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Actions and metabolism of histamine in glomeruli and tubules of the human kidney

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Actions and metabolism of histamine in glomeruli and tubules of the human kidney. The effects of histamine on cAMP and cGMP accumulation and the intrarenal metabolism of histamine were studied in glomeruli and cortical tubules of nine human kidneys. Histamine stimulated cAMP but not cGMP accumulation in glomeruli (Δ + 100% to + 265%) in a dose- (10^{-6} to 10^{-4} M range) and time-dependent manner. This effect of histamine was inhibited by the histamine H_2 antagonist cimetidine but not the H_1 antagonist diphenhydramine. Moreover, the H_2 agonist dimparit but not the H_1 agonist 2-pyridylethylamine stimulated cAMP accumulation. Histamine had no effect on cAMP or cGMP accumulation in tubules. Because the content of histamine ($\simeq 2 \times 10^{-6}$ M) in glomeruli was far above the circulating levels of plasma histamine in humans ($<10^{-8}$ M), we explored whether histamine is formed in human renal tissue. Incubation of glomeruli with 1 mM of the histamine precursor L-histidine resulted in an increase in histamine levels ($+\Delta 6.08 \pm 0.5$ pmoles/mg protein, $N = 7$ kidneys) while a marked drop in histamine levels was observed in tubules ($-\Delta 13.8 \pm 2.4$ pmoles/mg protein, $N = 7$ kidneys). The increase in histamine levels in glomeruli was abolished by the histidine decarboxylase inhibitor bromocresine. These results indicate that human glomeruli have histamine H_2 receptors, which mediate enhanced cAMP accumulation, and that glomeruli are major sites of histamine production in the human kidney. Histamine acting via cAMP may influence glomerular function of the human kidney.

Effets et métabolisme de l'histamine dans les glomérules et les tubules de rein humain. Les effets de l'histamine sur l'accumulation de cAMP et de cGMP, et le métabolisme intra-rénal de l'histamine ont été étudiés dans les glomérules et les tubules corticaux de neuf reins humains. L'histamine stimulait l'accumulation de cAMP mais non celle de cGMP dans les glomérules (Δ + 100% à 265%) de façon dose- (de 10^{-6} à 10^{-4} M) et temps-dépendante. Cet effet de l'histamine était inhibé par la cimétidine, antagoniste H_2 de l'histamine mais non par la diphenhydramine, un antagoniste H_1. En outre, le dimparit, un agoniste H_2, mais non la 2-pyridyléthylamine, un agoniste H_1, a stimulé l'accumulation de cAMP. L'histamine était sans effet sur l'accumulation tissulaire de cAMP ou de cGMP. Parce que le contenu d'histamine dans les glomérules ($\simeq 2 \times 10^{-6}$ M) était bien supérieur aux niveaux circulants d'histamine plasmatique chez l'homme ($<10^{-8}$ M), nous avons exploré si l'histamine se forme dans le tissu humain normal. L'incubation de glomérules avec 1 mM d'histidine, le précurseur de l'histamine, a entrainé une augmentation des niveaux d'histamine ($+\Delta 6{,}08 \pm 0{,}5$ pmoles/mg protéines, $N = 7$ reins) alors qu'une chute marquée des niveaux d'histamine était observée dans les tubules ($-\Delta 13{,}8 \pm 2{,}4$ pmoles/mg protéines, $N = 7$ reins). L'augmentation des niveaux d'histamine dans les glomérules a été abolie par la bromocrésine, un inhibiteur de l'histidinedécarboxylase. Ces résultats indiquent que les glomérules humains possèdent des récepteurs H_2 pour l'histamine qui médient la stimulation de l'accumulation de cAMP, et que les glomérules sont les sites principaux de production d'histamine dans le rein humain. L'histamine agissant par l'intermédiaire du cAMP pourrait influencer la fonction glomérulaire dans le rein humain.

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Several recent observations suggest that the biogenic amine histamine may play a role in renal pathophysiology [1–9]. In the rat kidney, histamine has been shown to alter renal hemodynamics and glomerular microcirculation [2] and to stimulate renin release [3]. In rat glomeruli, histamine elicits a marked increase in 3′,5′-adenosine cyclic monophosphate (cAMP) accumulation [4, 5], and histamine receptors linked to adenylate cyclase are present on glomerular cells [6]. We and others have recently shown that glomeruli are the major sites of histamine production in this species [7]. In addition, studies in some experimental models of glomerular disease strongly suggest a role for histamine in modulating the renal injury [7, 8]. Very little information is available on the metabolism and cellular actions of histamine in the human kidney (HK) [1, 9]. Lindberg, Lindell, and Westling [10] found that minced tissue of human fetal kidney converted labeled histidine to labeled histamine. Zeller et al [11] described a diamine oxidase activity in the human renal cortex and medulla. Lindell [12] found that whole renal tissue produces radiolabeled methylimidazole acetic acid from ^{14}C-histamine suggesting that the methylation pathway is also present in the human kidney. More recent studies provide indirect evidence that histamine may be involved in renal pathophysiology in humans. In normal subjects, loading studies with the histamine substrate L–histidine show an increase in urine but not blood histamine [13] and provide indirect evidence that the human kidney is capable of histamine production. Moore, Thompson, and Glassock [14] found that the urinary and blood levels of histamine are elevated following renal transplantation in humans. The histamine H_2 antagonist cimetidine has been reported to cause actual reduction in glomerular filtration rate (GFR) in patients with impaired renal function [15], implying a role for histamine in modulating renal function in humans.

Because certain metabolic properties of isolated nephron segments have been reported to differ in humans compared to other species [16, 17], the present studies were undertaken to examine the cellular actions of histamine in isolated glomeruli

and nonglomerular cortical tissue (tubules) of the human renal cortex and to localize the site(s) of histamine production in the human kidney.

