Histamine Modulates Contraction and Cyclic Nucleotides in Cultured Rat Mesangial Cells

Differential Effects Mediated by Histamine H_1 and H_2 Receptors

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Abstract

Histamine influences the glomerular microcirculation and modulates immune-inflammatory responses. In the rat kidney, histamine is synthesized by glomeruli and stimulates cyclic nucleotide production specifically in glomeruli. We investigated the in vitro effect of histamine on cyclic nucleotide accumulation in rat cultured glomerular mesangial and epithelial cells. Histamine stimulated cyclic AMP (cAMP) accumulation in cultured mesangial cells (64.0±22.1 to 511.4±86.6 pmol/mg protein, n = 9) but had no effect on cAMP accumulation in epithelial cells. This effect was dose-dependent and time-dependent. Stimulation of cAMP accumulation occurred in the range of 5×10^{-6} M–10^{-4} M histamine with a half maximal stimulatory effect of 2×10^{-5} M. Initial stimulation was noted by 30 s, and maximum stimulation was observed at 5 min. The H_2 antagonist cimetidine (10^{-4} M) abolished the stimulatory effect of histamine (10^{-4} M), while equimolar concentrations of the H_1 antagonist diphenhydramine had no significant effect on cAMP accumulation. Moreover, the specific H_2 agonist dimaprit, but not the H_1 agonist 2-pyridylethylamine, stimulated cAMP accumulation. Histamine had no effect on cAMP accumulation in epithelial cells or on cyclic guanosine monophosphate accumulation in epithelial or mesangial cells.

Since the in vivo infusion of histamine reduces ultrafiltration coefficient and since mesangial cell contraction is thought to be responsible for the reduction in the ultrafiltration coefficient, we examined the effect of histamine on the contractile property of mesangial cells. Histamine (5×10^{-6}–10^{-4} M) contracted mesangial cells, and the H_1 antagonist diphenhydramine (10^{-4} M) but not the H_2 antagonist cimetidine (10^{-4} M) prevented histamine (10^{-4} M) induced contraction. In addition, the H_1 agonist 2-pyridylethylamine, but not the H_2 agonist dimaprit, contracted mesangial cells. Histamine and its specific agonists and antagonists induced contraction of isolated glomeruli as assessed by glomerular planar surface area in a manner parallel to their effect on mesangial cells. Cinnarizine (10^{-5} M), a Ca^{++} channel blocker, or Ca^{++}, Mg^{++}-free medium prevented histamine (10^{-4} M) induced mesangial cell and glomerular contraction. Thus, histamine enhances cAMP accumulation specifically in mesangial cells via an H_2 receptor. In contrast, histamine contracts mesangial cells and glomeruli via an H_1 receptor, an effect that is dependent on extracellular Ca^{++} entry. These findings show that histamine potentially influences intraglomerular hemodynamics via effects on mesangial cell contraction. Moreover, our findings considered with the in vivo observation that histamine reduces k_f via an H_1 receptor provide further support for the hypothesis that mesangial cell contraction regulates the glomerular capillary surface area available for filtration. Our studies also show that this contractile effect of histamine is dependent on extracellular calcium.

The presence of a cAMP system sensitive to histamine may have major implications in the pathogenesis of inflammatory glomerulopathies. Mesangial cells possess characteristics similar to circulating and tissue immune effector cells, including lysosomal enzyme release, oxygen radical production, and release of a number of immunomodulatory factors. Histamine and cAMP have been shown to modulate such characteristics of inflammatory cells. It is therefore conceivable that histamine, via its interaction with H_2 receptors and subsequent generation of cAMP, may have profound effects on such properties of mesangial cells, suggesting that this autacoid may modulate not only glomerular hemodynamics but also immune, inflammatory responses within the glomerulus.

Introduction

Recent in vivo and in vitro studies show that renal glomeruli actively participate in the regulation of the glomerular microcirculation under physiological conditions or in the course of renal injury (1–11). It is well recognized that the renal glomerulus is a target organ for a number of circulating hormones and locally synthesized compounds with potent biologic activity (12, 13). In vivo micropuncture studies have shown that the infusion of the peptide hormone angiotensin II (Ang II)[1] causes a reduction in the ultrafiltration coefficient (k_f), a major determinant of single nephron glomerular filtration rate, and that this reduction results from a decline in the glomerular capillary surface area (5, 11). Since Ang II contracts glomeruli (8, 9) and mesangial cells (14) in vitro, it is believed that the contraction of mesangial cells leads to a reduction in glomerular capillary surface area and hence k_f. Hormones potentially influence glomerular and mesangial cell contraction by at least two mechanisms. The first is a direct hormonal effect that requires extracellular calcium. The in vivo effect of Ang II to

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1. *Abbreviations used in this paper:* Ang II, angiotensin II; cGMP, cyclic guanosine monophosphate; DMEM, Dulbecco's modified eagle medium; ETYA, 5,8,11,14 eicosatetraynoic acid; GPSA, glomerular planar surface area; k_f, ultrafiltration coefficient; MIX, 1-methyl-3-isobutylxanthine; MPMC, Millipore particle measurement computer; PAN, aminonucleoside of puromycin; PEA, pyridylethylamine; TCA, trichloroacetic acid.

reduce k_f is completely reversed by the infusion of verapamil, a calcium entry blocker, suggesting that this effect is dependent on extracellular Ca^{++} (11, 15). The second mechanism seems to be mediated by a cyclic AMP (cAMP)-induced activation of the renin-angiotensin system. The in vivo effect of dibutyl cAMP, parathyroid hormone, and vasodilator prostaglandins to reduce k_f is reversed by the Ang II antagonist saralasin (3). Since several of these autacoids increase glomerular cAMP production (12) and also stimulate renin release (1, 16), local generation of Ang II is most likely responsible for the reduction of k_f observed in the in vivo micropuncture studies.

Histamine is a renal autacoid synthesized in rat (17) and human glomeruli (18) that has also been shown to influence glomerular metabolism in vitro (19) and renal hemodynamics, including the glomerular microcirculation, in vivo (20, 21). Torres et al. (19) were the first to describe an H_2 receptor-mediated, stimulatory effect of histamine on cAMP production in rat glomeruli. Studies using labeled histamine H_1 and H_2 receptor antagonists show that both receptors are present in glomeruli (22) and that a hormone-sensitive adenylate cyclase primarily linked to the H_2 receptor is present on glomerular cells (22, 23). The glomerular cell type(s) bearing histamine receptors is not known. Ichikawa et al. (21), using micropuncture technique, found that histamine reduces k_f in vivo in the rat and that this effect is blocked by an H_1 receptor antagonist. More recently, histamine has been shown to stimulate renin release by the isolated perfused rat kidney, an effect primarily mediated by an H_2 receptor (24). In addition to its hemodynamic effects on the renal and other regional circulations, histamine is an important regulator of the immune-inflammatory response in many tissues (25–27).

The present studies were designed (*a*) to determine the glomerular cell type in which histamine exerts its effects on cAMP accumulation; and (*b*) to study the effects of histamine on glomerular and mesangial cell contraction.

