Biochemical Pharmacology, Vol. 35, No. 11, pp. 1825–1834, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
© 1986 Pergamon Journals Ltd.

PHARMACOLOGY OF HISTAMINE H_2 RECEPTOR ANTAGONISTS IN THE HUMAN GASTRIC CANCER CELL LINE HGT-1

STRUCTURE–ACTIVITY RELATIONSHIP OF ISOCYTOSINE-FURAN AND IMIDAZOLE DERIVATIVES RELATED TO CIMETIDINE

SHAHIN EMAMI and CHRISTIAN GESPACH
INSERM U 55, Hôpital Saint-Antoine, 184 rue du Faubourg Saint-Antoine, 75571 Paris Cedex 12, France

(*Received* 24 *June* 1985; *accepted* 25 *November* 1985)

Abstract—Imidazole and isocytosine-furan derivatives inhibited H_2 receptor activity in HGT-1 cells, in accordance with the following relative potencies (IC_{50} = 2.3 μM cimetidine as reference): SKF 93479 = cimetidine = 100 > metiamide = 62 > SKF 92408 = 2 > SKF 91581 = 0.07). The Schild plot for cimetidine was linear (slope = 0.97) with a pA_2 value of 6.72 ± 0.12 (K_i = 0.18 μM cimetidine), suggesting competitive inhibition. Preincubation of HGT-1 cells for 10 min with H_2 antagonists at 2 μM concentration resulted in 90–100% inactivation (SKF 93479 and oxmetidine) and 65% inactivation (ranitidine) which persisted for 30 min, even after a washout period. Accordingly, the kinetics of 2 μM [^{3}H] SKF 93479 binding to HGT-1 cells revealed a half-time for association of 10 min and a dissociation half-time of 120 min. There was a good correlation between the kinetics and relative potencies of cimetidine and SKF 93479 in inhibiting H_2 receptor activity in purified plasma membranes (40 nM) as well as in intact HGT-1 cells preincubated for 2 hr with SKF 93479 before histamine addition (45 nM). Chronic treatment of HGT-1 cells for 6 days with 2 μM SKF 93479 specifically blocked H_2 receptor activity since cyclic AMP generation induced by other hormones and agents such as VIP, glucagon, GIP and sodium fluoride was unaltered. In contrast, short term and chronic treatment by cimetidine was readily reversible.

The isocytosine-furan derivative SKF 93479 differs from the imidazole analogue cimetidine by its apparent irreversible action, due to the slow onset of association from HGT-1 cells. The isocytosine ring in SKF 93479 and oxmetidine seems to play a preponderant role in their apparent long-lasting, irreversible actions.

Gastric acid secretion is partially controlled by histamine, acting through the H_2 receptor in parietal cells [1]. The direct action of histamine on these acid-secreting cells results in binding to specific recognition sites [2, 3], leading in turn to adenylate cyclase and cyclic AMP-dependent protein kinase activation [4, 5]. These biochemical events are directly related to oxygen consumption [6], aminopyrine uptake (as an index of acid formation), and to the morphological transformations of the parietal cells associated with the onset of acid secretion ([7, 8] and Review in ref. 9). Previous investigations [1–20] indicated that gastric H_2 receptor activity may be blocked by a variety of compounds bearing an imidazole (burimamide, metiamide, cimetidine, oxmetidine), a triazole (AH 2216, AH 23844), a thiazole (tiotidine, YM 11170), a thiadiazole (L-643,441), furan and/or isocytosine rings (SKF 93479, ranitidine, oxmetidine). Compared to the classical H_2 antihistamine cimetidine, a number of these antagonists exhibit the following pharmacological differences *in vivo* and *in vitro*: (1) increased inhibitory potency [3, 7, 12–19], (2) more prolonged action [14, 16, 18, 19], (3) noncompetitive antagonism [14] and, (4) apparently irreversible blockade [14, 16].