Methods

Studies were performed on tissue obtained from nine adult human kidneys (HK): six cadaver donor kidneys judged unsuitable for transplantation, and normal renal tissue obtained from three surgical nephrectomy samples. All donor kidneys were initially flushed with ice-cold Collin's solution and then perfused via the renal artery with purified protein fraction and immersed in the same solution (in the perfusion apparatus) up until the time of transfer to the laboratory. The surgical samples were obtained immediately following nephrectomy. The data available on the nine patients includes: HK 1 was from a 51-year-old patient who underwent nephrectomy for a large hematoma involving the middle portion and the upper pole of the kidney; the uninvolved lower pole of the kidney was used. HK 2 was a cadaver donor solitary kidney from a 56-year-old patient who died in an auto accident. HK 3 was a cadaver donor kidney from a 44-year-old patient; the cause of death was not listed. HK 4 was a donor kidney from a 41-year-old patient who died from a stroke. HK 5 was from a 58-year-old patient who underwent nephrectomy for a nonobstructing tumor of the renal pelvis. The renal parenchyma was not involved. HK 6 was a cadaver donor kidney from a 35-year-old patient who also died in an auto accident. HK 7 was a cadaver donor kidney from a 31-year-old patient who died from head injuries in an auto accident. HK 8 was a donor kidney from a 5-year-old patient who died from a traumatic subdural hematoma. HK 9 was from a patient who underwent nephrectomy for a large hypernephroma. An uninvolved piece (as judged by light microscopy on a frozen section) of the renal cortex most distant from the tumor was used.

As soon as the tissue samples became available, they were immersed in ice-cold modified Kreb's buffer (KRB) 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 sodium acetate, 2 mM sodium phosphate, and 20 mM Tris (pH = 7.4), and transferred to the laboratory. The renal capsule was stripped off the parenchyma, the kidney was bisected, and the cortical tissue was dissected with a pair of scissors. All subsequent steps were performed at 4°C. Glomeruli and nonglomerular cortical tissue samples referred to here as "tubules" were prepared by a sieving technique as described [5, 18] with minor modifications. The cortical tissue samples were minced and then pressed through a stainless steel sieve (212 μm) with a metal spoon. The resulting suspension was passed in succession through 390-, 250-, and 150-μm nylon sieves; glomeruli were collected from the top of the 150-μm sieve. The glomerular suspension was then allowed to settle by gravity for 5 min and the supernatant containing small tubular fragments or portions of glomeruli was aspirated with a Pasteur pipette. Glomeruli were then resuspended in fresh KRB. The glomerular suspension was greater than 95% pure as judged by light microscopy. Unlike rat cortical tissue [5, 7], the human nonglomerular fraction of the renal cortex fragmented into small tubular segments that passed through all the sieves, with relatively little material collecting on top of the sieves. Therefore, all the washings subsequent to the first sieving (212 μm) were collected and combined with the material that settled on top of the sieves. The suspension was pelleted by centrifugation at $\times 1500g$ for 20 min and resuspended in ice-cold KRB. This nonglomerular suspension is referred to as "tubules." The resulting glomerular and tubular preparations were used for these studies. The bulk of the cortical and glomerular tissue from HK 1, 2, and 3 was used for enzyme (histidine decarboxylase) studies (to be reported elsewhere).

Cyclic nucleotide studies. These studies were performed on glomerular preparations from six separate kidneys. Incubations were carried out in a manner analogous to our studies in the rat [5, 18]. Freshly prepared glomeruli and cortical tubules (5 to 10 mg wet wt) were resuspended in 100 μl of ice-cold KRB and distributed into 10-ml glass homogenization tubes (size A, Thomas Scientific Products, Philadelphia, Pennsylvania) containing appropriate volumes of KRB and kept on ice. Tubes containing the various suspensions were then preincubated at 37°C for 20 min in a shaking water bath (120 cycles/min, Dubnoff). At the end of the preincubation, test agents dissolved in KRB were added in 50-μl volumes. The addition of test agents was made within a time interval of less than 10 sec. The final volume of each incubation was 500 μl, and the final concentration of test agents was as indicted in **Results.** Tubes were then incubated for an additional 5 min or as specified in **Results** at 37°C in the metabolic shaking water bath. Drugs and buffer were freshly prepared prior to each experiment. At the end of the incubation, the reaction was terminated by the addition of 500 μl of ice-cold 10% TCA; the tissue samples were immediately homogenized. A small aliquot of the whole homogenate was taken for protein determination, and the rest of the homogenate was centrifuged to precipitate the proteins. TCA was removed from the supernate by repeated extraction ($\times 5$) with water-saturated ether. The remaining ether was then evaporated by heating and samples were adjusted to neutral pH with 0.5 N NaOH. Aliquots of these extracts were used for determination of cAMP and cGMP. In one experiment, the homogenate after the incubation was divided into two separate aliquots for cAMP and cGMP determination. Tracer amount of ^{3}[H]-cAMP was added to the respective aliquot for monitoring recovery. The quantity of ^{3}[H]-cAMP tracer added for the determination of recovery and that contributed to the final concentration of cAMP was calculated and subtracted from each sample. Added ^{3}H-cAMP (95% ± 4.0) was recovered in the final extract immediately prior to RIA. Therefore, this step of monitoring the recovery was omitted from the rest of the experiments.

Cyclic nucleotide determination by RIA was carried out as originally described by Steiner, Parker, and Kipnis [19] and modified to increase the sensitivity of cAMP [20] and cGMP [21] by acetylation and succinylation, respectively. Drugs used were tested for possible interference with RIA. At the highest concentration used in this study, none of the drugs interfered with either cAMP or cGMP assay.

In each experiment, glomeruli and tubules from the same kidney were incubated on the same day and under identical conditions. Samples from the same kidney were also processed for extraction, RIA, and protein determination at the same time with the same batch of reagents. Protein was determined by the method of Lowry et al [22] and cyclic nucleotide data were expressed as picomoles per milligram of protein.

Histamine content. Tissue samples from two separate kidneys (one surgical sample and another cadaver donor kidney) were used for the determination of histamine content. As soon as the tissue became available, a small piece of the renal cortex, freshly prepared glomeruli, and tubules were homogenized in ice-cold 0.05 M sodium phosphate buffer pH 7.9. A small aliquot of the homogenate was taken from protein determination; the rest of the homogenate was heated in boiling water for 5 min and centrifuged at $\times 12{,}800g$ for 30 min in a microcentrifuge (Eppendorf). The supernatant was frozen and stored at −20°C for histamine determination by radioenzymatic microassay [23] (see below).