Methods

Histamine dihydrochloride, 1-methyl-3-isobutylxanthine (MIX), diphenhydramine HCl, and cinnarizine were purchased from Sigma Chemical Co. (St. Louis, MO). Ang II and forskolin were purchased from Calbiochem Corp. (San Diego, CA). Cimetidine, dimaprit, and 2-pyridylethylamine were kindly supplied by Mr. C. Porter of Smith, Kline, and French (Philadelphia, PA). Indomethacin, meclofenamate, and 5,8,11,14 eicosatetraynoic acid (ETYA) were kindly provided by Dr. M. Dunn, Case Western Reserve University, Cleveland, OH. [^{3}H]cAMP for recovery measurement was purchased from New England Nuclear (Boston, MA). Antibodies and ^{125}I-labeled antigens for cAMP were from radioimmunoassay (RIA) kits purchased from Schwartz/Mann, Div. of Becton-Dickinson & Co. (Oxnard, CA). Antibodies and ^{3}H-labeled antigens for cyclic guanosine monophosphate (cGMP) were purchased from Amersham Corp. (Arlington Heights, IL).

Glomerular cell cultures. Glomerular isolation, mesangial, and epithelial cell cultures were performed as described (28–30), with minor modifications. 75–125 g male Sprague-Dawley rats (Charles River, North Wilmington, MA) were anesthetized with ether, and the kidneys were removed. All subsequent steps were performed at 0–4°C under a laminar flow hood. Renal cortices from 4–6 rats were pooled, minced with a sterile razor blade, and pressed gently through a 212-μm stainless steel sieve. The resulting tissue was collected and suspended in Dulbecco's phosphate-buffered saline (PBS; Gibco Laboratories, Grand Island, NY) containing penicillin, streptomycin, and fungizone. After vigorous pipetting to disperse the tissue, the glomerular suspension was passed through successive Nitex nylon sieves of 250, 211, 150, and 50 μm pore size (Tetko Inc., Rolling Meadows, IL). The glomeruli were then collected from the top of the 50-μm sieve, resuspended, and washed twice with PBS. After the final washing, the glomerular suspension was centrifuged at 1,000 rpm for 5 min and resuspended in RPMI 1640 medium (M.A. Bioproducts, Walkerville, MD), buffered with 15 mM Hepes at pH 7.4, supplemented with 15% decomplemented fetal calf serum (Gibco Laboratories), 0.66 U/ml insulin (Sigma Chemical Co.), penicillin 100 U/ml, streptomycin 100 μg/ml, and fungizone 250 ng/ml (Gibco Laboratories), and diluted in half with conditioned medium from Swiss 3T3 cells in log phase growth (kindly provided by Dr. Richard Miller from Case Western Reserve University, Cleveland, OH). Culture dishes were incubated in 95% air and 5% CO_2 in a humidified incubator at 37°C. 20–30% of glomeruli attached, and epithelial cells started to grow within 24–48 h and reached confluence 6–9 d after culture. Experiments on epithelial cell cultures were performed at this time.

To obtain mesangial cells, glomeruli were plated as above, and cultures were allowed to grow for 21–28 d. At this time the cultured cells are confluent mesangial cells. Subcultures were then performed using 0.025% trypsin–0.5 mM EDTA in calcium and magnesium-free PBS. Harvested cells suspended in culture medium were then plated onto 35-mm dishes (Costar, Cambridge, MA) and placed in a humidified incubator containing 5% CO_2 and 95% air. 24 h later, medium containing nonadherent cells and occasional glomerular tufts was aspirated and replaced with fresh culture medium. Mesangial cells grew and reached confluence 10–14 d after subculture. All cultures were fed every 48 h.

Several criteria were used to establish the identity of the cultured cells. First, morphologic studies were done. Cell monolayers were examined by phase-contrast microscopy using an inverted microscope equipped with a SLR 35 mm camera (E. Leitz, Inc., Rockleigh, NJ). For electron-microscopy (29, 30), monolayers were washed free of media and fixed in a 2.5% glutaraldehyde, 4% sucrose, 0.05 M cacodylate buffer, pH 7.4, for 2–6 h. Cells were then washed, scraped, and pelleted in 0.1 M cacodylate buffer and post-fixed in 1.0% osmium tetroxide in 0.2 M S-collidine buffer. Cell pellet was then rinsed with distilled water and stained in block in 1% aqueous uranyl acetate, rinsed again, and dehydrated in an ascending series of acetone and embedded in Spurr (a mixture of vinylcyclohexane dioxide, D.E.R. 736, nonenyl succinic anhydride, and dimethylamino ethanol). The resulting blocks were cut on an ultramicrotome. 1-μm thick sections were studied by light microscopy. Thin sections were examined with a Joel-JEM-100 CX electron microscope with magnification ranging from 2,000 to 30,000. Second, growth in L-valine deficient medium was examined (31). Fibroblast contamination was assessed by the ability of mesangial cells to grow in medium in which L-valine was substituted by D-valine (Gibco). Third, the effect of aminonucleoside of puromycin (PAN) and mitomycin C was examined. Cultured epithelial and mesangial cells were treated with PAN, a previously described epithelial cell cytotoxin (32), or mitomycin C, a mesangial cell cytotoxin (33), and cell growth was evaluated periodically by phase contract microscopy.

To exclude endothelial cell contamination, mesangial cell cultures grown on glass cover slips were stained for Factor VIII antigen using immunoperoxidase technique. Specific antiserum for Factor VIII antigen (rabbit antiserum against human Factor VIII antigen), and other reagents were purchased from Dako Corp., Santa Barbara, CA. Control tissue included human umbilical vein endothelial cells (kindly provided by Dr. Paul DiCorleto, Cleveland Clinic Foundation) and cryostat sections of snap frozen rat renal cortical tissue. Negative controls were performed by incubating cultured human endothelial and mesangial cells and the rat cortical tissue with normal (nonimmune) rabbit serum.

Cyclic nucleotide incubations. To test the effect of histamine and its specific agonists and antagonists on cyclic nucleotides, incubations were performed with cell monolayers in 35-mm culture wells. At incubation time, medium was aspirated and cell monolayers were rapidly washed twice with PBS. Incubation medium was Hank's balanced saline solution (HBSS) containing 1.2 mM Ca^{++} and 1.25

mM Mg^{++} (pH 7.4) or modified Kreb's buffer of the following composition: 140 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 2.0 mM $CaCl_2$, 10 mM glucose, 10 mM Na acetate, 2.0 mM Na phosphate, and 20 mM Tris buffer (pH 7.4). The medium was warmed to 37°C immediately before its addition to the dishes. In the experiments where the effect of histamine antagonists were tested, the cells were preincubated with medium containing the antagonists or medium alone for 5 min. Medium was then aspirated and replaced with medium alone or medium containing histamine plus or minus the antagonist. Cells were incubated in a final volume of 1.0 ml at 37°C. The reaction was terminated by the addition of 0.5 ml. 15% ice-cold trichloroacetic acid (TCA) and cell dishes were placed at 0°C on ice. Cells were then scraped from the dishes using a rubber policeman; they were transferred to microcentrifuge Eppendorf tubes, and cell suspensions were sonicated. The homogenate was then centrifuged (12,000 *g*) for 30 min in the cold to precipitate the proteins, and the supernatant was separated. TCA was removed from the supernate by repeated extraction (×4) with water-saturated ether, and the remaining ether was evaporated by heating. The samples were then adjusted to neutral pH with 0.5 N NaOH. Aliquots of these extracts were diluted and used for determination of cAMP or cGMP by RIA as described previously (34, 35). Briefly, cyclic nucleotides determination by RIA was carried out as originally described by Steiner et al. (36) and modified to increase the sensitivity of cAMP and cGMP determination by acetylation and succinylation, respectively (37, 38). The pellet was solubilized in 1.0 N NaOH and the protein determination was carried out by the method of Lowry et al. (39). In two experiments, at the end of the incubation, the medium was rapidly separated from the cells and frozen, and 5% ice-cold TCA was added to the cells to end the reaction. cAMP was then determined on aliquots from the medium and cells separately.

