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COMMENTARY

DETECTION OF HISTAMINE RECEPTORS AT CELLULAR LEVEL

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Ash and Schild [1] have suggested that the pharmacological activities of histamine are mediated by at least two different types of receptors. The *concept of two receptors for histamine* as well as the characteristics of histamine H_1 and H_2 receptors have been defined on a pharmacological basis: H_1 receptors are specifically inhibited by classical antihistamines such as mepyramine, while H_2 receptors are blocked by antagonists such as cimetidine [2, 3]. Both H_1 and H_2 receptors are widely distributed in the gastrointestinal, respiratory and cardiovascular systems, nervous system, mast cells, smooth muscle cells, and leukocytes [3–8]. Histamine receptors play an important role in physiological, immunological and immunopathological processes. There is now sufficient evidence that these actions of histamine are caused by its direct action on specific receptors. The nature of histamine receptors varies not only from species to species but also from tissue to tissue [9].

The availability of selective receptor agonists and antagonists has stimulated new interest in the study of the nature of responses to histamine in many systems, including the cardiovascular system [4, 9]. The response to small doses of vasoamine could be abolished by treatment with H_1 receptor antagonists, whereas the responses to larger doses of histamine were refractory to histamine H_1 receptor blockade. With the introduction of histamine H_2 receptor antagonists, as reported for cimetidine [2, 10–12], the depressor responses to histamine, which persisted after histamine H_1 receptor blockade, were shown to be due to interaction with histamine H_2 receptors.

The aim of this commentary is to discuss some of the problems and the recent data related to the detection of histamine receptors on different mammalian cells. A wide variety of physiological reactions mediated by H_1 and H_2 receptors which are distributed on the cell membrane of mammalian tissue have been reported [1, 13–16]. The evidence for histamine receptors and their subclasses on diverse cells, including those of the cardiovascular system, has relied primarily on the target responses to histamine and its specific agonists and antagonists.

Smooth muscle cells seem to possess both H_1 and H_2 histamine receptors. They mediate vasoconstrictor and vasodilator actions of histamine, depending on the species, although the dual effect of vasoactive amine is unmasked by the presence of only an antagonist to one of the receptors. Presumably, the net effect of histamine depends upon the relative number and distribution of the two types of receptors on the membrane [3, 9]. Membrane fraction of small intestinal smooth cells contains histamine receptors, and attempts have been made to purify them, using an indirect method, namely the binding of ^{14}C-labeled dibenamine. Unfortunately, this binds to other receptor types, being a nonspecific drug, in a chemical and pharmacological sense. In experiments aimed to evidentiate histamine receptors, in addition to [^{14}C]dibenamine, 5′-nucleotidase was used as a plasma membrane marker. Distribution of this marker is closely paralleled by radioactivity, suggesting that labeled receptor was associated with the membrane [6]. Another indirect detection of histamine receptors was achieved by determining the [^3H]mepyramine specific binding—as a selective high-affinity H_1 receptor antagonist—to homogenates of intestinal smooth muscle from guinea pig [17] or mammalian brain membranes [5]. The affinity constant of [^3H]mepyramine for histamine H_1 receptor found to be $1.6 \pm 0.4 \times 10^{-9}$ M was deduced from the parallel shift of the log dose–response (contraction) curve [1]. As shown before, using suitable protecting agents, the histamine receptor in smooth muscle could be labeled with the relatively nonspecific antagonist [^{14}C]dibenamine. The amount of labeled receptor material seems, however, remarkably large, being some 200-fold greater than that bound by [^3H]mepyramine (68 pmoles/g protein). Even allowing for species differences (cat vs guinea pig), there should be some suspicion that the bulk of the dibendiamine was bound to nonreceptor sites. These observations indicate that [^3H]mepyramine might be used to investigate the number of histamine receptors in various tissues and in various conditions and to study the binding characteristics of H_1 agonists and antagonists [6, 17].