Histamine production studies. These studies were performed on glomerular and tubular preparations from seven separate kidneys. The incubation of freshly prepared glomeruli and cortical tubules was carried out in a manner similar in principle to our previous studies in the rat [7]. Freshly prepared glomeruli (50-μl aliquots) and cortical tubules (15 to 25 mg wet wt) suspended in KRB were distributed into 1.5-ml microcentrifuge tubes (Eppendorf) kept on crushed ice. The incubation mixture also contained 0.1 mM pyridoxal phosphate, a coenzyme of histidine decarboxylase, the histaminase inhibitor aminoguanidine (0.01 mM), and 1.0 mM L-histidine, the histamine substrate. The final volume of each incubation was brought up to 200 μl with KRB buffer pH 7.4. In each experiment, control incubations were made using glomerular and tubular suspensions heated in boiling water for 5 min before reagents were added. In some experiments, additional control incubations were carried out in the presence of D-histidine (not a histamine substrate). Three experiments included incubations carried out in the presence or absence of the histidine decarboxylase inhibitor bromocresine. Tubes were shaken manually and incubated for 180 min at 37°C in a water bath. The tubes were additionally shaken every 30 min during the incubation. At the end of the incubation, the reaction was stopped by heating the tubes in boiling water for 5 min. The tubes were then frozen and stored at −20°C. Prior to the determination of histamine, suspensions of glomeruli and tubules were frozen and thawed in an ethanol/dry ice bath (four times) to insure release of histamine. Tubes were then centrifuged at $\times 12{,}800g$ for 30 min in a microcentrifuge (Eppendorf). The supernate was transferred to clean tubes. pH was adjusted to 7.9 to 8.0 by the addition of 5 μl of 0.5 N NaOH (optimum pH for the histamine assay). Protein was determined in the pellets which were solubilized in 1.0 N NaOH. Histamine was determined on 20 μl aliquots of the supernate by a specific and sensitive radioenzymatic microassay [23].

Briefly, this assay uses histamine methyltransferase (HMT), which methylates histamine to form Nτ-methylhistamine. The sample or histamine standard (20 μl) is incubated with 10 μl of guinea pig HMT (2 mg/ml), containing 0.22 pmoles of ^{3}H-histamine, and 0.28 nmoles of ^{14}C SAMe. The final concentrations of the reactants were as follows: ^{3}H-histamine 7.35 nM and ^{14}C-SAMe 9.35 μM. The total reaction volume was 30 μl. The mixture was incubated for 1 hr at 37°C. The reaction was terminated by adding 10 μl 2.4 N perchloric acid. The reaction product Nτ-methylhistamine was extracted as described [23] with minor modifications [24]. NaCL (20 μl) saturated 3.3 N NaOH, and 400 μl of chloroform were added to the tubes. Tubes were then tightly capped and shaken on a mechanical shaker (Eppendorf, Brinkmann) for 20 min. Then they were centrifuged at $\times 12{,}800g$ for 2 min in a microcentrifuge (Eppendorf 5412). The upper aqueous phase was removed by aspiration, and to the organic phase 100 μl of NaCl saturated 1 N NaOH were added. Tubes were shaken for an additional 5 min and centrifuged. The NaOH layer was removed carefully by aspiration. A 300-μl aliquot of the chloroform phase was transferred to a counting vial; chloroform was evaporated to dryness in a stream of air. Then 5 ml of Aquasol (New England Nuclear Corp., Boston, Massachusetts) were added, and the radioactivity of ^{14}C and ^{3}H was counted in a scintillation spectrometer (Searle Mark III Liquid Scintillation System, Model 6880, Searle Analytical, Chicago, Illinois) until the SE of counting was 5% or for 20 min, whichever was shorter. A standard curve for authentc histamine base was determined for each assay. The ratio of ^{14}C/^{3}H is directly proportional to the amount of unlabeled histamine present in the incubation mixture. The assay used was linear between 2.5 and 50 pmoles histamine per sample or standard. Histamine added to boiled tissue extract was recovered to the extent of 85 to 95%. Values for histamine were expressed as picomoles per milligrams of protein.

Materials

Histamine dihydrochloride, L-histidine, D-histidine, 1-methyl-3-isobutylxanthine (MIX), and diphenhydramine HCl were purchased from Sigma Chemical Co., St. Louis, Missouri. Aminoguanidine sulphate was purchased from Eastman Organic Chemicals, Eastman Kodak Co., Rochester, New York, Bromocresine (4-bromo-3-hydroxybenzylozyamine diphydrogen phosphate) was kindly supplied by Lederle Laboratories, Division of American Cyanamid Co., Pearl River, New York. Cimetidine, dimaprit, and 2-pyridylethylamine were kindly supplied by Mr. C. Porter of Smith, Kline and French, Philadelphia, Pennsylvania.

[^{3}H]-cAMP for the measurement of recoveries was purchased from New England Nuclear Corp. Antibodies and ^{125}I-labeled antigens for cAMP were from RIA kits purchased from Schwartz/Mann, Division of Becton, Dickinson and Co., Orangeburg, New York. Antibodies and ^{3}H-labeled antigens for cGMP and S-Adenosyl-L-methionine-[^{14}C-methyl], 55 to 60 mCi/mmole, were purchased from Amersham Corp., Arlington Heights, Illinois [(β-side chain label)-^{3}H] histamine was prepared from [(β-side chain label)-^{3}H] L-histidine 10 Ci/mmole (New England Nuclear Corp.) as described [7, 25]. Statistical significance was determined by analysis of variance.