In a preliminary experiment, tracer amounts of [^{3}H]cAMP were added to some dishes for monitoring recovery. Recovery of [^{3}H]cAMP in the final extract immediately before RIA exceeded 95%. Therefore, this step of monitoring the recovery was omitted from the rest of the experiments. Drugs used in this study were tested for possible interference with the RIA. At the concentration used in this study, none of the drugs interfered with either cAMP or cGMP determination.

Mesangial cell contraction. The contractile response of mesangial cells was evaluated using phase contrast microscopy (40, 41). At the time of the experiment culture medium was aspirated, and cells were washed twice with PBS. Cells were then incubated with fresh HBSS (with Ca^{++} and Mg^{++}) in the presence or absence of the tested agents and contraction was evaluated in cells incubated at room temperature (22°C) or after incubation at 37°C in a humidified incubator containing 5% CO_2 and 95% air. Timed control incubations were included in each experiment. When the effect of histamine antagonists or Ca^{++} channel blockers was studied, cells were preincubated in HBSS with or without the antagonist or the Ca^{++} blocker for 5 min. This medium was then replaced with fresh medium alone or medium containing histamine plus or minus the antagonist or the Ca^{++} channel blocker. In some experiments the effect of histamine was studied using each dish as its own control. Washed cells were incubated with HBSS (with Ca^{++} and Mg^{++}), and histamine was then added in small volumes (10–25 μl) to the final concentration desired.

Glomerular contraction. The contractile response of isolated rat glomeruli to histamine and its specific agonists and antagonists was determined as follows. Rat glomeruli were isolated by the sieving technique as described earlier and suspended in Dulbecco's modified eagle medium (DMEM, Gibco). 100-μl aliquots of the isolated glomeruli (200–250 glomeruli/100 μl) were distributed into test tubes kept at room temperature and drugs dissolved in DMEM were added to the tubes in 50-μl volumes. DMEM was then added for a final incubation volume of 500 μl and the final concentration of drugs as specified in Results. When histamine antagonists or Ca^{++} channel blockers were used, the glomerular suspensions were first incubated with the antagonist or the Ca^{++} blocker for 5 min before adding histamine. Tubes were then incubated at room temperature for 30 min. In each experiment, control incubations of glomerular suspensions without drugs were included. At the end of the incubation, 500 μl of 2.5% glutaraldehyde was added to each incubation tube to fix the glomeruli. Glomeruli were then allowed to settle by gravity. Glomerular contraction was assessed by measuring the change in glomerular planar surface area (GPSA) using a Millipore Particle Measurement Computer (MPMC) system (Millipore Corp., Bedford, MA) (42, 43). The components of this system include a microscope, video camera, video display, and computer. An aliquot of the glomerular suspension is placed on a glass slide and covered with a glass cover slip. The glass slide is placed under the objective of the light microscope, and the image of the glomerulus is transmitted to the monitor. The image of the glomerulus on the monitor appears as a dark area compared with the white contrasting background. The module accurately measures the two-dimensional surface area that the object (glomerulus) occupies and calculates GPSA in square micrometers (μm^2). The probable error for measuring surface area by MPMC is ±2.4%. At least 50 glomeruli were counted from each incubation sample. In each experiment, tubes containing glomerular suspensions were coded to avoid experimental bias. In some experiments, values were also expressed as percent of control glomerular surface area (incubation without drugs).

Results

Characteristics of cultured glomerular cells. The majority of the glomerular cell outgrowth at 7–9 d after glomerular cultivation were identified as epithelial cells using morphologic criteria (29, 32). By phase contract light microscopy, the cells grew in monolayers, appeared small and polyhedral, and had a pavement-cobblestone appearance when confluency was reached (Fig. 1 *A*). On electron microscopy, this cell seemed to have a large rounded nucleus with prominent nucleoli. The cytoplasm contained elongated mitochondria, rough and smooth endoplasmic reticulum, and abundant free polysomes. In addition, the cells appeared to have intercellular junctions and microvilli on their surface, a characteristic of glomerular epithelial cells. When incubated in the presence of PAN (100 μg/ml), 50% of the cells were detached by 12 h, while the attached cells rounded up and showed spindle-shaped processes. By 24 h, >90% of the cells were detached and floating in the medium. In contrast, identical incubation with mitomycin C (10 μg/ml) had no effect on epithelial cells as judged by phase contrast light microscopy.

The identity of mesangial cells was confirmed by the following criteria. Using phase contrast microscopy, the cells appeared large, stellate, or fusiform and contained prominent intracellular fibrillar structures that ran parallel to the plasma membrane similar to cultured mesangial cells described by other investigators (Fig. 1 *B*) (14, 30, 32). By electron microscopy, the cells displayed numerous bundles of microfilaments, elongated nuclei, and dense patches (Fig. 1 *C*). In some preparations, at least 20 consecutive cell micrographs were examined (× 22,500). All cells had the characteristic features of mesangial cells described. Moreover, the cells contracted in the presence of angiotensin II. Fibroblast contamination was excluded by the cells' ability to grow and reach confluence in D-valine containing medium. When mesangial cells were incubated with 10 μg/ml mitomycin C, cells lost their flat appearance and the majority were detached by 24 h. PAN (100 μg/ml), on the other hand, induced no morphological changes. Moreover, mesangial cell cultures showed no staining for Factor VIII antigen using antiserum that stained both human cultured endothelial cells and endothelial cells of rat renal vessels including glomerular endothelial cells, thereby excluding endothelial cell contamination.