Gastric mucosal cells from guinea pig were shown to possess only histamine H_2 receptors. The interaction of histamine with specific membrane receptors on this type of cells was performed using a direct method of binding, namely by incubation of dispersed mucosal cells with [^3H]histamine. Specific binding was determined by competitive interaction with histamine. The affinity constant was $5–25 \times 10^{-6}$ M as determined by either equilibrium or kinetic analysis. Maximal binding capacity found was 62 ± 29 pmoles/10^6 cells. The binding was localized

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to membrane components that are probably the H_2 receptors on the plasma membrane of parietal cells. Kinetic and thermodynamic analysis of binding indicated the presence of a single class of receptors with no evidence for cooperative interaction between [^{3}H]histamine and the receptors. The histamine binding to the receptor is followed by activation of adenylate cyclase and an increase in cellular cyclic AMP. In terms of cyclic AMP and [^{3}H]histamine binding, these receptors have higher affinity for H_2 agonists or H_2 antagonists than for the respective H_1 drugs [18]. The use of [^{3}H]cimetidine for affinity binding studies on the same type of cells (fundic mucosal cell membranes) showed a specific radioligand binding which might not represent the labeling of a particular pharmacological H_2 receptor but a specific recognition site of the imidazole moiety only. Obviously, this radioantagonist is not an ideal ligand for binding studies at the H_2 receptor [19, 20].

Calf thymocyte membrane fragments were incubated with [^{3}H]histamine: maximal specific binding occurred after incubation at a concentration of 5×10^{-6} M [^{3}H]histamine. By saturable binding of [^{3}H]histamine to these membrane fragments and by appropriate incubations with H_1 and H_2 receptor antagonists, the binding characteristics of H_1 and H_2 receptors in this system have been characterized by the fact that the H_1 site appears to be of higher affinity but less numerous than the H_2 receptors [21]. From experiments on gastric and thymocyte cells, [^{3}H]histamine appears to be a useful tool for studying histamine receptors and the structural-functional relationships of newly synthetized histamine antagonists that may have important therapeutic applications. Furthermore, [^{3}H]histamine could be useful in the solubilization and isolation of histamine receptors [18].

During recent years it has been demonstrated that mixed *leukocyte* populations possess specific receptors for a variety of hormonal substances, e.g. insulin, beta-adrenergic catecholamines, prostaglandins and histamine. These agents stimulate production of cyclic AMP [7, 22, 23]. Recent evidence indicates that a certain lymphocyte subpopulation carries histamine receptors having an important regulatory function both *in vitro* and *in vivo* [16, 24]. Murine T cells which are implied in the regulation of antibody secretion, and the precursors of T cells which are capable of mediating cytotoxic effector cells, carry histamine receptors [24, 25]. Receptors for histamine on cell membranes have been identified by the effect of histamine on intracellular cyclic AMP [24, 26], by using [^{3}H]histamine, histamine-coated beads or histamine-coated red cells [7, 27, 28]. Receptors for histamine and other small hormones on human leukocytes were also studied by insolubilizing the hormones and incubating them with the cells. The insolubilized hormones could not enter cells and the existence of histamine receptors on plasma membranes was accordingly established, because it was sufficient for the insoluble substances to combine with receptor sites in order to produce intracellular effects [29]. The receptors must recognize an extraordinarily small chemical determinant of the histamine but must be specific since the interaction with the insolubilized histamine is prevented by antagonists [22]. Columns of histamine-conjugated beads (such as histamine-BSA-Sepharose and histamine-synthetic polymer-Sepharose) were used to determine the distribution of receptors in mixed leukocyte populations [7]. The histamine-coated beads bound specifically a majority of human leukocytes but not platelets or erythrocytes [22]. Insolubilized histamine can be used to separate morphologically similar cells, to test whether they are biologically different. With insolubilized drugs, further characteristics of the drug–cell interaction can be deduced [7, 22].