Results

The effect of histamine on cAMP production was examined in glomeruli isolated from six separate human kidneys (Table 1). In the presence of 0.5 mM MIX, a phosphodiesterase inhibitor, 10^{-4} M histamine increased cAMP accumulation in all six glomerular preparations tested. After a 5-min incubation, the stimulatory effect of histamine on cAMP accumulation ranged between 100 to 263% and did not appear to depend on the duration of the perfusion or the source of the tissue (Table 1). The basal concentration of cAMP and cGMP in glomeruli incubated without MIX was much lower than in the presence of MIX (0.5 mM). However, even when glomeruli were incubated with histamine alone (in the absence of MIX), an increase in cAMP was also observed but was of lower magnitude than in

Table 1. Effect of histamine on cAMP and cGMP accumulation in isolated human glomeruli[a]

Kidney source/no.	cAMP, *pmoles/mg protein*			cGMP, *pmoles/mg protein*		
	Basal	Histamine	Δ%	Basal	Histamine	Δ%
1. Surgical sample		NT			NT	
2. Perfused donor kidney (12 hr)[b]	5.18 ± 0.1[c]	13.46 ± 1.2	+160%	3.73 ± 0.12	5.52 ± 2.41	+48%
3. Perfused donor kidney (18 hr)[b]	2.34 ± 0.1	6.16 ± 0.1	+263%	4.81 ± 0.1	4.70 ± 0.1	−2%
4. Perfused donor kidney (46 hr)[b]		NT			NT	
5. Surgical sample	2.98 ± 0.2	7.82 ± 0.9	+162%	4.22 ± 0.8	4.10 ± 0.3	−3%
6. Perfused donor kidney (16 hr)[b]	7.42 ± 0.3	25.76 ± 0.8	+247%	8.33 ± 0.2	8.89 ± 1.1	+7%
7. Perfused donor kidney (36 hr)[b]	5.80 ± 0.4	11.61 ± 0.1	+100%	5.89 ± 1.9	5.31 ± 1.6	−10%
8. Perfused donor kidney (12 hr)[b]	4.48 ± 0.1	11.44 ± 1.3	+155%	5.19 ± 1.5	6.74 ± 0.3	+30%
9. Surgical sample		NT			NT	
	4.70 ± 0.3[c]	12.7 ± 1.2[d]	181 ± 10%	5.36 ± 0.3	5.87 ± 0.3	12 ± 4%

Abbreviation: NT, not tested.
[a] Isolated glomeruli were incubated for 5 min in the presence of 0.5 mM MIX.
[b] The number of hours in parentheses denotes perfusion time.
[c] Values represent sample means ± SEM of duplicate incubation samples each assayed in duplicate.
[d] The value is significantly different from basal values (without histamine), $P < 0.05$ or higher degree of significance (paired t test).

Table 2. Effect of histamine on cAMP and cGMP accumulation in isolated glomeruli and tubules of HK 3[a]

	Glomeruli			Tubules		
	Basal	Histamine (10^{-4} M)	Δ %	Basal	Histamine (10^{-4} M)	Δ %
cAMP, *pmoles/mg protein*	2.34[b] ± 0.031	6.163 ± 0.103	+263%	1.18	1.425	+21%
cGMP, *pmoles/mg protein*	4.813 ± 0.093	4.70 ± 0.10	−2.3%	7.52 ± 0.39	5.73 ± 0.31	−23.8%

[a] Isolated glomeruli and tubules were incubated for 5 min at 37°C in the presence of 0.5 mM MIX.
[b] The value represents the mean ± range of duplicate incubation samples that were each assayed in duplicate.

identical incubations carried out in the presence of 0.5 mM MIX (Δ% + 34%, + 54% and + 74% in HK, 2, 3 and 7, respectively). Histamine did not influence cGMP accumulation in glomeruli (Table 1), and cAMP nor cGMP in one tubular preparation tested (Table 2).

Histamine stimulated cAMP accumulation in glomeruli in a time- and dose-dependent manner. Stimulation was seen as early as 30 sec and reached a maximum at 5 min (Fig. 1**A**). In three separate glomerular preparations, total cAMP was determined at 5 min as a function of histamine concentration. There was dose-dependent increase in cAMP accumulation between 10^{-6} to 10^{-3} M histamine (Fig. 1**B**), with half-normal stimulation at 5×10^{-5} M.

The effect of histamine specific agonists and antagonists on cAMP accumulation was tested in three separate glomerular preparations (Fig. 2**A**). The histamine (10^{-4} M) stimulated cAMP accumulation was abolished by cimetidine (10^{-4} M), an H_2 antagonist, but not by equimolar concentrations of the H_1 antagonist diphenhydramine. Cimetidine (10^{-4} M) and diphenhydramine (10^{-4} M) alone, had no sigificant effect on cAMP accumulation. In one glomerular preparation tested (HK 8), the β-adrenergic blocker propranolol (10^{-4} M) also had no effect on basal cAMP levels or on the stimulatory effect of histamine (10^{-4} M) on cAMP accumulation (data not shown). The effects of the specific H_1 agonist 2-pyridylethylamine and the H_2 agonist dimaprit were also compared in the same three separate glomerular preparations (Fig. 2**A**). Dimaprit (10^{-4} M) caused a more than twofold increase in cAMP accumulation, while 2-pyridylethylamine (10^{-4} M) had no effect. Neither histamine nor histamine specific agonists or antagonists influenced cGMP accumulation in glomeruli (Fig. 2**B**). The stimulatory effect of dimaprit on cAMP accumulation, similar to the effect of histamine, was dose-dependent (Fig. 3**A**) with half-maximal stimulation achieved at $\approx 10^{-5}$ M. Figure 3**B** shows that cimetidine inhibits cAMP accumulation in a dose-dependent manner, with 50% inhibition achieved at 10^{-5} M, a drug level achieved in the blood after clinically employed oral doses [26]. Equimolar concentrations of diphenhydramine, on the other hand, had no effect on cAMP accumulation.

