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HISTAMINE H_2 RECEPTORS IN RAT RENAL GLOMERULI

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Abstract—The aim of this study was to demonstrate histamine-H_2 receptors in glomeruli isolated from rat renal cortex and to correlate binding to stimulation by histamine of glomerular cyclic AMP concentration. Binding studies were performed at 10–12°C using [^{3}H]cimetidine as a tracer. Specificity of binding relies on the following: inhibition of [^{3}H]cimetidine binding by the unlabelled drug, other H_2-antagonists and agonists in contrast with the very weak inhibitory effects of H_1 agonists and antagonists; reversibility of steady-state binding after addition of unlabelled drug; half inhibition of the glomerular cyclic AMP response to histamine at concentrations of cimetidine close to the K_D value derived from the binding studies (3 μM); calculated K_D value in agreement with the therapeutical concentration of cimetidine and the physiological concentration of histamine. [^{3}H]Cimetidine binding strikingly increased in the presence of copper chloride (20–300 μM) due to an increase both in number of sites and affinity. However this greater binding did not influence either the inhibitory effect of cimetidine on histamine-induced glomerular cyclic AMP concentration or the stimulatory effect of histamine itself. [^{3}H]Cimetidine binding was temperature-dependent since it progressively diminished from 0 to 37°. This was not due to [^{3}H]cimetidine degradation as shown by thin layer chromatography but rather to a change in drug–receptor interaction at higher temperatures. Glomerular concentration of cyclic AMP increased progressively in the presence of histamine (0.1–1000 μM). This stimulatory effect was markedly inhibited by H_2 antagonists. These data demonstrate the presence in rat glomeruli of H_2 receptors linked to adenylate cyclase.

Two types of receptors for histamine (H_1 and H_2) have been described in several vascular beds [1, 2] and particularly in the renal vessels of the rabbit [3] and of the dog [4]. Most investigations have reported that histamine either does not modify [5], or slightly increases [4], glomerular filtration rate. These results, however, were compatible with a possible direct effect of histamine on the glomerular capillary cells since glomerular filtration rate depends on combination of multiple parameters. Such a direct effect was subsequently demonstrated both *in vivo* and *in vitro*: histamine infused intravenously in the rat resulted in reduction of the glomerular capillary coefficient, K_f [6], and histamine added to the incubation medium of glomeruli isolated from rat renal cortex caused a striking increase in the intracellular concentration of cAMP which was suppressed in the presence of H_2- but not of H_1 histamine antagonists [7]. In view of these considerations, the present study was performed in order to characterize, in binding experiments, H_2-glomerular receptors for histamine.

MATERIALS AND METHODS

Materials. [N-methyl-^{3}H]Cimetidine (17 Ci/ was purchased from the Radiochemical Centre (Amersham, U.K.) and stored at −20° as an ethanol solution under nitrogen. Histamine was purchased from Sigma Chemical Co. (St. Louis, MO). Compounds were donated by the following sources: cimetidine, metiamide, diphenylpyraline, 4-methylhistamine, 2-pyridylethylamine, dimaprit and impromidine (Smith, Kline & French Ltd, Welwyn Garden City, U.K.); triprolidine and chlorcyclizine (Wellcome SA, Paris, France); chlorpromazine and promethazine (Specia, Paris); clonidine (Boehringer, Paris). Other chemicals, all of highest purity grade, were purchased from standard suppliers.

[^{3}H]-Cyclic AMP for measurement of recovery and ^{125}I-labelled succinyl cAMP for radioimmunoassay were purchased from Commissariat à l'Energie Atomique (Gif sur Yvette, France) and Institut Pasteur (Paris, France) respectively. Antibodies against cAMP have been raised in the rabbit in our laboratory after injection of succinyl cAMP coupled to hemocyanin.

Isolation of glomeruli. Glomeruli were isolated from male Sprague–Dawley rats weighing 150–220 g as described in detail in a previous report [8]. Briefly, the kidneys of two rats were removed after perfusion with 0.16 M NaCl supplemented with heparin (50 U/ml) until surfaces were completely blanched. The cortex from the four kidneys was dissected and minced to a paste-like consistency. The homogenate suspended in 20 mM Tris–HCl buffer (pH 7.4) containing 5 mM glucose, 135 mM NaCl, 10 mM KCl and 10 mM $NaCH_3COO$ was pushed successively through a 106 μm-sieve which excluded the tubules and a 50 μm-sieve which retained the glomeruli. The suspension was then passed through a 25-gauge needle and centrifuged at 120 *g* for 90 sec. The supernatant was discarded, the pellet resuspended in the same buffer solution and passed again through the needle and centrifuged. This operation was repeated 5 times. Each individual preparation was checked for purity under light microscopy. Tubular

fragments were always below 2% of the total number of glomeruli. Glomerular protein concentration was measured according to Lowry *et al.* [9].