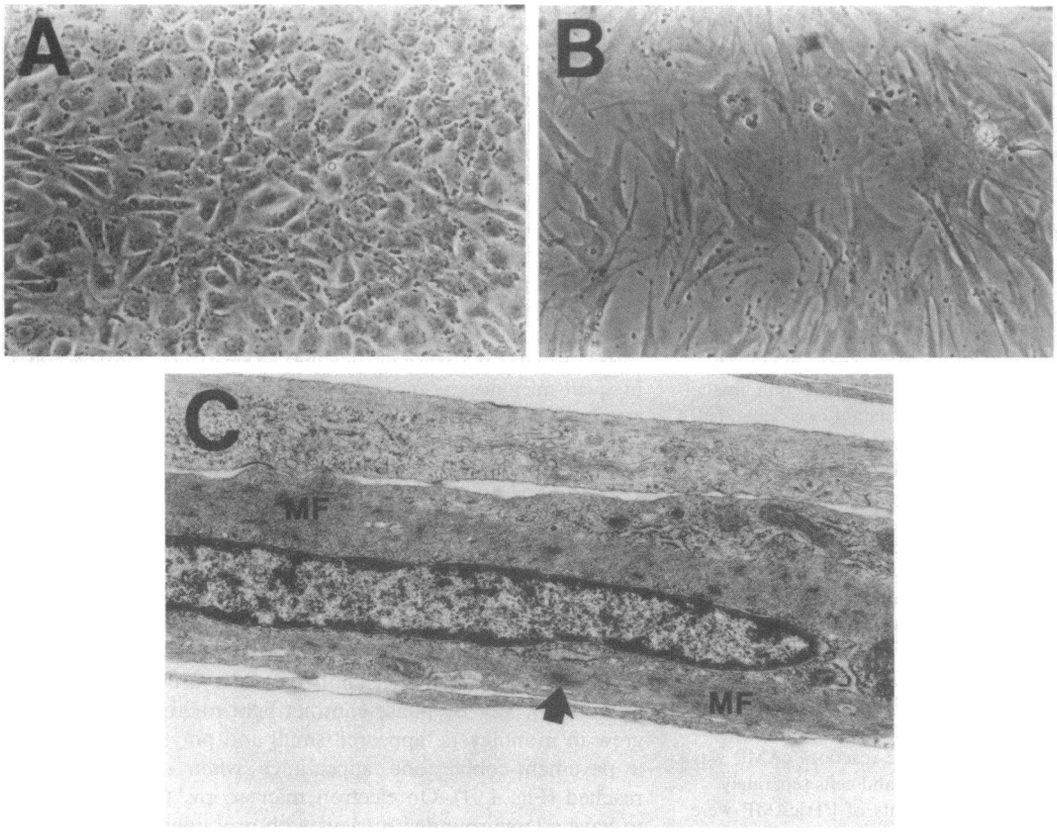

Figure 1. (*A*) Phase contrast photomicrograph of glomerular epithelial cells in culture (× 200); (*B*) phase contrast photomicrograph of glomerular mesangial cells in culture (× 200); and (*C*) transmission electron micrograph of a mesangial cell. Note the oval nucleus, myofilaments (MF), and the dense patch (arrow).

Effect of histamine and histamine agonists and antagonists on cyclic nucleotides. In cultured rat mesangial cells, in the presence of 0.5 mM MIX, histamine (10^{-4} M) caused from threefold to 21-fold increase in cAMP accumulation (Fig. 2). In the absence of MIX, histamine also stimulated cAMP accumulation. However, the stimulatory effect of histamine (10^{-4} M) was much less prominent than in the presence of MIX (Fig. 2). The enhanced cAMP accumulation was primarily intracellular. <5% of total cAMP was released into the medium under basal conditions or in response to histamine (data not shown). In contrast to its effects on cAMP, histamine had no effect on cGMP accumulation in the same cell cultures (Fig. 2). However, nitroprusside, a nonspecific stimulant of guanylate cyclase (44), enhanced cGMP accumulation in the same cell cultures, suggesting the presence of an intact guanylate cyclase system. Histamine stimulated cAMP but not cGMP accumulation in mesangial cells in a time-dependent fashion (Fig. 3). The stimulation was observed as early as 30 s and reached maximum at 5 min, after which it started to decline. When total cAMP production was determined at 5 min as a function of histamine concentration, there was a dose-dependent increase in cAMP accumulation between 5×10^{-6} and 10^{-4} M histamine (Fig. 4 *A*).

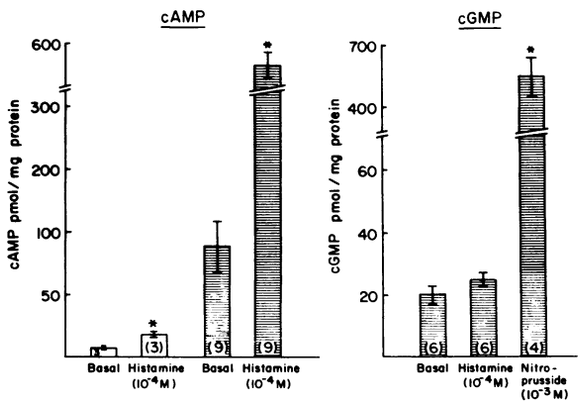

Figure 2. Effects of histamine on cAMP and cGMP accumulation in cultured mesangial cells. Incubations were performed for 5 min at 37°C. Each bar represents mean±SEM of the number of experiments indicated in parenthesis at the bottom of each bar. In each experiment, incubations were performed on 2–5 dishes. Asterisks (*) denote values significantly different from corresponding basal values (without agonists) at $P < 0.05$ or better (t test). □, without MIX; ▤, with MIX.

To determine the histamine receptor subclass involved in the enhanced production of cAMP in mesangial cells, we next examined the effects of specific histamine receptor agonists and antagonists (45). The histamine H_2 receptor agonist dimaprit stimulated cAMP but not cGMP accumulation in a dose-dependent manner, while 2-pyridylethylamine, a histamine

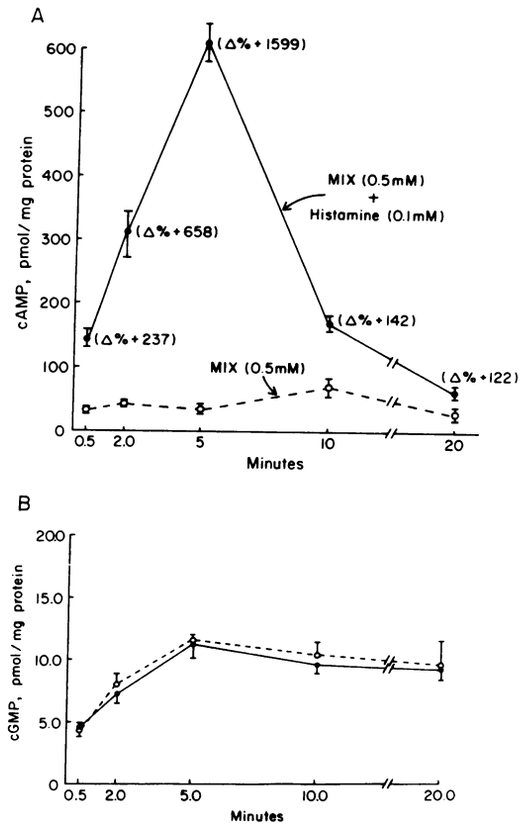

Figure 3. Representative time course of the effects of histamine on (10^{-4} M) cAMP accumulation (*A*) or cGMP accumulation (*B*) in cultured mesangial cells. Incubations were performed at 37°C (in the presence of 0.5 mM MIX). Points indicate the mean±SEM for duplicate determinations on each of three culture dishes. Relative changes (%) represent differences from corresponding control values (in the presence of MIX alone). - - - o - - -, MIX (0.5 mM); — • —, MIX (0.05 mM) plus histamine (0.1 mM).