The concentration of circulating histamine in human plasma ($<10^{-8}$ M) is more than two orders of magnitude lower than the concentration (10^{-6} M) that elicited enhanced cAMP accumulation in glomeruli. Therefore, histamine content was determined in the renal cortex, glomeruli, and tubules of the same kidney. Histamine content averaged 17.62 ± 2.5 in the cortex, 22.55 ± 1.5 in glomeruli, and 23.29 ± 3.0 in tubules (pmoles/mg protein; $N = 4$ samples from HK 1) and 18.34 ± 3.6 in the cortex, 27.8 ± 3.1 in glomeruli, and 31 ± 6.5 in tubules (pmoles/mg protein; $N = 4$ samples from HK 2). These tissue levels of histamine markedly exceed plasma levels. To determine the source of histamine in the human renal cortex, glomeruli and tubules isolated from the same kidneys were incubated with the histamine substrate L-histidine and histamine levels were determined. Incubations with 1 mM L-histidine (Fig. 4**A**) resulted in a significant increase in histamine levels in seven separate glomerular preparations tested (+Δ6.08 ± 0.5 pmoles/mg protein). Unlike incubations with L-histidine, incubations of glomeruli with D-histidine (not a precursor of

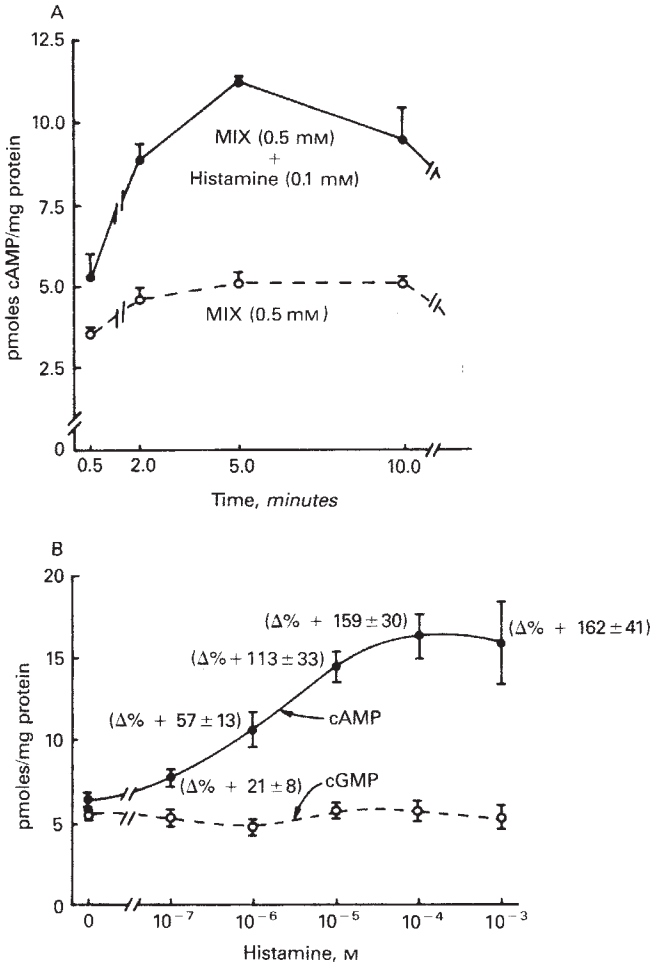

Fig. 1. A *Time course of the effect of histamine on cAMP accumulation.* Isolated glomeruli from HK 7 and 8 were incubated for various time periods at 37°C. Total cAMP was determined by RIA. For details see **Methods.** *Points* indicate the mean ± SEM of data from four incubation samples (two from each kidney). Each sample was assayed in duplicate. **B** *Dose-response curve for the effect of histamine on cAMP and cGMP accumulation.* Isolated glomeruli from HK 5, 6, and 7 were incubated for 5 min at 37°C. Total cAMP and cGMP were determined by RIA. For details see **Methods.** *Points* indicate the mean ± SEM of data from six separate incubation samples (two from each kidney), with the exception of the 10^{-3} M (four separate incubation samples). Each sample was assayed in duplicate.

histamine) did not result in change in histamine levels (Fig. 4**B**). In contrast to glomeruli, suspension of cortical tubules incubated with L-histidine (Fig. 4**A**) under identical conditions as glomeruli showed no increase but rather a sharp decline in histamine levels ($-\Delta 13.8 \pm 2.4$ pmoles/mg protein). A similar drop in histamine levels were observed in tubules incubated with D-histidine (Fig. 4**B**).

To test whether the differences in the ability of glomeruli or tubules to accumulate histamine was due to a different rate of histamine synthesis rather than to differential catabolism or other factors that may influence histamine levels, the effect of bromocresine, an inhibitor of histidine decarboxylase [27], on histamine accumulation was examined. In three separate preparations tested (Fig. 5), the addition of bromocresine abolished the accumulation of histamine in glomeruli incubated with L-histidine but had no effect on histamine levels in tubules incubated under identical conditions.

Discussion

These studies demonstrate that the cAMP system in isolated human glomeruli is sensitive to stimulation by histamine and that glomeruli are sites of active histamine synthesis within the human renal cortex. The magnitude of the stimulatory effect of histamine on cAMP accumulation does not appear to be related to the source of tissue (surgical sample vs. perfused cadaver donor kidney) nor to the duration of the perfusion. Of interest is that glomeruli isolated from kidneys perfused for as long as 18 hr showed similar or higher responsiveness in terms of cAMP accumulation as glomeruli isolated from freshly obtained surgical samples. Whether this difference in responsiveness to histamine represents inherent individual variation, alteration in the levels of the cAMP substrate ATP or changes in the activities of adenylate cyclase or cAMP phosphodiesterase [28] during the perfusion procedure or clamping of the renal artery prior to the nephrectomy cannot be determined from our studies.

The effect of histamine on cAMP accumulation is dose-dependent; the concentration of histamine ($\simeq 5 \times 10^{-5}$ M) eliciting a half-maximal elevation in cAMP levels (reflecting indirectly the affinity of the receptor for histamine) approximates the concentration required for half-maximal stimulation of cAMP accumulation in human parathyroid tissue [29] and the apparent K_m of histamine-sensitive adenylate cyclase in human gastric mucosa [30]. Several criteria suggest that the effect of histamine is mediated by distinct histamine receptors. Inhibition of the stimulatory effect of histamine on cAMP accumulation by cimetidine but not by diphenhydramine or propranolol indicates that the effect of histamine is mediated by an H_2 receptor linked to adenylate cyclase that is also distinct from the B-adrenergic receptor. This conclusion is supported further by the finding that the H_2 receptor agonist dimaprit also stimulates cAMP production in glomeruli in a dose-dependent manner. In contrast, the histamine H_1 agonist 2-pyridylethylamine at equimolar concentrations had no effect on cAMP accumulation. While the present studies focused on glomeruli, the limited observations showing no effect of histamine on cAMP or cGMP accumulation in cortical tubules suggest that the presence of histamine receptors in the human renal cortex is a distinct feature of glomeruli. The cell type or types within the renal glomerulus bearing histamine receptors remains to be identified.