The first evidence of histamine receptors at the light microscope level has been produced by using fluorescein isothiocyanate-bovine serum albumin-histamine conjugate (FITC-BSA-H). Rabbit *blastocyst* (containing two types of cells, the inner cell mass, and the trophoblast) and uterine *endometrium* (containing epithelial, stromal and glandular cells) were incubated *in vitro* with FITC-BSA-H and examined under a fluorescence microscope. The control cells, incubated with FITC-BSA in the absence of histamine, were negative. The specificity of binding was determined by incubating cell suspensions with free histamine or H_1 and/or H_2 receptor antagonist before incubation with the biologically active fluorescent conjugate. More work is necessary to elucidate the possible role of histamine receptors in blastocyst–endometrium metabolism and in implantation and acceptance of the blastocyst allograft by the maternal tissues. The bimodal function of histamine through H_1 and H_2 receptors is feasible, both of them having a possible importance in ovum implantation and in suppressing *in situ* maternal capacity to reject the implanting embryo carrying alloantigens [14].

We have reported recently the *in situ* evidence, at the light and electron microscope level, of histamine receptors on the *vascular endothelium* by using new electron-opaque conjugates of histamine and its H_1 and H_2 receptor agonists 2-pyridylethylamine and 4-methylhistamine respectively [30–33]. The biologically active conjugates were obtained by covalently coupling glutaraldehyde-activated ferritin to histamine (histamine–ferritin conjugate [HF]), to 2-pyridylethylamine (pyridylethylamine–ferritin conjugate [PEF]) and to 4-methylhistamine (methylhistamine–ferritin conjugate [MHF]) (Table 1).

Histamine–ferritin (HF) was prepared as monomers or small aggregates (~0.05 to 0.2 μm diameter), whereas PEF and MHF were prepared as small aggregates only. The HF monomers were separated from unbound histamine by chromatography on a Sepharose 6B column followed by dialysis against Tris–HCl buffer. Through experiments using [^{3}H]histamine, the molar ratio of HF was estimated in the range of ~70:1. The small aggregates of HF, PEF or MHF, obtained by dialyzing the crude conjugate against 0.15 M NaCl, facilitated a large sampling of vessels, especially those vascular segments containing numerous high-affinity binding sites for histamine or its agonists. The purity of the conjugates was assessed by polyacrylamide gel electrophoresis and thin-layer chromatography.

Two different tests were used to check the biological activity of HF. The conjugate maintained its

Table 1. Electron-opaque conjugates prepared, and antagonists used, for the detection of the histamine receptors*

Electron-opaque conjugates		Antagonists	Histamine receptors
Hormone	Agonists		
Histamine–ferritin (HF)			H
	2-Pyridylethylamine–ferritin (PEF)	Mepyramine	H_1
	4-Methylhistamine–ferritin (MHF)	Cimetidine	H_2

* Modified from Ref. 33.

ability to produce contraction of the smooth muscle prepared from the fundal portion of the rat stomach [34]. The same preparation was not sensitive to native ferritin or to 0.15 M NaCl solution added to the organ bath. The capability to induce venular leakage was tested for HF monomers applied topically or HF aggregates injected intravascularly. Results demonstrated that HF conjugate is able to induce the extravasation of previously injected carbon particles or of HF aggregates themselves, as a result of typical focal separation of endothelial junctions in venules. Neither native ferritin nor activated ferritin induced detectable vascular leakage.

The distribution of histamine receptors along the luminal surface of the endothelium in several vascular segments including arteries, arterioles, capillaries, venules and veins was explored *in situ* with HF aggregates or monomers. The inquiry was carried out mostly on mouse diaphragm, heart, lung and pancreas. The general screening of histamine receptors revealed their high density on the microvascular endothelium, particularly concentrated in venules (both pericytic and muscular). The lowest values have been found in capillaries and arteries (both elastic and muscular) and quite a few binding sites in arterioles and veins. For further detailed study we concentrated on well defined bipolar microvascular fields recognizable in some areas of the mouse and rat diaphragm [35–37].