The present study was undertaken to establish a pharmacological basis for these differences by using cimetidine and its imidazole analogues metiamide, SKF 92408, SKF 91581 and the isocytosine-furan derivatives ranitidine, oxmetidine and SKF 93479 (Fig. 1). We therefore measured cyclic AMP production after adding histamine or its antagonists separately or together to intact HGT-1 cells or purified plasma membrane preparations. The HGT-1 cell line, derived from a human gastric cancer localized in fundus [20], possesses histamine H_2 receptors [13, 20] with pharmacological properties remarkably similar to those we established in human fundic glands using selective histamine agonists or antagonists [9, 12]. The specificity of the action of the H_2 antagonists studied here on gastric receptors was determined with regard to their immediate or prolonged effects (3 hours–6 days) on the receptors sensitive to vasoactive intestinal peptide (VIP), glucagon and gastric inhibitory peptide (GIP) in HGT-1 cells [13, 21]. These peptide hormone receptors have already been characterized in gastric glands isolated from man and laboratory animals [11, 22–25]. A portion of this work has been described as abstracts [26, 27].

* Abbreviations used: I, impromidine; PEA, 2-(2-pyridyl)ethylamine; H, histamine; VIP, vasoactive intestinal peptide; GIP, gastric inhibitory peptide; IBMX, 3-isobutyl-1-methylxanthine; PMSF, phenylmethyl-sulfonyl-fluoride; KRP, Krebs Ringer phosphate buffer; BSA, bovine serum albumin; HMT, histamine methyltransferase.

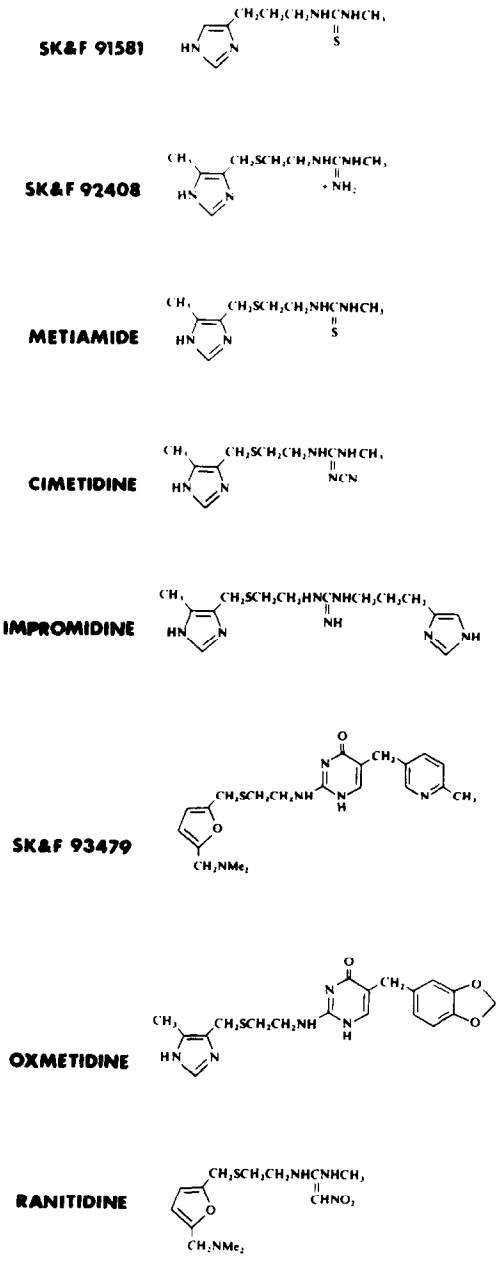

Fig. 1. Chemical structure of SKF 91581, SKF 92408, metiamide, cimetidine, impromidine, SKF 93479, oxmetidine and ranitidine.

MATERIALS AND METHODS

Chemicals. SKF 93479, [^{3}H] SKF 93479 (41 μCi/μmole), SKF 92408, SKF 91581, cimetidine, impromidine (I), metiamide, 2-(2-pyridyl) ethylamine (PEA), and oxmetidine were generously donated by Dr R. Brimblecombe of Smith, Kline and French Laboratories Ltd. (Welwyn Garden City, Herts, U.K.). Ranitidine was a generous gift from Dr R. T. Brittain (Glaxo Group Research Ltd., Welwyn Garden City, Herts., U.K.). Histamine dihydrochloride (H), cyclic AMP, 3-isobutyl-1-methylxanthine (IBMX), dithiothreitol, phenylmethyl-sulfonyl fluoride (PMSF), GTP and pure ATP were from Sigma Chemical Company (St. Louis, MO). Creatine phosphate (A grade) was from Calbiochem and phosphocreatine kinase from Boehringer. Purified natural porcine VIP (lot 79.4.25) and GIP were purchased from Pr. V. Mutt (GHI Laboratory Stockholm, Sweden). Crystallized, highly purified porcine glucagon (lot 42306) was from Novo Research Institute (Bagsvaerd, Denmark). *N*-methyl-[^{3}H] cimetidine (24 Ci/mmol), [^{3}H]cyclic AMP (20–30 Ci/mmol) and [^{125}I] Na (IMS 300, 600–800 mCi/mmol) came from the Radiochemical Centre (Amersham, U.K.). All other chemicals were of the purest available grade.