Binding studies. Freshly prepared glomeruli (400 μg glomerular protein per tube) were incubated at 10–12° with [^{3}H]cimetidine at a final concentration of 2 nM (1 pmole per tube) in 500 μl of the same buffer as that used in the preparation of isolated glomeruli in the presence or absence of 50 μM $CuCl_2$. The effect of copper was systematically studied because in preliminary experiments on the effects of cations we observed a marked stimulatory effect of this agent on [^{3}H]cimetidine binding. Incubation usually took place during 20 min in a shaking water-bath and was terminated by the addition of 5 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.4). Glomerular bound and unbound radioactivities were then immediately separated by filtration through a filter (Whatman GF/B, 1 μm pore diameter) positioned over a vacuum. After three successive washings with 5 ml of this same buffer, the filter was dried and ^{3}H radioactivity was counted in 10 ml Aquasol (New England Nuclear) at an efficiency of 33% with a liquid scintillation spectrometer (Packard, model 3003 apparatus). Specific binding was determined by subtracting from the total bound radioactivity (obtained from the incubation with [^{3}H]cimetidine) the non specifically bound radioactivity (obtained from the parallel incubation with [^{3}H]cimetidine and an excess, 100 μM, of unlabelled cimetidine). Specific binding was expressed as fmoles per mg of glomerular protein.

Glomerular cAMP determinations. In order to correlate binding with a physiological event, we studied the effect of histamine on cAMP glomerular concentration according to a slight modification of the technique described by Torres *et al.* [7]. Briefly, freshly prepared glomeruli (0.3–0.8 mg glomerular protein) were resuspended in 350 μl of Krebs–Ringer phosphate 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 sodium acetate, 2 mM sodium phosphate, and 20 mM Tris (pH 7.4). These suspensions were first preincubated at 37° for 20 min in a shaking water-bath. At the end of the preincubation period, 50 μl of 5 mM 1-methyl-3-isobutylxanthine (MIX) dissolved in the same buffer and either 100 μl of buffer (controls) or histamine and drugs dissolved in 100 μl of buffer were added. Here also, we studied the effects of 50 μM $CuCl_2$ in parallel experiments. The final volume of each suspension was 500 μl. After the addition of the various test agents and MIX the tubes were incubated for 2 min at 37° under continuous agitation. The incubation was terminated by the addition of 500 μl of ice-cold 10% trichloroacetic acid (TCA) and immediate homogenization. After addition of a tracer amount of [^{3}H]-cAMP for recovery, the precipitated proteins were removed by centrifugation and the TCA was removed from the supernatant by 3 extractions with ethylether. Samples adjusted to neutral pH with 0.5 M NaOH were directly used for measurement of recovery and radioimmunoassay according to the slightly modified technique of Steiner *et al.* [10]. Extracted samples were incubated in 50 mM sodium acetate buffer (pH 6.2) with anti-cAMP antibodies at a final dilution of 1 : 50,000 and [^{125}I]succinyl cAMP methyl ester for 18 hr at 4°, and then the free and bound radioactivities were separated using the charcoal–dextran method. ^{125}I radioactivity was counted in a gamma counter (Searle, model 1185). The lower limit of sensitivity of RIA was 50 fmoles per tube. This made unnecessary previous acetylation or succinylation of the tissue extract. cGMP even at 100 pmoles per tube did not interfere with the determination of cAMP. After correction for recovery and after subtraction of the added tracer, the contents of cAMP were expressed as pmoles per mg of glomerular protein.

Since the more appropriate conditions for cAMP determinations were different from those used in binding studies, we also measured the binding of [^{3}H]cimetidine to glomeruli under identical incubation conditions in terms of time (short periods), temperature (37°) and composition of the incubation medium (Krebs–Ringer phosphate buffer).

Degradation studies. Degradation of [^{3}H]cimetidine present in the incubation medium was studied by thin layer chromatography. Aliquots of the incubation medium collected at increasing times of incubation were spotted on silicic acid thin layer plates together with 20 μg of unlabelled cimetidine for each sample and developed over 20 min in ethyl acetate: methanol: concentrated ammonia (5 : 1 : 1). A similar amount of unlabelled cimetidine was treated in parallel and visualized by exposing the gel to ninhydrin in order to measure the R_f of cimetidine. The gels containing [^{3}H]cimetidine were divided into 12 segments and each segment was transferred to a scintillation vial in order to count ^{3}H radioactivity. Undamaged cimetidine was estimated as the peak of radioactivity corresponding to cimetidine R_f and expressed as percentage of the total radioactivity of the gel.