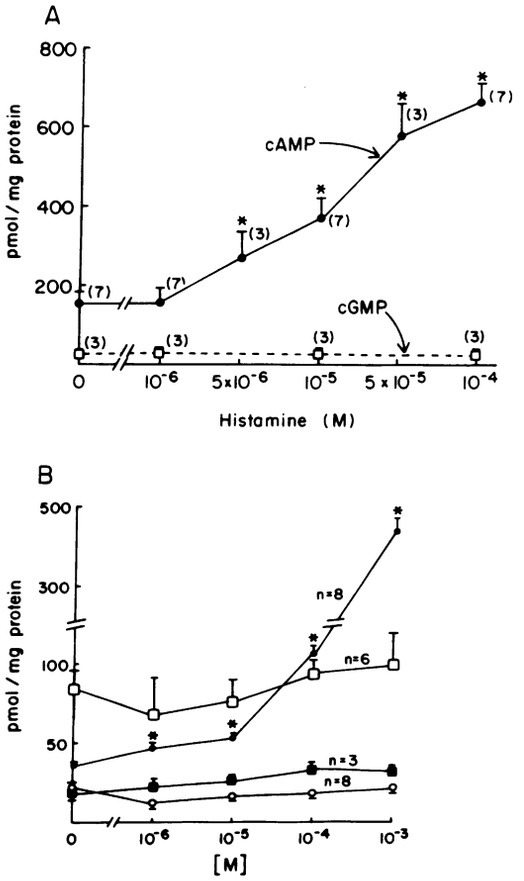

Figure 4. Dose response curve of the effects of histamine (*A*) or the histamine specific H_1 agonist (PEA) and the H_2 agonist (dimaprit) (*B*) on cAMP and cGMP accumulation in cultured mesangial cells. All incubations were performed for 5 min at 37°C (in the presence of 0.5 mM MIX). Each point represents mean±SEM for duplicate determinations on each of the number of dishes, indicated in parenthesis (from three separate cell cultures). Asterisks (*) denote values significantly different from basal values at $P < 0.05$ or better (analysis of variance). — • —, dimaprit, cAMP; — □ —, PEA, cAMP; — ■ —, dimaprit, cGMP; — o —, PEA, cGMP.

H_1 receptor agonist, had no significant effect on cAMP or cGMP accumulation (Fig. 4 *B*). Moreover, the H_2 receptor antagonist cimetidine (0.1 mM) but not equimolar concentrations of the H_1 receptor antagonist diphenhydramine abolished the stimulatory effect of histamine (0.1 mM) on cAMP accumulation (Fig. 5). The mild inhibitory effect of the H_1 antagonist on cAMP accumulation in response to histamine may have resulted from its weak binding to the H_2 receptor at this concentration (45). Cimetidine and diphenhydramine alone had no effect on cAMP accumulation (Fig. 5).

In contrast to its effects on mesangial cells, histamine did not influence either cAMP or cGMP accumulation in epithelial cell cultures (Fig. 6). Enhanced accumulation of cAMP in response to forskolin, a diterpene that directly activates adenylate cyclase (46), shows that the adenylate cyclase system of the epithelial cells is intact and suggests that epithelial cells do not contain histamine receptors. Therefore, the presence of histamine H_2 receptors is a distinct feature of glomerular mesangial cells.

To explore the role of arachidonic acid metabolites in mediating the histamine-induced increase in cAMP accumulation, we next examined the effect of the arachidonate metabolism inhibitors indomethacin, meclofenamate, and ETYA. The cyclooxygenase inhibitors indomethacin (10^{-5} M) and meclofenamate (3×10^{-6} M) (concentrations that inhibited prostaglandin E_2 production by mesangial cells > 90%, data not shown) did not influence histamine-stimulated cAMP accumulation. In addition, the cyclooxygenase and lipooxygenase inhibitor ETYA, in a concentration (10^{-5} M) that has been shown to markedly inhibit lipooxygenase pathway in other cell systems (47), also had no significant effect on cAMP production by mesangial cells (Table 1).

Effect of histamine and histamine specific agonists and antagonists on mesangial cell contraction (Table II). Mesangial cells incubated with HBSS containing Ca^{++} and Mg^{++} retained their flat, spindle-shaped appearance. The addition of histamine to the incubation medium elicited cell contraction. Contracted cells lost their flat appearance, became rounded, and their cytoplasmic processes moved closer to the cell body (14, 40, 41) (Fig. 7). When the contraction studies were performed at

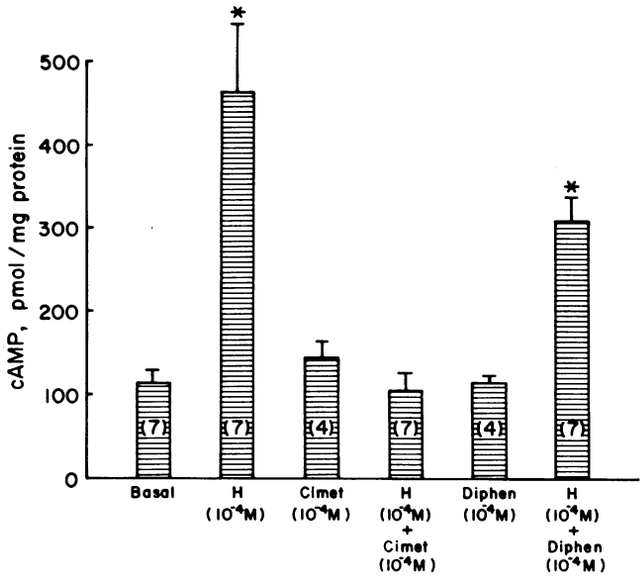

Figure 5. Effects of histamine H_2 antagonist cimetidine and H_1 antagonist diphenhydramine on cAMP accumulation in cultured mesangial cells. All incubations were performed for 5 min at 37°C (in the presence of 0.5 mM MIX). Each bar represents mean±SEM for duplicate determinations on each of the number of dishes indicated in parenthesis. Asterisks (*) denote values significantly different from basal values of $P < 0.05$ or better (t test).

37°C, the contractile response was observed within 2 min after the addition of histamine. At room temperature (22°C), the contractile response was observed 5–10 min after adding histamine. Mesangial cells remained contracted in response to 10^{-4} M histamine as long as 60 min after its addition. When the cells were washed free of drug and reincubated with fresh medium, the cells regained their usual appearance. In the presence of 10^{-4} M histamine, 30–40% of cells within a field contracted. Lower concentration of histamine caused fewer

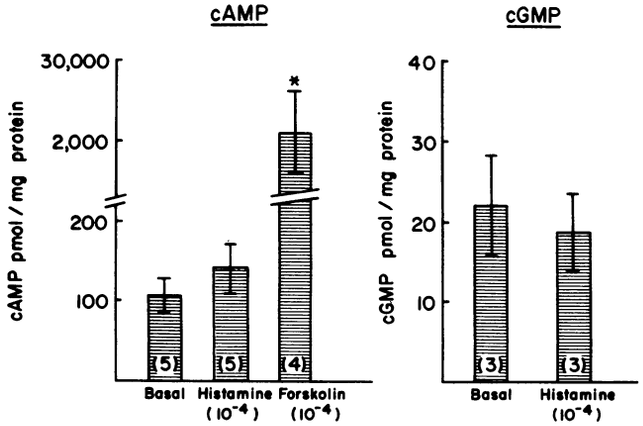

Figure 6. Effects of histamine on cAMP and cGMP accumulation in cultured epithelial cells. All incubations were performed for 5 min at 37°C (in the presence of 0.5 mM MIX). Each bar represents the mean±SEM of the number of experiments indicated in parenthesis at the bottom of each bar. In each experiment, incubations were performed on 2–3 dishes. Asterisks (*) denote values significantly different from corresponding basal values (without agonists) at $P < 0.05$ or better (t test).