HGT-1 cells. The HGT-1 cells, generously provided by Dr C. Laboisse (INSERM U.239, Faculté de Médecine Xavier Bichat, Paris, France), were routinely cultured in Dulbecco's modified Eagle medium, as described previously [20]. Cultured cells between passages 63 and 105 were washed (×3 at 37°) with calcium-free phosphate buffer saline and removed from culture flasks after cell exposure to 0.02% EDTA for 1 min at 37°. Isolated cells were then washed three times at 20° with 40 ml Krebs Ringer phosphate buffer (KRP, pH 7.4) and resuspended after centrifugation (200 *g*, 3 min). Cell viability, determined by trypan blue exclusion, was about 95%.

Cyclic AMP assay. (1) In a standard assay, 150 μl from the HGT-1 cell suspension (1–2 × 10^6 cells/ml) was preincubated at 20° for 10 min in 250 μl KRP buffer containing 1% Bovine Serum Albumin (BSA, Fraction V) and 2 mM of the cyclic AMP phosphodiesterase inhibitor, IBMX [13]. The reaction was initiated by the addition of 100 μl of appropriate hormones or chemicals. It was stopped at the time indicated by adding 50 μl 11 N $HClO_4$, and cyclic AMP was determined by the radioimmunoassay method already described in detail [11, 13]. None of the agents tested in the present study interfered with the assay of cyclic AMP. Recovery of tritiated cyclic AMP added to the cells before extraction was 83%. Absolute values are expressed as picomoles of cyclic AMP produced per 10^6 HGT-1 cells.

(2) Prior to the addition of histamine or VIP, HGT-1 cells were preincubated with cimetidine, ranitidine, oxmetidine or SKF 93479 under the following conditions: Cell suspensions of 150 μl were preincubated for 10–180 min at 20° in 250 μl of KRP buffer containing 1% BSA and 2 mM IBMX, either in the presence of 50 μl of H_2 antagonists or with an additional 50 μl of buffer. Cyclic AMP generation was initiated by addition of histamine, either alone or mixed with the final concentration of the preincubated antagonists. Incubation was terminated as described above.

(3) Cultured HGT-1 cells between passages 82 and 100 were exposed to either 10 μM cimetidine or 2 μM SKF 93479 during 6 days. Concentration of drugs for culture in sterile conditions has been monitored by addition of [^{3}H] SKF 93479 and [^{3}H] cimetidine during filtration of nonradioactive drugs on 0.2 μm disposable syringe filters (Sartorius). Results were corrected for recovery of radioactivity after filtration. From seeding to confluency, the culture medium was changed every 24 hr.

(4) To eliminate the H_2 receptor antagonists cim-

etidine, ranitidine, oxmetidine and SKF 93479 pre-incubated or cultured with HGT-1 cells, we washed the cells at 20° by four serial additions of 40 ml of KRP buffer and centrifugations (200 *g* for 3 min). Those cells as well as the control HGT-1 cells were then exposed to histamine for the standard cyclic AMP assay described above. At the concentrations and under the conditions described, none of the agents tested modified HGT-1 cell viability.