RESULTS

Binding studies

The [^{3}H]cimetidine binding was measured as a function of time under basal conditions and in the presence of 50 μM copper chloride (Fig. 1). Specific binding was markedly greater (12 times more) when copper had been added. The amount of drug specifically bound increased with time. Equilibrium was reached more slowly with (20 min) than without (10 min) copper. Non-specific binding was low in both conditions. It represented 6 and 13 per cent of total binding with and without copper respectively. Addition of an excess of unlabelled cimetidine (100 μM) produced dissociation of the drug–receptor complex, since bound radioactivity decreased until a value equal to non-specific binding, 20 (with copper) and 10 (without copper) min later. This decrease in bound radioactivity represented release of [^{3}H]cimetidine and not degradation of the tracer or receptor, or both, as demonstrated by the persistence of the steady state in the absence of an excess of unlabelled molecules. Specific binding at 20 min under basal conditions was linear with the concentration of glomerular protein in the range tested (50–850 ng/tube). Specific binding in the presence of 50 μM copper chloride was also linear but

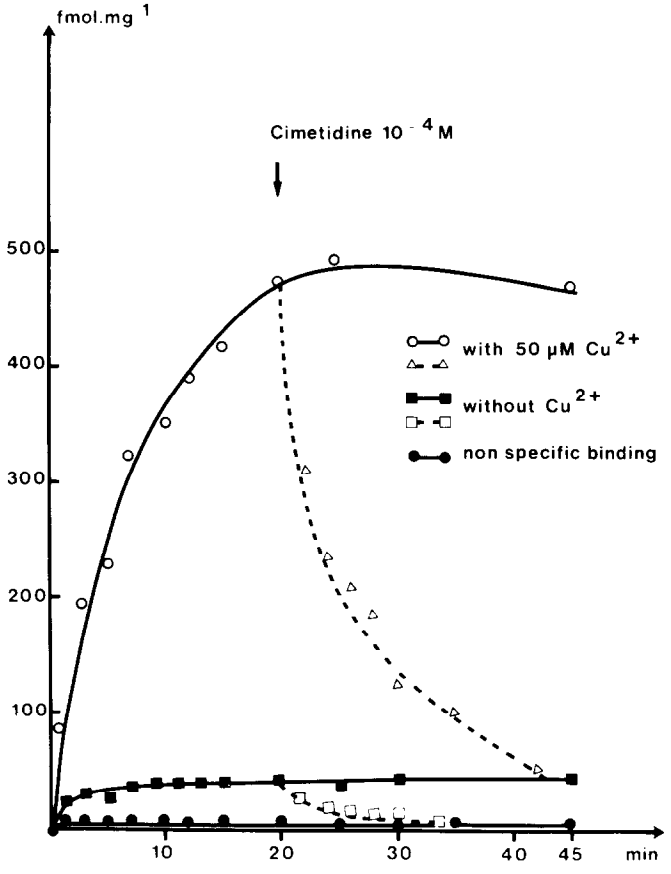

Fig. 1. Time-course of [^{3}H]cimetidine binding to isolated glomeruli in the presence (upper curves) or absence (lower curves) of 50 μM $CuCl_2$. The dotted lines correspond to the dissociation curves obtained after addition of 100 μM unlabelled cimetidine at equilibrium (20 min incubation). The closed circles represent non-specific binding without Cu^{2+} whereas the other symbols represent total binding.