cells to contract. The lowest concentration of histamine that elicited identifiable contraction was 5×10^{-6} M. Preincubation of mesangial cells with the histamine H_1 receptor antagonist, diphenhydramine (10^{-4} M) for 5 min before the addition of histamine (10^{-4} M), prevented the histamine-induced cell contraction. Similar incubations with the histamine H_2 receptor antagonist cimetidine (10^{-4} M) had no effect on histamine-(10^{-4} M) induced cell contraction. To further characterize the histamine receptor involved in mediating the contractile response, we tested the effect of specific histamine receptor agonists. The H_1 agonist pyridylethylamine (PEA) (10^{-5} M and 10^{-4} M) elicited a contractile response while equimolar concentration of the H_2 agonist dimaprit had no effect. To determine the role of extracellular Ca^{++} in the contractile response to histamine, contraction studies were carried out in Ca^{++}-free and Mg^{++}-free medium. No cell contraction was observed when histamine (10^{-4} M) was added to cells in Ca^{++}-free medium. Similarly, when cells were preincubated with medium containing Ca^{++} and the calcium channel blocker cinnarizine (48) for 5 min and then challenged with histamine, no contractile response was elicited.

Effects of histamine and histamine-specific agonists and antagonists on GPSA. Quantitation of mesangial cell contraction is difficult. Since glomeruli contract and mesangial cells are the only cells within the renal glomerulus that have been shown to contract (32, 40, 49), we studied glomerular contraction in response to histamine and its specific agonists and antagonists. GPSA was assessed after a 30-min incubation and subsequent fixation with glutaraldehyde. Histamine (10^{-4} M) reduced GPSA in every glomerular preparation tested. Similar to findings by others, Ang II (10^{-9} M) incubated under identical conditions also reduced glomerular size (data not shown) (8, 9, 50). This effect of histamine was dose-dependent with reduction of GPSA observed at between 5×10^{-6} and 10^{-3} M (Fig. 8). To determine the specificity of histamine's effect and to define the type of histamine receptor involved, the effect of histamine-specific antagonists and agonists was studied. The H_1 receptor antagonist diphenhydramine abolished the contractile effect of histamine and restored glomerular size to base-line values (Fig. 9). In contrast, cimetidine, an H_2 receptor antagonist, did not interfere with the histamine-induced glomerular contraction. Neither cimetidine nor diphenhydramine alone altered glomerular size. Moreover, the histamine H_1 receptor agonist PEA significantly reduced glomerular size, while the histamine H_2 agonist dimaprit had no effect (Fig. 9). To determine the role of extracellular Ca^{++} in the histamine-induced glomerular contraction, the effect of histamine was examined in glomerular suspensions in the presence and absence of the Ca^{++} channel blockers cinnarizine. Cinnarizine alone had no effect on glomerular size. However, cinnarizine abolished the contractile effect of histamine (Fig. 9).

Discussion

These studies show that histamine stimulates cAMP production in rat mesangial cells primarily by an H_2 receptor and that histamine contracts mesangial cells and glomeruli via an H_1 receptor. These latter effects require extracellular calcium. Several criteria suggest that the effect of histamine on cAMP accumulation is mediated by distinct histamine receptors. Inhibition of the stimulatory effect of histamine on cAMP accumulation by cimetidine but not by diphenhydramine

Table I. Effect of Cyclooxygenase and Lipooxygenase Inhibitors on Histamine-stimulated cAMP Production in Mesangial Cells

Inhibitor	No.	Control	Inhibitor	Histamine (10^{-4} M)	Histamine (10^{-4} M) + inhibitor
Indomethacin (10^{-5} M)	3	91.5±23*	93.9±35	491.8±44‡	475.9±53‡
Meclofenamate (3×10^{-6} M)	4	89.2±17	71.3±7.2	428±61	402.5±50‡
ETYA (10^{-5} M)	3	94.4±19	70.9±13	471.4±44‡	382±72‡

* Mean±SEM of values from the number of separate cell cultures indicated (at least triplicate wells from each culture); data are expressed as picomoles cAMP per milligram protein. Mesangial cells were preincubated with medium alone or medium containing the inhibitor for 5 min. This medium was then aspirated and replaced with medium alone (basal), medium containing the inhibitor, or medium containing histamine with or without the inhibitor. For details of the subsequent steps, see Methods. ‡ Significantly higher compared with basal ($P < 0.05$, t test). Values for cAMP in the presence of histamine were not significantly different from those in the presence of histamine plus the inhibitor.

indicates that the effect of histamine is mediated by an H_2 receptor linked to adenylate cyclase. The stimulatory effect of the histamine H_2 agonist dimaprit but not the H_1 agonist PEA on cAMP accumulation also supports this conclusion. Moreover, distinct agonists (histamine, adenosine, isoproterenol, PGE_2, and vasopressin) elicited variable degrees of cAMP accumulation within the same cell culture (unpublished observations), implying the presence of distinct autacoid receptors on the mesangial cell.

The absence of an effect of histamine on cAMP accumulation in epithelial cells points to the mesangial cell as the primary site of histamine receptors linked to cAMP accumulation. These were first described in intact rat glomeruli (19) and more recently in human glomeruli (18). Using specific histamine H_1 and H_2 receptor antagonists in the rat, Torres et al. (19) identified the glomerular histamine receptor that is associated with enhanced cAMP accumulation as H_2 in type. While our studies do not exclude an effect of histamine on