In specimens collected from animals perfused with HF monomers, the binding occurred on restricted areas of the luminal surface of endothelial cell membrane mostly on cell regions rich in cytoplasmic filaments and particularly in the parajunctional zones. In experiments with HF aggregates, the binding pattern was similar to that recorded for HF monomers.

The localization of the two classes of histamine receptors was carried out by perfusion of either pyridylethylamine-ferritin as specific agonist conjugate for H_1 receptors or methylhistamine-ferritin for H_2 receptors. The two conjugates seem to mimic the localization of HF on the endothelial cell plasmalemma; their binding on the endothelium of different microvessels has been recorded as the average number of affinity sites per square micrometer and presented characteristic high values in venules (Table 2).

MHF binding (~62%) was approximately twice as intensive as PEF binding (~32%), presumably corresponding to the fractional distribution of H_2 and H_1 receptors in venular endothelium.

The specificity of histamine-ferritin binding to the luminal surface of the microvascular endothelium was assessed by controls with native ferritin or glutaraldehyde-activated ferritin. None of them bound specifically to the endothelial cell membrane. Occasional association of the conjugates or native

Table 2. Specific binding of HF, MHF and PEF in the microvessels of the mouse diaphragm*

	Average binding sites/μm^2 endothelial surface			
Conjugate	Arterioles	Capillaries	Venules	Total
(as aggregates)	(%)	(%)	(%)	
HF	1.2 ± 0.66	4.5 ± 0.85	12.0 ± 2.20	17.7 ± 1.24
	(6.8)	(25.4)	(67.8)	
MHF	1.4 ± 0.25	3.5 ± 0.38	6.6 ± 0.94	11.5 ± 0.52
	(12.2)	(30.4)	(57.4)	
PEF	1.3	2.0 ± 0.26	3.1 ± 1.38	6.4 ± 0.82
	(20.3)	(31.3)	(48.4)	
MHF + PEF†	2.7	5.5 ± 0.32	9.7 ± 1.16	17.9 ± 0.67
	(15.1)	(30.7)	(54.2)	

* For each type of conjugate, the average numbers of vascular profiles examined were: 2–4 arterioles, 4–12 capillaries, and 6–12 venules. In each vascular profile, the endothelial cell surface (luminal surface) explored was in the range of 0.3 to 3.0 μm^2. Values are means ± S.E.M.; the percentage of binding in a given type of vessel out of the total recorded for that microvascular bed is given in parentheses. The table is modified from Ref. 32.

† Values represent the sum of the binding figures recorded separately in MHF and PEF experiments.

and glutaraldehyde-activated ferritin with plasmalemmal vesicles was interpreted as an uptake process not significantly influenced either by the histamine antagonists or by histamine. In competitive inhibition experiments, previous perfusion with histamine abolished the HF binding. The inhibitory effect of mepyramine (H_1 receptor antagonist) and cimetidine (H_2 receptor antagonist) on the HF binding emphasized the specific H_1 and H_2 antagonists respectively. These types of experiments also support the observation mentioned above, in terms of the fractional distribution of the two types of histamine receptors. At least in the vasculature we have explored, venules are particularly rich in H_2 receptors and it appears that the H_2 receptor-mediated mechanism might be a major component of the inflammatory condition. It has been shown that in postcapillary (pericytic) venules, endothelial junctions have a very loose organization [36] and about 30% are open to a gap of ~6 nm [37]. It was demonstrated that venules are the "leaky" segment of the microvasculature which, in inflammation or after administration of mediators such as histamine, undergo focal opening of endothelial junctions that allows extravasation of colloidal carbon and blood cells [38–40].

These studies represent the first localization of histamine receptors at the ultrastructural level. The new electron-opaque conjugates appear to be suitable tools for the detection of histamine-binding sites on the cell membrane of different types of cells from various tissues. The search for histamine receptors on the albuminal surface of vascular endothelium is under way. The new techniques applied to the study of affinity binding sites for histamine together with the pharmacological methods could lead to the merging of cell biology with pharmacology in their efforts for more comprehensive studies on hormone receptors.

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