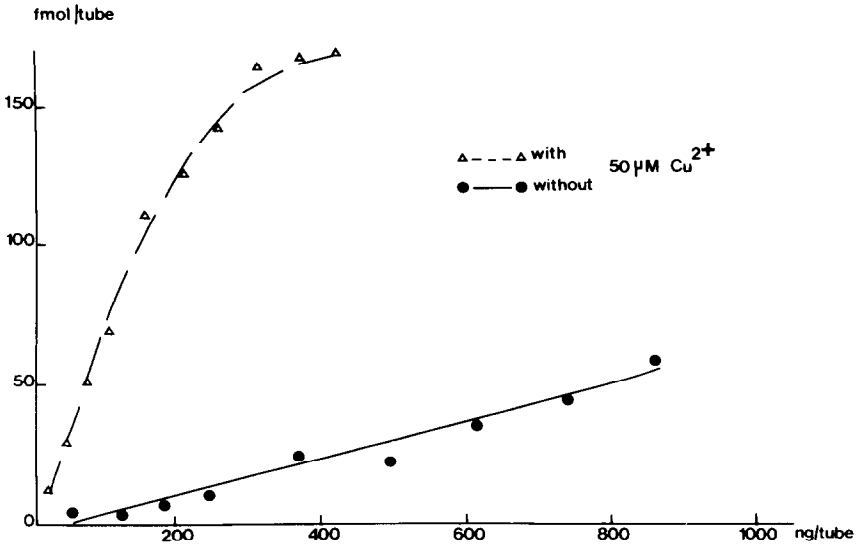

Fig. 2. [^{3}H]Cimetidine specific binding to isolated glomeruli as a function of the amount of glomerular protein in the presence (open triangles) or absence (closed circles) of 50 μM $CuCl_2$.

in a narrower range (100–800 μg/ml). The slope was markedly greater with (530 fmoles/mg protein) than without (61 fmoles/mg protein) copper (Fig. 2). These linear relationships allowed us to express binding as femtomoles per mg of glomerular protein. Competitive inhibition of binding of [^{3}H]cimetidine was observed in the presence of increasing concentrations of unlabelled drug. The concentration of cimetidine corresponding to half inhibition of binding of [^{3}H]cimetidine was 0.6 and 2 μM with and without 50 μM copper respectively (Fig. 3). The Scatchard transformation of the data (bound-to-free drug versus the concentration of bound drug) provided a straight line under both conditions (Fig. 3, inset). The abscissa intercept corresponding to the number of sites was greater with (40 pmoles/mg protein) than without (20 pmoles/mg protein) copper. The slope allowing calculation of the K_D value was also greater with than without copper, indicating thus that addition of chloride copper enhanced the affinity of cimetidine for its receptors. The specificity of [^{3}H]cimetidine binding sites was evaluated by competitive inhibition studies in the presence of increasing concentrations of H_1- and H_2 agonists and antagonists with and without copper (Table 1). Under both conditions, the more marked inhibitions of binding were observed with H_2 antagonists (cimetidine and metiamide) and agonists (impromidine). For these three drugs, half inhibition of binding was observed at 1 μM or less. Histamine and 4-CH_3-histamine also inhibited [^{3}H]cimetidine binding but to a lesser degree. Dimaprit, considered as an H_2 agonist, was inactive. Addition of copper increased the inhibitory potency of the H_2 agonists and antagonists tested except for dimaprit. This appears clearly from comparison between the percentages of binding observed in the presence of 10 or 100 μM of the same drug with and without copper respectively (Table 1). Statistical evaluation (two-way analysis of variance) confirmed that this difference was significant ($P < 0.01$). H_1 antagonists and agonists were very weak inhibitors and the effect of copper was not significant. Promethazine was tested and behaved also as a weak inhibitor. Specific binding of [^{3}H]cimetidine was temperature-dependent (Fig. 4). Both with and without copper, specific binding was maximum at low temperatures. For example, binding at 8° was four (with copper) and two (without copper) times greater than at 24°. Only specific binding decreased with temperature. Non-specific binding remained unchanged in the range of temperatures tested. Finally, the effects of divalent cations on the binding of [^{3}H]cimetidine were investigated. The binding was chiefly copper-dependent. Specific binding linearly increased with the concentration of copper chloride between 0 and 300 μM and reached a plateau above