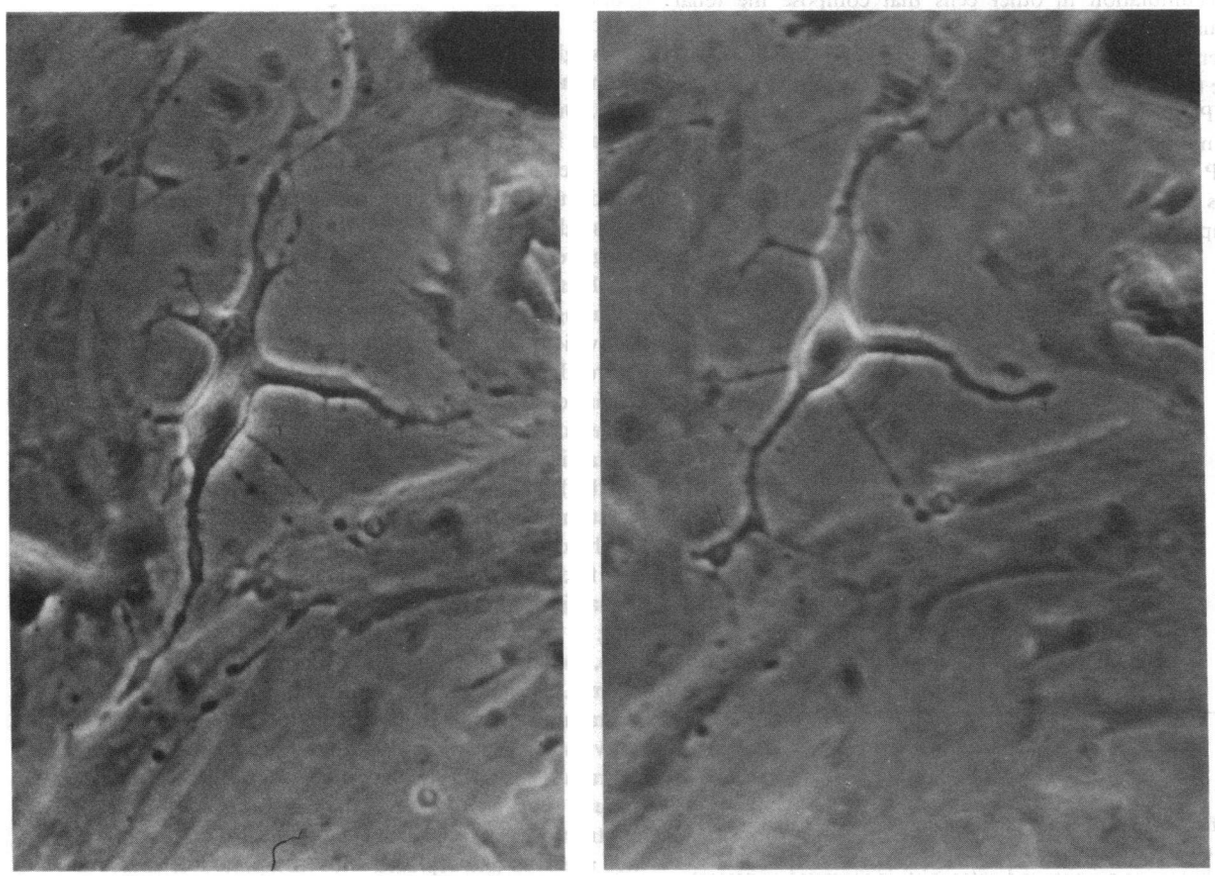

Figure 7. Phase contrast micrograph of a relaxed (*left*) and contracted (*right*) mesangial cell (× 320). 5 min after the addition of HBSS (with Ca^{++} and Mg^{++}) alone, note that the relaxed cell is well spread with long processes. However, 5 min after incubation with HBSS with Ca^{++} and Mg^{++} and histamine (0.1 mM), note the morphological change in the same cell. The cell body is rounded and the cell processes are retracted. Qualitatively similar changes were observed in the presence of lower concentrations of histamine and in the presence of the H_1 agonist, pyridylethylamine.

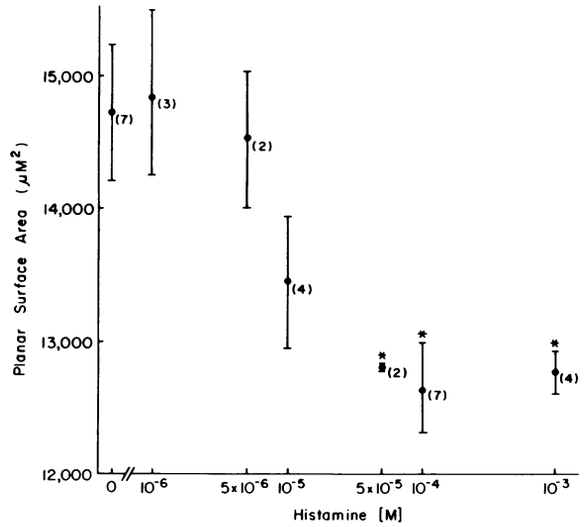

Figure 8. Dose response curve of the effect of histamine on GPSA, which is expressed in square micrometers (μm^2). Each point indicates mean±SEM of the number of experiments in brackets. * denotes significantly different from control glomeruli, $P < 0.05$ (analysis of variance). Note that vertical axis is not continuous with zero.

Table II. Effect of Histamine and its Specific Agonists and Antagonists on Mesangial Cell Contraction

Effector	Cell contraction
H (10^{-4} M)	6/6
H (10^{-4} M) + diphenhydramine (10^{-4} M)	0/3
H (10^{-4} M) + cimetidine (10^{-4} M)	3/3
Pyridylethylamine (10^{-4} M)	3/3
Dimaprit (10^{-4} M)	0/3
H (10^{-4} M) + cinnarizine (10^{-5} M)	0/2
H (10^{-4} M) + Ca^{++}-free medium	0/2

Mesangial cells were incubated with the tested agents in HBSS (with Ca^{++} and Mg^{++}) at 22°C, and morphologic changes were assessed by phase contrast microscopy. When histamine antagonists or calcium channel blockers were used, the cells were first preincubated with the antagonist or the Ca^{++} channel blocker for 5 min. Data represent the ratio of the number of experiments in which cells contracted (at least 30% of cells within a field showed typical morphological changes). Timed control incubations were included in each experiment. H, histamine.

cAMP accumulation in other cells that compose the renal glomerulus, such as endothelial cells, note that histamine does not influence cAMP accumulation in vascular endothelial cells in culture (51). The absence of a stimulatory effect of histamine on cGMP accumulation in mesangial and epithelial cells suggests indirectly that the stimulatory effect of the histamine on cGMP accumulation observed in glomeruli by Torres et al. (19) is an effect on glomerular endothelial cells or other cells composing the renal glomerulus. Indeed, histamine has been shown to stimulate cGMP production in vascular endothelial cells in culture (51).

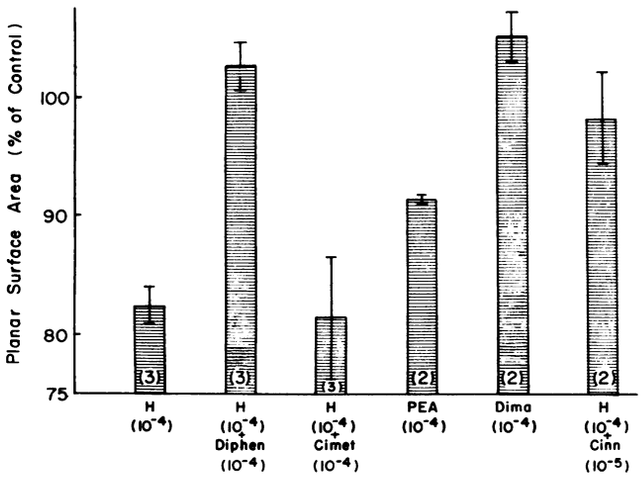

Figure 9. Effect of histamine (H) and histamine specific agonists and antagonists on glomerular planar surface area (GPSA). GPSA in the presence of histamine agonists and antagonists is expressed as percent of control (GPSA of glomeruli incubated under identical conditions but in the absence of drugs). Bars denote mean±SEM. Numbers between brackets denote the number of experiments. For details see methods. Note that the vertical axis is not continuous with zero. Dima, dimaprit.