These studies in human glomeruli differ from previous observations [4, 5] on the effect of histamine on cyclic nucleotides in rat glomeruli in two respects. First, the increase in cAMP accumulation in response to histamine is lower in human glomeruli as compared to rat glomeruli. Second, histamine had no effect on cGMP accumulation in human glomeruli even after 2-min incubations (data not shown), unlike the small but consistent increase in cGMP levels in response to histamine observed in the rat. Whether these differences could be accounted for by species or age differences or the manner in which the kidneys were handled prior to the glomerular isolation cannot be determined from our experiments. If the effect of histamine on cGMP in rat glomeruli is secondary to the inhibitory effect of generated cAMP on cGMP phosphodiester-

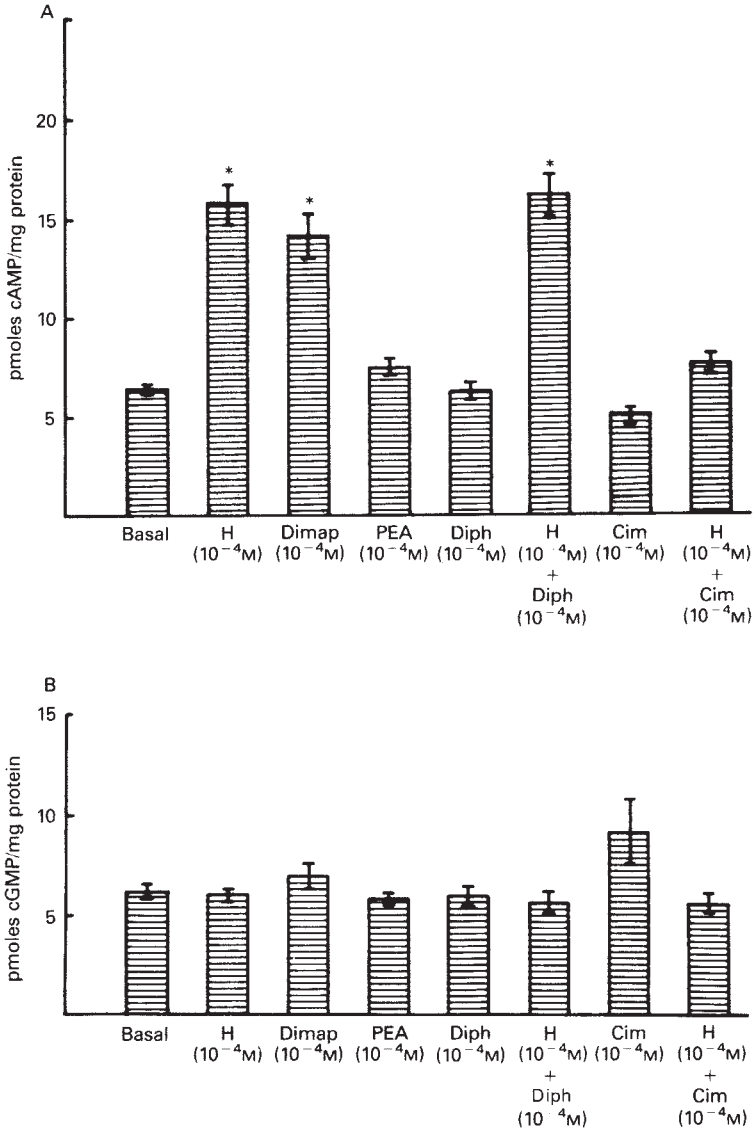

Fig. 2. A and **B** *Effects of histamine and histamine-specific agonists and antagonists on cAMP* **(A)** *and cGMP* **(B)** *accumulation.* Isolated glomeruli from HK 5, 6, and 7 were incubated for 5 min at 37°C (in the presence of 0.5 mM MIX). Total cAMP and cGMP were measured by RIA. For details see **Methods.** Each *bar* represents mean ± SEM of data from six separate incubation samples (two from each kidney). Each sample was assayed in duplicate. *Asterisks* denote values significantly different from basal value (MIX alone) at $P < 0.05$ or higher degree of significance (analysis of variance). Abbreviations are: H, histamine; Dimap, dimaprit (H_2 agonist); PEA, 2-pyridylethylamine (H_1 agonist); Diph, diphenhydramine (H_1 antagonist); Cim, cimetidine (H_2 antagonist).

ase, as suggested by Torres et al [4], the absence of such an effect in human glomeruli may be accounted for by the lesser increase in cAMP in response to histamine.

In addition to the presence of histamine receptors, human renal cortical tissue contains relatively high levels of histamine.[1] While these concentrations of histamine [1] probably underestimate its in situ concentration, they are still within the range capable of eliciting an increase in cAMP in glomeruli. Normal circulating levels of plasma histamine ($< 10^{-8}$ M) [32] on the other hand are at least 100 times lower than the minimal concentration of histamine capable of stimulating cAMP accumulation in this tissue in vitro, and plasma levels sufficient to have a stimulatory effect on glomerular cAMP system are unlikely to be achieved in plasma since small elevations in plasma histamine evoke numerous and severe systemic reactions in humans [13, 33]. Endogenous histamine is more likely therefore to influence glomerular function than circulating histamine. However, the extent to which endogenous histamine is quantitatively accessible to histamine receptors remains to be determined.

There are two potential sources of histamine in renal tissue [27]. Histamine may be synthesized locally from L-histidine or it may conceivably be extracted from the arterial blood and concentrated in the tissue. Our finding that incubation with L-histidine but not D-histidine increases histamine levels in glomeruli but not in cortical tubules incubated under identical conditions suggests that local synthesis in glomeruli is the major source of histamine in this nephron structure. This conclusion is supported by the observation that the histidine decarboxylase inhibitor bromocresine abolished the increase in histamine levels observed in glomeruli. The relatively high levels of histamine in cortical tubules varies with our recent findings in the rat [7] where the histamine levels in cortical tubules

[1] Assuming that tissue content of water is about 80% of wet weight [31], the estimated histamine concentration in human glomerular tissue fluid would be about 2 nmoles/ml of tissue water or 2×10^{-6} M.