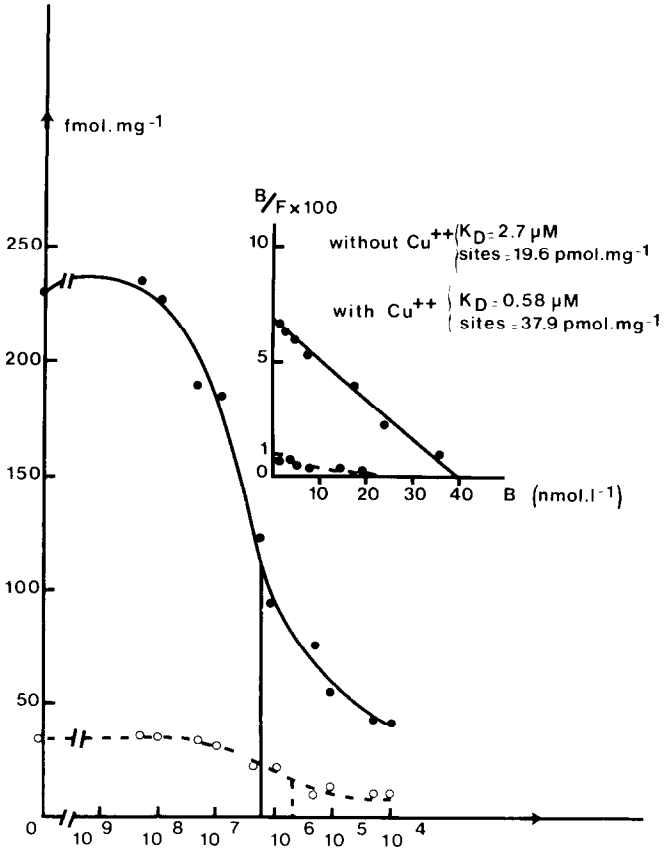

Fig. 3. Competitive inhibition of binding of [^{3}H]cimetidine to isolated glomeruli by increasing concentrations of unlabelled cimetidine in the presence (upper curve) or absence (lower curve) of 50 μM $CuCl_2$. Concentrations corresponding to 50% of maximum bindings are indicated. The Scatchard transformation of the data is given in the inset.

Table 1. Binding of [^{3}H]cimetidine to isolated glomeruli in the presence of increasing concentrations of H_1 and H_2 agonists and antagonists with and without 50 μM copper

Drugs tested	Concentrations (μM)							
	0.1		1		10		100	
	without Cu^{2+}	with Cu^{2+}	without Cu^{2+}	with Cu^{2+}	without Cu^{2+}	with Cu^{2+}	without Cu^{2+}	with Cu^{2+}
Histamine (4)	93.9 ± 5.6	92.7 ± 3.1	93.6 ± 5.3	86.9 ± 2.1	94.5 ± 5.6	78.7 ± 4.7	77.6 ± 6.8	36.7 ± 1.9
H_2 antagonists								
Cimetidine (18)	74.7 ± 3.7	74.5 ± 2.6	39.6 ± 2.9	36.4 ± 4.6	26.3 ± 2.3	21.8 ± 2.5	22.0 ± 3.0	15.3 ± 2.7
Metiamide (4)	68.4 ± 6.3	46.7 ± 1.6	43.8 ± 2.6	13.4 ± 2.4	34.0 ± 7.6	4.4 ± 0.6	24.6 ± 2.8	2.1 ± 0.5
H_2 agonists								
Dimaprit (2)	96.0	100.0	100.2	100.3	89.7	89.3	84.6	75.5
Impromidine (3)	77.5 ± 4.4	72.2 ± 6.5	49.1 ± 6.9	50.7 ± 0.8	28.3 ± 5.2	19.2 ± 4.6	12.1 ± 4.3	9.5 ± 3.2
4-CH_3-Histamine (4)	102.4 ± 2.8	79.9 ± 6.2	90.2 ± 5.5	69.1 ± 5.4	85.9 ± 2.0	51.4 ± 5.6	48.6 ± 5.9	26.4 ± 4.7
H_1 antagonists								
Mepyramine (3)	102.1 ± 8.5	99.0 ± 3.2	87.7 ± 3.3	88.0 ± 2.1	82.7 ± 2.4	77.7 ± 3.1	82.9 ± 3.2	78.9 ± 2.3
Triprolidine (4)	101.4 ± 1.6	95.7 ± 2.1	98.0 ± 4.8	102.1 ± 3.2	89.1 ± 6.2	92.6 ± 4.2	90.2 ± 4.2	85.3 ± 3.2
Chlorcyclizine (2)	100.3	101.0	99.8	100.2	93.4	93.9	80.2	89.0
H_1 agonist								
2-Pyridylethylamine (2)	101.2	96.8	103.1	95.3	103.1	91.5	94.2	85.6
Miscellaneous								
Promethazine (2)	94.4	96.7	86.2	85.8	84.2	78.2	78.2	75.7

Results are expressed as percentages of binding in the absence of any drug. Means ± S.E.M. are given when the number of individual values which is indicated in brackets is greater than two. Means only are given elsewhere.