In several tissues including vascular endothelium (52) and rabbit lung (53), histamine has been shown to stimulate prostaglandin synthesis. Moreover, prostaglandins (PGE_2) have been shown to stimulate cAMP production in mesangial cells (14). Our experiments using the cyclooxygenase and lipooxygenase inhibitors suggest that arachidonic acid metabolites do not mediate the stimulatory effect of histamine on cAMP production in mesangial cells. Note that the histamine-induced prostaglandin synthesis in both endothelial cells and rabbit lung are H_1 receptor mediated events.

Histamine has been reported to decrease k_f in vivo by reducing glomerular capillary surface area (21). The mechanism by which hormones or vasoactive substances reduce k_f is currently thought to involve glomerular and specifically mesangial cell contraction (1–11). Therefore, we next examined the effect of histamine on mesangial and glomerular contraction. Histamine contracted mesangial cells, an effect that was blocked by the H_1 antagonist diphenhydramine but not the H_2 antagonist cimetidine. The involvement of the H_1 histamine receptor in the contractile effect of histamine is further supported by the finding that the H_1 agonist PEA but not the H_2 agonist dimaprit contracted the cells. Unlike the contractile effect of Ang II on mesangial cells, which lasts for only 10–15 min (14), the contractile effect of histamine persisted for as long as 60 min. This effect may result from a slower rate of histamine degradation by mesangial cells. This possibility is very likely, in view of our recent findings of a rather low histamine degradative capacity within glomerular cells as compared with tubular cells (54). However, histamine-induced mesangial cell contraction was reversible when histamine-containing medium was removed and replaced with histamine-free medium.

Since mesangial cell contraction is difficult to quantitate, we next examined the effect of histamine and histamine agonists and antagonists on GPSA. A reduction in GPSA, glomerular contraction, most likely reflects mesangial cell contraction since mesangial cells are the only cells within the

renal glomerulus that have been shown to contract (1, 32, 40). Similar to its effects on mesangial cells, histamine also contracted glomeruli by an H_1 receptor. The contractile effect of histamine was dose-dependent, mimicked by the H_1 agonist PEA and blocked by the H_1 antagonist diphenhydramine. These studies on isolated glomeruli confirm our observations in the cultured mesangial cells and suggest that the in vitro effect of histamine on mesangial cell contraction is not appreciably modified by the presence of the endothelial or the epithelial cells that compose the glomerular capillary wall. This finding differs from previous observations on the effect of histamine and vasoactive hormones on other vascular beds where the presence of endothelial cells exert a modulating role on the contractile response (55, 56).

The concentrations of histamine needed to induce mesangial cell or glomerular contraction and to stimulate cAMP in mesangial cells approximate the endogenous content of histamine in glomeruli (17) and are far above the circulating levels of plasma histamine (17). Endogenous histamine produced by glomeruli (17) or histamine released by infiltrating inflammatory cells is therefore more likely than circulating histamine to influence glomerular cell function. However, the cellular site of histamine synthesis within the glomerulus, the quantitative distribution of histamine within the various cell types, and the accessibility of the histamine receptor to locally produced histamine remain to be determined.

There is considerable evidence that in smooth muscle cells, an increase in free cytosolic Ca^{++} induces contraction and a decrease leads to relaxation (57, 58). Hormones that induce contraction in smooth muscles require Ca^{++} for their contractile activity (48, 57, 58). Histamine has been shown to enhance Ca^{++} entry into several tissues, including vascular endothelium (59), bronchial smooth muscle (60), and brain (61). Moreover, Ang II-elicited contraction in mesangial cells was dependent on extracellular Ca^{++} (62). We therefore explored the role of Ca^{++} in mediating the action of histamine on mesangial cell and glomerular contraction. The contractile response of mesangial cells and glomeruli observed with histamine was dependent on extracellular Ca^{++}. Both isolated glomeruli and mesangial cell cultures treated with histamine in Ca^{++} free medium or in calcium containing medium in the presence of the calcium channel blocker cinnarizine did not contract. Since cinnarizine does not directly affect intracellular Ca^{++} translocation, the above studies do not exclude the possibility that histamine-induced mesangial cell contraction is also dependent on changes of Ca^{++} within the cell.

Histamine has been shown to stimulate cGMP accumulation in several tissues via an H_1 receptor and by a Ca^{++} dependent mechanism (57, 58). The relationship between the enhanced cGMP accumulation and the actions of histamine in these tissues, however, has not been established (63). The absence of a stimulatory effect of histamine and the histamine H_1 agonist PEA on cGMP accumulation suggest that the effect of histamine on mesangial cell contraction, although Ca^{++} dependent, is independent of cGMP. It has been shown in the smooth muscle of the guinea pig ileum that the H_1 receptor is coupled to the phosphatidyl-inositol cycle, which is thought to act as a calcium gating mechanism in the cell membrane (64). It remains to be determined whether the H_1 receptor on the mesangial cell is also coupled to the phosphatidyl-inositol cycle.

These in vitro studies suggest that the in vivo effect of histamine to reduce glomerular capillary surface area is mediated by an H_1 receptor, and is dependent on the availability of extracellular Ca^{++}. Since the infusion of histamine in the isolated perfused kidney stimulates renin release via an H_2 receptor (24), it remains possible that activation of the renin-angiotensin system may at least partially mediate the observed in vivo effect of histamine. This possibility, however, is very unlikely in view of the findings by Ichikawa et al. (21) that the in vivo effect of histamine to reduce k_f is totally reversed by the administration of H_1 antagonists. These findings agree with our in vitro studies. The in vivo effect of Ca^{++} channel blockers on the hemodynamic changes induced by histamine is not known.

These findings show that histamine potentially influences glomerular hemodynamics via mesangial cell contraction and provide further support for the hypothesis that the contractile property of the mesangial cell is the underlying mechanism for the reduction in glomerular capillary surface area observed in the in vivo studies in respone to vasoactive hormones. In this context, note that histamine (present studies), Ang II, (8, 14, 41), and arginine vasopressin (14) are the only hormones that have been shown to reduce k_f in vivo and to simultaneously contract glomeruli or mesangial cells in vitro. Whether altered intraglomerular histamine metabolism in renal disease may similarly influence glomerular hemodynamics remains to be determined. It is conceivable that the enhanced production of histamine found in glomeruli isolated from rats with PAN nephrosis (17) may be responsible, at least partially, for the decline in k_f observed in this experimental model (65).

The implications of our findings of the presence of histamine H_2 receptors linked to adenylate cyclase and cAMP accumulation in mesangial cells remain speculative. Recent studies have shown that mesangial cells possess characteristics similar to those of immune effector cells. This is exemplified by their enrichment with high activity of lysosomal enzymes (66), their ability to release neutral proteinases in culture (67), and their capacity to synthesize mediators such as arachidonic acid metabolites (30, 68). In addition, mesangial cells are capable of phagocytosis (68, 69), production of oxygen-derived radicals (68, 70), and production of immunomodulatory factors (71). In a number of inflammatory cells, such properties have been shown to be influenced by histamine H_2 receptor induced increase in cAMP (25, 72, 73). Our present finding that the cAMP system in mesangial cells is responsive to histamine suggests that this mediator synthesized locally within glomerular cells or released from infiltrating inflammatory cells may play an important role in modulating mesangial cell function in the course of renal and specifically glomerular diseases.

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