Conditions of the electrophoretic run are given under Materials and Methods.
Tests for the Biological Activity of HF Conjugate

CONTRACTION OF SMOOTH MUSCLE: It has been shown by Black et al. (8) that histamine stimulates the contraction of smooth muscle from various organs, such as ileum, uterus, and stomach. Such biological effect was assessed for HF through Vane's method using the fundal portion of the rat stomach (44). The fundal strip was suspended in a 10-ml bath containing Krebs' bicarbonate solution (composition in mM: NaCl, 118.4; KCl, 4.7; CaCl$_2$-2H$_2$O, 1.3; NaHCO$_3$, 25.0; MgSO$_4$.H$_2$O, 1.2; KM$_2$PO$_4$.2H$_2$O, 0.1; glucose, 11.7) warmed up at 37°C and gassed with 5% CO$_2$ and 95% O$_2$. After a 30-min stabilization period, the following compounds were tested by adding one of them, once every 10-15 min, to the organ bath: histamine diphosphate prepared immediately before use (5 ml; 2.7 x 10$^{-4}$ M as base), HF conjugate (50 µl; 0.5 mg protein), native ferritin (50 µl; 0.5 mg protein) and NaCl (50 µl; 0.15 M). The last two were used as controls. The contractions were recorded on a polygraph and allowed to develop for 2-3 min until resting to the base level. After each test, the organ bath was washed out by overflow (Fig. 4).

VENULE LEAKAGE: As shown by Majno et al. (24-26) the topical or systemic administration of histamine is followed by vascular leakage characteristics restricted to venules. The capability of HF to induce a histaminelike effect was assessed through two lines of experiments testing: (a) HF monomers' capability to induce the extravasation of previously injected carbon black particles, and (b) extravasation at the level of venules of some of the intravascularly administered HF aggregates (HFAs).

(a) Under general anesthesia, RAP mice were given an i.v. injection of 0.1 ml/100 g body weight carbon black (Pelikan Special Ink Black C11/1431/A Günter Wagner, Pelikan Werke, Hanover, Germany). After 2 min, a HF monomers solution (0.15 mg bound histamine/100 g body weight) was administered intravenously. 30 min later, the diaphragm was fixed in situ with 5% formaldehyde and 2% glutaraldehyde in 0.1 M HCl-Na arsenate buffer, pH 7.2. Under the dissecting microscope, the diaphragm was screened for black vascular "tattoo" and the labeled regions were photographed for further measurements (38).

(b) From experiments in which HF aggregates were injected (see Experimental Procedure), regions of diaphragm containing well identified bipolar microvascular fields (BMFs) were collected, processed for electron microscopy, and examined for opened endothelial junctions (36, 37).

Experimental Procedure

Mice were anesthetized with chloral hydrate (50 mg/100 g body weight) given intraperitoneally; the abdominal aorta was cannulated with a # 7405 polyethylene tubing (I.D.: 0.38 mm; Clay Adams-Becton Dickinson & Co., Sharon, MA) connected to a Harvard infusion pump securing a 2-3 ml/min perfusion flow. The caudal vena cava was incised and used as outlet. Blood was washed out by perfusion of ~20 ml of Dulbecco's phosphate-buffered saline (PBS) containing 1 mM Ca$^{2+}$ and supplemented with 14 mM glucose. Small HFAs or HFm (20 mg protein/100 g body weight) were perfused for 3 min. The unbound conjugate was washed out of vessels with ~10 ml of the same buffer. Fixation was carried out for 5 min by perfusion of 2% glutaraldehyde in 0.1 M HCl-Na arsenate buffer, pH 7.2. All solutions used were prewarmed to 37°C. The observations were focused primarily on the diaphragm at the level of which sequential vascular segments (arteries, arterioles, capillaries, venules, and veins) can be reliably identified under the dissecting microscope. Diaphragm was collected, photographed, and BMFs were excised and further processed for electron microscopy as indicated in references 36-38.

Controls

The specificity of HF binding was assessed by perfusing the vasculature with one of the following: (a) native ferritin; (b) activated ferritin (both solutions were made in 0.15 M NaCl, at a concentration of 20 mg protein/100 g body weight, and were given under the same conditions used for the basic experiments); (c) competitive inhibition with histamine: a solution of 4 x 10$^{-3}$ M/100 g body weight was made in 0.15 M NaCl and perfused for 4 min before the administration of HF conjugate.