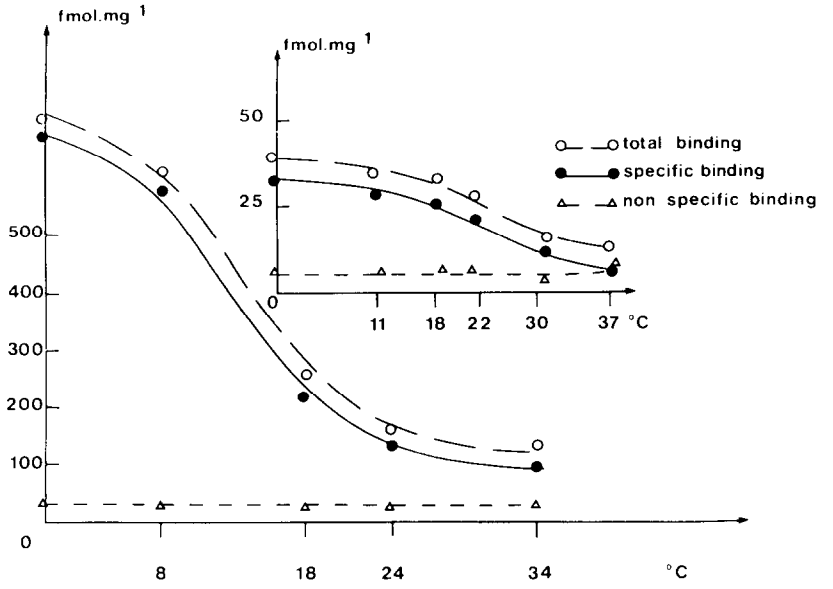

Fig. 4. Effects of temperature on [^{3}H]cimetidine binding to isolated glomeruli in the presence or absence (inset) of 50 μM $CuCl_2$.

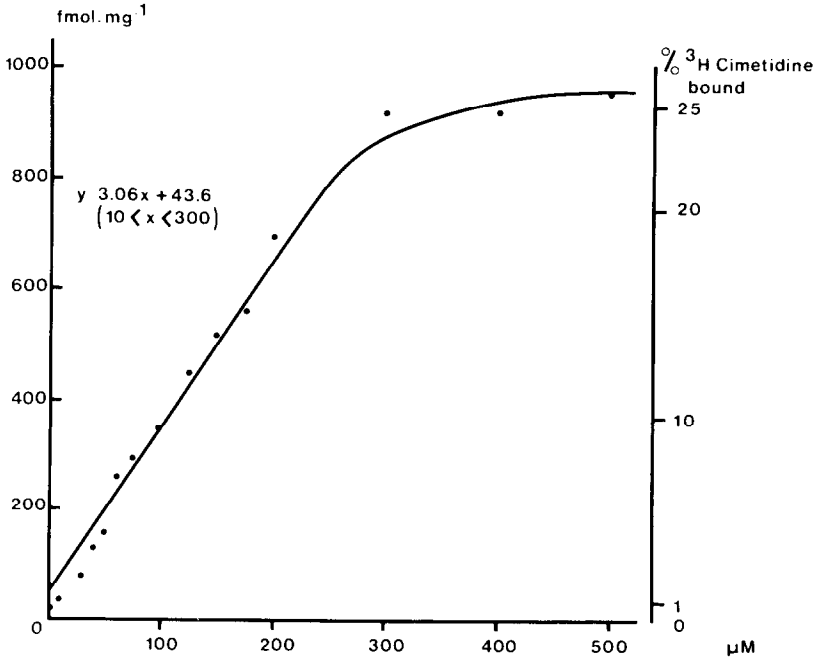

Fig. 5. Effects of increasing concentrations of $CuCl_2$ on [^{3}H]cimetidine binding to isolated glomeruli. Binding is expressed both as fmoles·mg^{-1} and percentage bound of [^{3}H]cimetidine present in the medium.

this latter value (Fig. 5). The slope of the straight portion of the curve was 3 fmoles/mg per μM of copper. We chose 50 μm as concentration of chloride copper for the binding studies because this value corresponds to the physiological concentration of copper in rat plasma [11]. The other cations tested were either less active as mercury or totally inactive as calcium, magnesium and manganese. Mercury chloride was approximately three times less potent than copper chloride.

We also studied [^{3}H]cimetidine binding (2 nM [^{3}H]cimetidine in the presence of 400 μg glomerular protein per tube) under the conditions of stimulation of adenylate cyclase activity (incubation at 37° in Krebs–Ringer phosphate buffer). Specific binding was marked at 2 min (160 fmoles/mg) but diminished very rapidly with time. It was as low as 50 fmoles/mg at 5 min and became nil after 15 min incubation. Copper also stimulated specific binding under these conditions but did not prevent it from decreasing rapidly with time. The results which were obtained initially led us to choose different conditions of incubation for the binding studies and the glomerular cyclic AMP determinations.

Degradation studies of [^{3}H] cimetidine

Under the conditions of incubation selected and at 10°, degradation of [^{3}H]cimetidine estimated by thin layer chromatography was negligible. There was also no degradation at 37° (Table 2).