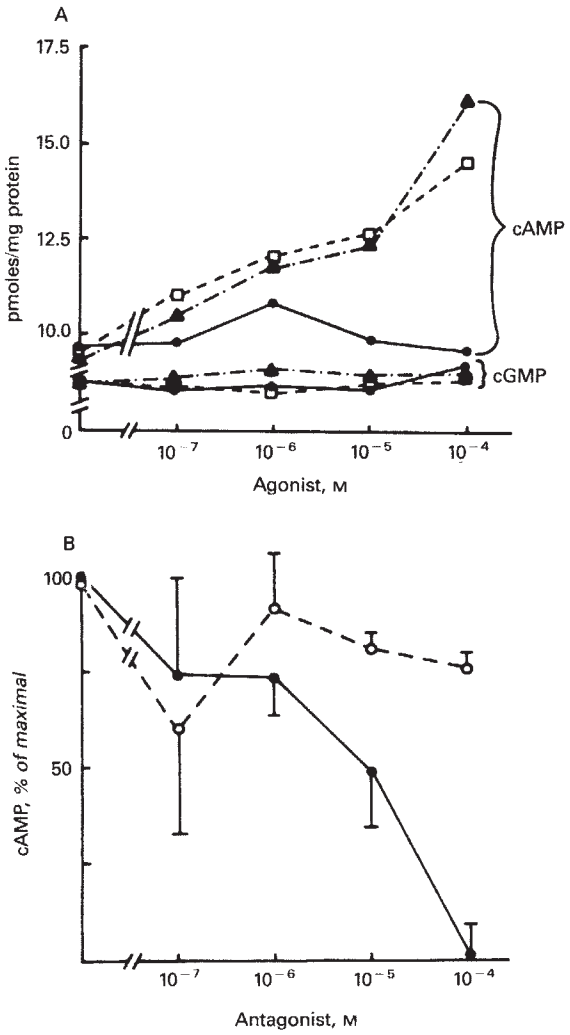

Fig. 3. **A** *Dose-response curve of the effects of histamine and histamine agonists on cAMP and cGMP accumulation.* Isolated glomeruli from HK 7 were incubated for 5 min at 37°C, with increasing concentration of agonists (in the presence of 0.5 mM MIX). Total cAMP and cGMP were determined by RIA. For details see **Methods.** *Points* represent the mean of four determinations from two incubation samples. The range for the incubation samples was ± 29% or less. Symbols are: □------□, histamine; ●——●, pyridylethylamine; ▲— • —▲, dimaprit. **B** *Dose-response curve of the effects of histamine antagonists on histamine-stimulated cAMP accumulation.* Isolated glomeruli from HK 8 were incubated for 2 min at 37°C with 10^{-5} M histamine and increasing concentration of antagonists (in the presence of 0.5 mM MIX). *Points* represent the mean ± range of four determinations from two incubation samples. Symbols are: ●——●, cimetidine; ○------○, diphenhydramine.

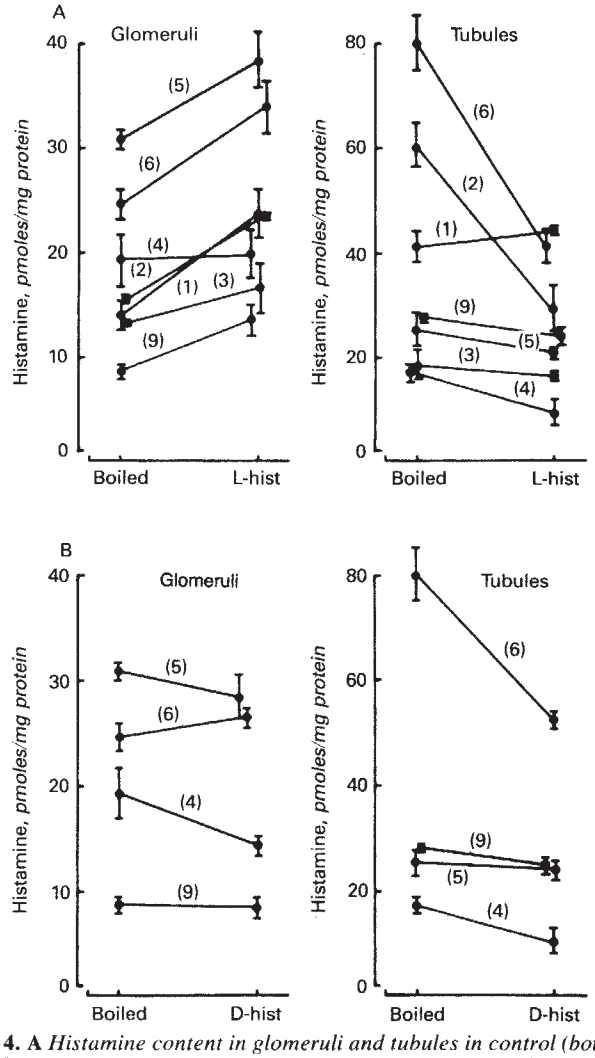

Fig. 4. **A** *Histamine content in glomeruli and tubules in control (boiled) incubations and after incubation with 1.0 mM L-histidine for 180 min at 37°C.* All incubations contained 0.1 mM pyridoxal phosphate and 0.01 mM aminoguanidine. Each *point* indicates mean ± range of at least two incubation samples from each kidney. Each sample was assayed in duplicate. *Numbers* between *parentheses* indicate kidney sample (for key see Table 1). The mean histamine level (pmoles/mg protein) in boiled glomeruli was 18.1 ± 1.1 and in incubated glomeruli 24.19 ± 1.3 (mean ± SEM of seven experiments; $P < 0.05$ analysis of variance). Corresponding levels in tubules (pmoles/mg protein) were 42.2 ± 3.4 and 26.43 ± 1.9 (mean ± SEM of seven experiments; $P < 0.05$ analysis of variance). **B** *Histamine content in glomeruli and tubules in control (boiled) incubations and after incubation with 1.0 mM D-histidine (not a histamine substrate) for 180 min at 37°C.* All incubations contained 0.1 mM pyridoxal phosphate and 0.01 mM aminoguanidine. Each *point* indicates mean ± range of data from two incubation samples from each kidney. Each sample was assayed in duplicate. *Numbers* between *parentheses* indicate kidney samples (for key see Table 1). Histamine levels (pmoles/mg protein) in boiled glomeruli was 21.07 ± 2.36 and in incubated glomeruli 20.45 ± 2.42 (mean ± SEM of four experiments). Corresponding levels in tubules (pmoles/mg protein) were 38.1 ± 7.2 and 29.3 ± 4.1 (mean ± SEM of four experiments).