Characterization of H$_1$ and/or H$_2$ Receptors Using Ferritin-Conjugated Agonists

H$_1$ and H$_2$ receptor agonists were used to produce two conjugates (as small aggregates ≤ 0.1 µm): 2-pyridylethylamine-ferritin (PF), and 4-methylhistamine-ferritin (MF). The coupling was achieved by incubating activated ferritin with one of the two drugs, using the method described for the HF aggregates (see above). The two conjugates stored at 4°C preserved their properties for up to 3 wk. As demonstrated by thin-layer chromatography, the suspensions of PF and MF did not contain detectable amounts of free 2-pyridylethylamine and 4-methylhistamine, respectively.

To characterize the H$_1$ and H$_2$ receptors, five groups of experiments (using 2-3 mice for each experiment) were carried out, in which one of the following substances was administered under the same conditions as described for the basic procedure: (a) perfusion of MF; (b) perfusion of PF; (c) perfusion of cimetidine before the administration of HFAs; (d) perfusion of cimetidine before MF administration; (e) perfusion of mepramine before administration of PF. HFAs, MF, and, PF were given at a concentration of 20 mg protein/100 g body weight. Mepramine maleate (as specific H$_1$ receptor antagonist) was prepared in isotonic saline and perfused before PF (experiment e) at a concentration of 4 x 10$^{-3}$ M/100 g body weight (as base) for 2 min. Cimetidine (as specific H$_2$ receptor antagonist) was prepared by dissolving 252 mg in 1.10 ml of 1 M HCl, neutralized with 2.0 ml of 0.1 M NaOH, and brought to the desired dilution with isotonic saline: the solution was perfused before HFa (experiment c) and MF (experiment d) at a concentration of 4 x 10$^{-3}$ M/100 g body weight for 2 min.

Tissue Processing for Electron Microscopy

Aorta, vena cava, renal artery, and the diaphragm were fixed in situ by perfusion of 2% glutaraldehyde in 0.1 M HCl-Na arsenate buffer, pH 7.2, warmed to 37°C. After 10 min, large vessels as well as bipolar microvascular fields of the diaphragm (36-38) were excised under the dissecting microscope and further immersed in the same fixative for an additional 80 min, and subsequently processed for electron microscopy as indicated in reference 36. Sections cut with a diamond knife on an American Optical Ultracut microtome (American Optical Corp., Buffalo, NY) or Reichert Ultramicrotome were stained with citrate and examined with a Philips 400 electron microscopy operating at 80 kV. Microvessels were identified according to the criteria used in reference 36.

RESULTS

HF Conjugates

Electrophoretic analysis showed that the native ferritin and the crude HF conjugate are resolved in three similar bands,
which in the case of the former compound were named α, β, and γ, and considered to correspond to apoferritin monomer, dimer, and trimer, respectively (48). HF monomers chromatographically separated on Sepharose 6B (peak A in Fig. 1), and subsequently checked by electron microscopy, appeared to consist of two analogous components (Fig. 2): a fast moving component (α-band) containing >90% of the protein is represented by HF monomers, and a slower moving constituent (β-band) containing <10% of protein is formed by HF dimers. As indicated by the chromatographic analysis of the crude [3H]HF conjugate, the molar ratio of [3H]histamine to ferritin in this conjugate is 350:1 to 80:1. Ferritin activation with more than a 340-fold molar excess of glutaraldehyde did not increase the histamine/ferritin molar ratio. 

As demonstrated by thin-layer chromatography, the solution of HF conjugates did not contain a detectable amount of free histamine (Fig. 3). As compared to the pI 4.6 of native ferritin, the isoelectric point of HF monomers is 5.3-5.5 when they have not been treated with NaBH₄; such treatment brings the pI to 6.8.

HF conjugates, in either monomeric or aggregate form, stored at 4°C could be used for at least 2 wk without significant loss of bound histamine or biological effects.²

HF aggregates produced by exhaustive dialysis against water, and sonicated for 10 min, represent a relatively heterogeneous population of small clusters ranging from ~0.05-0.15 μm; they sediment easily at 3,000 g. The availability of these small aggregates allowed a much larger sampling of vessels labeled by HF conjugate, especially those vascular segments provided with high-affinity binding sites for histamine. The HF aggregates obtained only by dialysis against distilled water presented large diameters ranging from ~0.2-0.5 μm. By perfusion of such HF large aggregates, one could produce false labeling due to artificial plugging of microvessels; to avoid this, the HFa suspension was again dialyzed against 0.15 M NaCl and sonicated for 10 min before use.