Glomerular cyclic AMP determinations

Histamine caused a striking increase of cyclic AMP in glomeruli. The concentration of this nucleotide which was close to 25 pmoles/mg protein under basal conditions reached 70 pmoles/mg in the presence of 100 μM histamine. Cimetidine and metiamide which are specific H_2-receptor antagonists blocked the histamine-induced increase in cyclic AMP levels in glomeruli whereas triprolidine, mepyramine and chlorcyclizine which are specific H_1-receptor antagonists were less potent. Cimetidine alone had no effect. Histamine increased cyclic AMP in glomeruli in a dose-dependent way. A plateau was reached with doses of 1 mM histamine but higher doses produced a lesser degree of stimulation. The concentration corresponding to 50 per cent of maximum stimulation was 7 μM. When increasing doses of cimetidine were added in the presence of 100 μM histamine, there was a progressive decrease in histamine-induced stimulation. The basal concentration was reached with 100 μM cimetidine and the concentration corresponding to 50 per cent inhibition was 4.2 μM. Addition of 50 μM copper did not modify the stimulatory effects of histamine and the inhibitory effects of cimetidine (Table 3).

Table 3. Stimulation of glomerular cyclic AMP (pmoles · mg^{-1}) by histamine associated or not to cimetidine in the presence or in the absence of 50 μM copper chloride

Drug tested	With 50 μM copper	Without 50 μM copper
No drug	24.7 ± 3.8	28.4 ± 3.5
Histamine (100 μM)	67.9 ± 11.6	80.4 ± 7.9
Histamine (10 μM)	48.4 ± 6.7	51.7 ± 5.7
Cimetidine (100 μM)	23.5 ± 4.0	24.7 ± 3.8
Histamine (100 μM) + cimetidine (100 μM)	35.1 ± 4.7	32.7 ± 4.3
Histamine (100 μM) + cimetidine (10 μM)	42.2 ± 8.9	54.5 ± 3.5

The results are means ± S.E.M. from 6 individual values. Two-factor (drug; presence or absence of copper) analysis of variance with replication was used to analyze this data. There is no significant difference between the results obtained with and without copper respectively.

DISCUSSION

This report demonstrates the presence of specific H_2 histamine receptors in rat renal glomeruli linked to adenylate cyclase. Specificity of binding relies on the following: specific inhibition of binding of [^{3}H]cimetidine by unlabelled drug, other H_2-antagonists and H_2-agonists, except dimaprit, in contrast with the very weak inhibitory effects of H_1-agonists and antagonists; reversibility of steady-state binding after addition of unlabelled drug independent of the degradation of either drug or receptor; half inhibition of the glomerular cyclic AMP response to histamine in the presence of concentrations of cimetidine (4 μM) close to the apparent K_D value derived from the binding studies (3 μM); calculated K_D value in agreement with the concentrations of cimetidine needed to obtain a biological or therapeutical effect [12]. The concentration of histamine corresponding to 50 per cent of maximum stimulation of glomerular cyclic AMP was also in the micromolar range but histamine behaved as a weak inhibitor of [^{3}H]cimetidine binding.

Our second main conclusion is the striking stimulatory potency of copper on [^{3}H]cimetidine binding.

Table 2. Thin layer chromatography of [^{3}H]cimetidine

Time of incubation (min)	Incubation with buffer		Incubation with glomeruli	
	12°	37°	12°	37°
10	93.6	93.9	92.9	90.2
30	93.6	94.5	94.5	93.4

The results are expressed as percentage of the total radioactivity of the gel corresponding to the peak of undamaged [^{3}H]cimetidine. (R_f = 0.42). These values are means of duplicates. The result found with fresh [^{3}H]cimetidine was 96.5% (mean of 6 individual values).

Copper increased both the number of sites and the affinity of [^{3}H]cimetidine binding to isolated glomeruli at concentrations close to its physiological levels in rat plasma. Copper clearly enhanced the potency of H_2 receptor agonists and antagonists whereas it did not affect that of H_1 receptor agonists and antagonists. However copper modified neither the histamine-induced stimulation of glomerular cyclic AMP nor the inhibitory potency of cimetidine on the latter. This suggests that the binding sites revealed by the addition of copper are not linked to adenylate cyclase and thus probably non-specific. This conclusion is different from that advanced by Kendall *et al.* [11] on rat brain membrane fractions. For these authors, copper may regulate the pharmacological specificity of H_2 receptors in brain since in its absence [^{3}H]cimetidine binding may be associated with the H_1 receptor system. In fact their report was limited to binding studies and thus made it difficult to formulate physiological hypotheses.