were much lower than that in glomeruli. The reason for this discrepancy is not clear. Several factors may influence histamine levels during the perfusion or the nephrectomy, such as changes in the activity of the synthesizing or degrading enzymes or even influx or efflux of histamine from the tissue. Furthermore, the nonglomerular fraction of the renal cortex referred to here as "tubules" represents not only material that sedimented on top of the sieves, but also all nonglomerular cortical tissue which might substantially differ in its cellular content (interstitial cells or mast cells) from the "tubular" fraction in the rat studies. Therefore, whether or not a similar pattern of distribution of histamine exists in situ in the human kidney tubules or glomeruli cannot be determined from these studies. The sharp decline in histamine levels observed when tubules

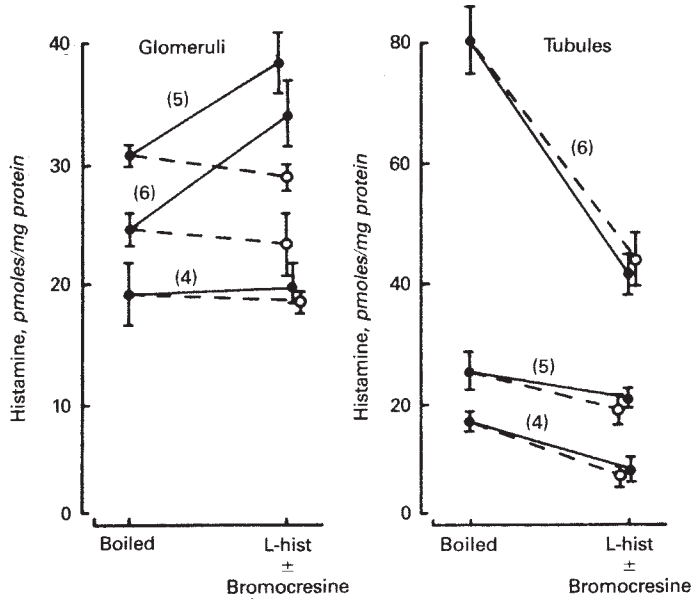

Fig. 5. *Changes in histamine content in glomeruli and tubules in control (boiled) incubations and after incubations with 1.0 mM L-histidine for 180 min at 37°C in the absence* (——) *and in the presence* (------) *of 0.1 mM bromocresine.* All incubations contained 0.1 mM pyridoxal phosphate and 0.01 mM aminoguanidine. Incubations were carried out for 180 min at 37°C. Each *point* indicates the mean ± range of two incubation samples from each kidney. Each sample was assayed in duplicate. Numbers between *parentheses* indicate kidney sample (for key see Table 1).

are incubated with L-histidine (similar to those with D-histidine) excludes tubular synthesis of histamine as a major source of histamine in the human kidney. Active uptake of circulating histamine by tubules has been demonstrated in the rat [34] or in addition histamine synthesized in glomeruli that may partially leak to the peritubular circulation may contribute to tubular histamine content. However, regardless of the presence and source of histamine in tubules, our studies show that glomeruli are not only targets for histamine's action on cAMP but also sites of active synthesis. The cell type(s) within the renal glomerulus capable of histamine synthesis is not known. Of major interest is that human glomeruli are devoid of mast cells [35], the major site of tissue histamine synthesis. It is conceivable that histamine synthesized in human glomeruli represents non-mast cell or nascent histamine. The presence of this pool of histamine was proposed several years ago [36, 37]. Recent observations in a mouse model deficient in mast cells [38] support the existence of this pool of non-mast cell histamine in many tissues including the kidney. The presence of histidine decarboxylase in isolated cultured endothelial and smooth muscles from rabbit aorta [39, 40] and the ability of human endothelial cells cultured from umbilical veins to synthesize histamine [41] suggest that glomerular endothelial and perhaps mesangial (smooth, muscle-like) cells are the source of glomerular histamine synthesis. Precise identification of the cell type responsible for histamine synthesis awaits studies utilizing glomerular cells in culture.

The presence of histamine H_2 receptors that mediate the enhanced cAMP accumulation in human glomeruli suggest that at least some actions of histamine in this nephron structure may be mediated by cAMP. Experimental evidence in rat as well as human glomeruli suggest that certain hormones acting via cAMP may influence the contractile properties of the glomerular mesangium thereby regulating renal blood flow and GFR [42]. A similar role of histamine and cAMP in modulating glomerular microcirculation may be operative in humans. It should also be emphasized that histamine acting via cAMP may have numerous other effects, besides its hemodynamic effects, on both intrinsic glomerular cells or cells infiltrating the glomerulus in the course of immune-mediated inflammatory injury [43]. For example, histamine may modulate lysosomal enzyme release, phagocytosis, release of other mediators, and metabolism of extracellular glomerular matrix by intrinsic glomerular cells, as has been demonstrated in extrarenal tissues [43–46]. Furthermore, since histamine receptors have been demonstrated on a number of inflammatory cells [47] that have been shown to infiltrate glomeruli in various forms of glomerulonephritis in experimental animals [48, 49] and humans [50], histamine may profoundly modulate various phases of glomerular immune inflammatory injury.

Recent studies [51] showing that inflammatory mediators such as lymphocyte products may enhance histamine production in target organs suggest that local synthesis of histamine may be an important mechanism of histamine availability in glomeruli, sites of histamine receptors. Pharmacologic manipulation of endogenous histamine and cyclic nucleotides may potentially prove to be effective in influencing the course of immune-mediated glomerular injury.

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