Biological Activity of HF Conjugate

HF conjugate maintained its capability to induce a histaminelike effect, namely the contraction of smooth muscle from rat stomach (Fig. 4). The preparation was sensitive neither to native ferritin nor activated ferritin-ferritin polymers during the first step of the reaction (23). The stability of the HF conjugate may be explained by the extensive cross-linkage introduced by glutaraldehyde, as a result of stable Michael-type additions formed between amino groups and α, β-unsaturated aldehydes (23, 31).

³ The excess of glutaraldehyde used for ferritin activation prevents the formation of ferritin-ferritin polymers during the first step of the reaction (23).

² The stability of the HF conjugate may be explained by the extensive cross-linkage introduced by glutaraldehyde, as a result of stable Michael-type additions formed between amino groups and α, β-unsaturated aldehydes (23, 31).

HF Binding on the Luminal Aspect of the Microvascular Endothelium

The existence of BMFs in the mouse diaphragm allowed the examination of the distribution of histamine receptors in well-identified sequential microvascular segments: i.e. arterioles, capillaries, and venules (36).

HF MONOMERS: In specimens obtained from animals perfused with HFM, the conjugate was found on restricted areas of the luminal front of endothelial cell plasmalemma. The conjugate appeared as monomers and, occasionally, as dimers (Figs. 6 and 7). The HF binding occurred mostly on those regions of the endothelial cells rich in filaments, especially the parajunctional areas (Fig. 8a, b). Occasionally, the HF monomers were found associated with plasmalemmal vesicles and coated pits, a process that was not significantly influenced either by the histamine antagonists or by histamine. In the BMFs of the diaphragm, HF binding was particularly prominent in venules, specially postcapillary (pericytic) venules, to a much lesser extent in arterioles and almost inconspicuous in capillaries. Aorta, veins, and muscular arteries from random specimens showed a low HF binding affinity.

HF AGGREGATES: In experiments with small aggregates, the electron microscope examination of thin sections from the arteriolar and venular level of BMFs specimens revealed the same localization of HFA as observed in the HFM experiments. The small aggregates appeared bound particularly to those parts of the venular endothelial cells which contained a relatively large number of cytoplasmic filaments or were located next to the parajunctional areas (Fig. 9). In specimens in which relatively large aggregates (0.2 to 0.5 μm Diam) have been used, the binding of the latter to venular endothelium produced a sharp decoration which could be detected by light microscope examination of cross sections (Fig. 11). The density of such labeling, however, was not always sufficient to render these vessels visible with the dissecting microscope. In cases in which such decoration occurred, it was frequently due to a combination of specific binding and, in addition, a partial plugging of the vessel lumen by large aggregates. For this reason we focused
our observations on experiments with HF monomers and HF small aggregates.

Control Experiments

Neither the native ferritin nor the activated ferritin bound specifically to endothelial cell plasmalemma. Preliminary estimates indicated that the number of HF monomers (or dimers) bound to the luminal aspect of vascular endothelium is ~12- to 15-fold more numerous than the nonspecific attachment of native or activated ferritin. Artificial aggregates of native or activated ferritin did not bind conspicuously to the endothelial cell membrane. Previous perfusion with 4 x 10^-5 M histamine resulted in the inhibition of HF conjugate binding to venular (high-affinity sites) and arteriolar (low-affinity sites) endothelium.

Characterization of H₁ and H₂ Receptors

The binding of PF and MF conjugates (as small aggregates) on endothelial cell membrane of different microvessels (BMFs) was recorded as the average number of binding sites per square micrometer (Table I). PF and MF distribution was found to mimic the localization of HF conjugate on the luminal front of the endothelial cell plasmalemma: postcapillary (pericytic) venules presented characteristic high-affinity sites for the two specific H₁ and H₂ receptor agonists (Fig. 10). In venules, MF binding (6.6 ± 0.94) was approximately twice as intensive as PF binding (3.1 ± 1.38). The percentage of MF and PF binding, out of the sum of MF and PF binding, was 68.0 and 31.9, respectively, presumably corresponding to the fractional distribution of H₂ and H₁ receptors in venular endothelium.

Cimetidine (H₂ receptor antagonist) perfused before HFa administration, inhibited almost completely the binding of the latter to the venular (and arteriolar) endothelium. When given before MF perfusion, cimetidine abolished completely the binding of this histamine agonist conjugate to the venular endothelium. The same results were also obtained when mepyramine was used before PF perfusion.