Different H_2 histamine receptor systems have been already studied using binding techniques. High affinity binding sites for [^{3}H]cimetidine were observed by Kendall *et al.* [13] and Smith *et al.* [14] in rat brain tissue and by Burkard [15] in guinea pig brain homogenate. Many findings concordant with ours were observed in these three studies. Burkard [15] observed that specific binding of [^{3}H]cimetidine decreased with increasing temperature of incubation from 0 to 37°. Kendall *et al.* [13] reported that copper dramatically increased specific [^{3}H]cimetidine binding but affected only the number of sites. In contradiction with our data these authors found that copper did not affect H_2 receptor agonist and antagonist potency on [^{3}H]cimetidine binding whereas it markedly decreased the potency of H_1 receptor agonists and antagonists. Kendall *et al.* [13] calculated, like us, a K_D value close to 1 μM and also observed that dimaprit was the only H_2 receptor antagonist inactive on [^{3}H]cimetidine binding. The same finding was observed by Rising *et al.* [16] in guinea pig brain and gastric mucosa preparations and by Smith *et al.* [14] in rat brain tissue and was attributed to the lack of an imidazole ring in the dimaprit molecule. [^{3}H]Cimetidine binding sites have also been demonstrated in homogenates of rat anterior pituitary [17]. None of these studies included determinations of cyclic AMP concentrations or of adenylate cyclase activity. It was thus not possible to relate the binding sites described with a physiological effect. H_2 histamine receptors have also been demonstrated in calf thymocyte membranes [18] using [^{3}H]histamine as a tracer and considering that H_2 receptors corresponded to the difference between total binding and binding in the presence of an excess of H_2 antagonist. In this study, the apparent K_D 3.2 μM was similar to that calculated from our experiments. This order of magnitude corresponds to the concentrations of cimetidine pharmacologically active in different systems [12] and to the histamine content of the renal tissue in the rat [19]. It is slightly greater than the plasma concentration of histamine in this species [19].

The temperature dependency of binding was particularly striking in the conditions used. This effect of temperature was not due to the degradation of the drug (Table 2) but to a change in the binding process itself. Similar results were observed in binding [15] and pharmacological [20, 21] studies with both H_1 and H_2 antagonists. Several explanations for these findings have been put forward. Rocha e Silva and Fernandes [22] suggested that the nature of the chemical bond between histamine and its receptor was different at low and high temperature whereas for Kenakin *et al.* [20] there is conversion of H_1- into H_2 receptors at low temperatures. The more likely explanation is that advanced by Wochten *et al.* [23]. These authors have shown that the pK_a value of cimetidine increased with increasing temperatures thus reducing the proportion of protonated cimetidine. This may explain the decrease in binding especially as glomerular capillaries are negatively charged [24].

Our experimental design detected changes in glomerular cyclic AMP concentration in response to histamine alone or associated to H_1 or H_2 antagonists. The incubation time (2 min) was short; but it corresponded to maximum [^{3}H]cimetidine binding at 37°. In the presence of MIX which inhibits cyclic AMP phosphodiesterase, any increase in cyclic AMP concentration can be assumed to result from adenylate cyclase stimulation. Some of the data reported in the present study were previously demonstrated by Torres *et al.* [7]. In agreement with their findings we observed that glomerular cyclic AMP concentration was progressively increased by histamine in a dose-dependent way (1 μM–1 mM). But, in addition, we also showed that cimetidine, used in the same range of concentrations, inhibited this histamine-stimulated increase and that the dose of cimetidine corresponding to half-inhibition of this phenomenon was identical to the K_D value derived from [^{3}H]cimetidine binding studies.

The role of H_2 histamine receptors in the glomerular physiology is not yet clear. Histamine interacting with the glomerular cells may come from the plasma and the blood cells or be synthesized by the glomeruli themselves as demonstrated by Heald and Hollis [25]. Histamine-stimulated cyclic AMP production probably modulates the inflammatory response at the initiation of various glomerular diseases. In this respect, Abboud *et al.* [26] have shown that administration to rats of dexamethasone, which is used as an anti-inflammatory drug, inhibited cyclic AMP increase in response to histamine in the glomeruli isolated from these rats. Histamine also modifies the parameters of glomerular filtration rate in the rat via a decrease in the glomerular capillary ultrafiltration coefficient (K_f) [6]. However this effect was not suppressed by metiamide, an H_2 antagonist, but by diphenydramine, an H_1 antagonist. This suggests that this action of histamine is mediated by H_1 receptors.

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