These findings support the idea that, at least in mouse diaphragm, the response of venular endothelium is mediated by both types of receptors, the H₂ receptors being predominant.

DISCUSSION

HF conjugate results from the coupling of the amino group of histamine to the glutaraldehyde-activated amino residues of ferritin. In the conjugate, histamine preserves its vasoactive activity probably by conversion to a secondary amine.

HF binding capacity cannot be ascribed to its slightly modified pI (5.6 as compared to 4.6 for the native ferritin) because, on the one hand, at this pI, ferritin itself does not bind to the endothelium, and on the other hand, it has been shown that both free histamine and histamine bound to a carrier display equipotent vasoactive effects independent of the charge characteristics of the conjugate (46, 47).
FIGURE 8  Venules of the mouse diaphragm, 3 min after perfusion of HF monomers. The conjugate is bound to a relatively high density on the luminal endothelial cell membrane in the parajunctional area (arrowheads) of both pericytic (a) and muscular venules (b). bl, basal lamina, e, endothelial cell, j, endothelial junction, l, lumen. (a) × 125,000; (b) × 138,000.

FIGURE 9  Pericytic venule of a mouse diaphragm, 3 min after perfusion of HFa: aggregates of relatively small size (a) are bound to the endothelial cell membrane, often corresponding to cell regions containing filaments (arrowhead). bl, basal lamina, e, endothelium, l, lumen. × 116,000.

FIGURE 10  Pericytic venule in a BMF of mouse diaphragm, 3 min after perfusion of 4-methylhistamine-ferritin conjugate: small aggregates of the conjugate (arrowheads), bound at relatively high density, on the luminal endothelial cell membrane. bl, basal lamina, l, lumen, ps, perivascular space, m, muscle, e, endothelium. × 104,000.
The fact that HF preserves the biological activity of the histamine suggests that the imidazol portion of the molecule—which is critical for recognition by receptor, particularly H2 receptor—was not changed by the coupling.

The results obtained indicate that the luminal aspect of vascular endothelium contains membrane receptors for histamine. They are especially well-represented in venules (both pericytic and muscular), at the level of which the aggregate area of histamine receptors represents a highly differentiated domain of the plasma-lemma. Hr are preferentially localized in cell regions rich in filaments, including the parajunctional zones. This might confirm Majno's original assumption that the opening of the venular junctions induced by histamine is due to the contraction of intracytoplasmic filaments (24-26). The possible implication of other factors (e.g., the contraction of pericytes and smooth muscle cells) has to be demonstrated. The search for HR on the abluminal front of vascular endothelium (presumably the first to "see" the histamine released by mast cells) will show whether the high density of HR detected on the luminal plasmalemma results at least in part from their migration in the plane of the membrane, with subsequent aggregation at the sites of histamine stimuli.

Despite the large representation of HF binding sites in the venules of various vascular beds, the distribution observed in mouse diaphragm may not represent a ubiquitous pattern; the particulars of the detailed HR distribution in other vasculature is now under investigation.

At least as revealed by the present findings, the receptor-mediated control of arterioles by histamine could be related to the endothelium but only by low-affinity sites. The marked chemical distinction between H1 and H2 receptor agonists (9, 13) and the considerable receptor selectivity of their antagonists (8, 33, 35) allows a reliable identification of the type of HR present in vascular endothelium. Our results indicate that histamine effects on the cardiovascular system are mediated by both types of HR; in venules the prevailing type is the H2 receptors. The mechanism of the cooperation between H1 and H2 receptors and the related cyclic AMP or GMP changes during the biphasic histamine effects on venules is still unclear. However, our observations indicate that histamine action on venular endothelium is receptor-mediated and that the H2 receptors appear to be an important component of the inflammatory condition. The opening of endothelial junctions in venules, as a prerequisite of extravasation and diapedesis, may be a complex process in which histamine participates by inducing the contraction of filaments (probably followed by relaxation) (4) which represent a common denominator in endothelial cells, pericytes, and smooth muscle cells.

Perfusion of specific histamine receptor antagonists impeded the binding and the subsequent formation of plugs of HF large aggregates. These findings support the idea that this kind of massive decoration implies an initial specific binding of HF conjugate. A procedure for the controlled decoration of vascular segments in mouse diaphragm (as a biological or pharmacological test for assessing the antihistaminic activity of different drugs) is now under investigation (16, 17).

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