Interaction of [^{3}H] *SKF 93479 with HGT-1 cells.* HGT-1 cells (2–2.5 × 10^6 cells) were incubated at 20° for various periods from 0 to 180 min in 0.5 ml KRP buffer containing 2 μM [^{3}H] SKF 93479 in the absence or presence of a 1000-fold excess of SKF 93479 or a 5000-fold excess of histamine. The assay was terminated by centrifugation for 30 sec of 300 μl aliquots of cell suspension through 1 ml ice-cold KRP buffer in a Beckman microfuge B. After washing the microfuge and the cell surface pellet by adding 1 ml ice-cold buffer, HGT-1 cells were solubilized in 200 μl 1 N NaOH for 20 min at 50°. The digested cells were transferred into 5 ml Aquasol (New England Nuclear) and counted in a Packard Model 3375 liquid-scintillation spectrometer. Each determination was performed in duplicate or triplicate. Total and nonspecific binding (i.e. binding not displaced by the 1000-fold excess of SKF 93479 or the 5000-fold excess of histamine) are indicated in the figures as the percentages of maximal binding observed during incubation. To assess the rate of dissociation of [^{3}H] SKF 93479 from HGT-1 cells, 2 μM [^{3}H] SKF 93479 was incubated at 20° with cell suspensions (20–25 × 10^6 cells/ml) for 1 hr in KRP buffer, pH 7.4. The HGT-1 cells were then washed 3 times at 4° with centrifugations at 200 *g* for 3 min and resuspended at 2–2.5 × 10^6 cells/ml in 500 μl fresh standard solution (see above) in the absence or presence of 2 mM SKF 93479 or of 0.1–10 mM histamine. About 97% of the [^{3}H] SKF 93479 associated with HGT-1 cells prior to the washing step remained bound after washing for the dissociation rate experiments. The decline in [^{3}H] SKF 93479 binding and recovery of H_2 receptor activity were followed at 20° from either 0 to 60 or 180 min after serial separations in microfuges, as described above.

Membrane-bound adenylate cyclase preparation and assay. After EDTA treatment, isolated HGT-1 cells were washed and resuspended (20 × 10^6 cells/ml) in 10 mM Tris–HCl buffer (pH 7.5) containing 1 mM EDTA, 30 mM NaCl, 1 mM dithiothreitol and 5 μM PMSF. Cells were disrupted with a Polytron homogenizer (Kinematica, Luzern, Switzerland) using three bursts of 5 sec. The homogenate was layered over 10 ml of a 41% sucrose solution dissolved in the homogenization buffer and centrifuged at 95,000 *g* for 1 hr at 4° in a Beckman SW27 swinging bucket rotor [28]. The white interfacial band of membranes was collected and diluted 4 times in homogenization buffer. Membranes were obtained by centrifugation at 4° (40,000 *g*, 30 min, 4°) in a Sorvall centrifuge RC-5B (Newton, CT). They were then stored frozen at −80° for 1 week. Adenylate cyclase activity was measured by a modified version of the method previously described [11, 28]. The standard incubation mixture (final volume: 250 μl) contained 1 mM ATP, 5 mM $MgCl_2$, the ATP-regenerating system (20 mM creatine phosphate, 1 mg/ml phosphocreatine kinase), 0.2 mM IBMX, 1 mg/ml BSA, 1 mM EGTA, 200 μg/ml bacitracin, 20 μM GTP and test substances in 25 mM Tris–HCl buffer (pH 7.5). The reaction was initiated by addition of 10 μg membrane protein per tube, and the mixture was incubated for 15 min at 20 or 30°. Data are expressed as picomole of cyclic AMP produced per min per mg membrane protein.

Processing of the data and statistical analysis. Data are derived from representative experiments, each of which was repeated at least 3 times in duplicate or triplicate. Results are expressed as means ± S.E.M. The significance of the differences observed was assessed using Student's *t*-test. Regression lines were fitted to the linear portions of the concentration-response curves derived from individual experiments and the apparent EC_{50} and IC_{50} potencies, i.e. the doses respectively required to produce half-maximal stimulation or inhibition, were calculated by the least square method. Antagonism against a fixed concentration of histamine (S) by different concentrations of SKF 93479, SKF 92408, SKF 91581, metiamide or cimetidine, was analyzed [29] according to the following equations: $K_i = IC_{50}/(1 + S/EC_{50})$ and $K_i = I/(K'a/Ka) - 1$, where Ka and $K'a$ are respectively the mean histamine concentrations required to produce half-maximal cyclic AMP stimulation in the absence and presence of the antagonist, and I is the concentration of antagonist. Histamine inhibition by cimetidine was analyzed by Schild plot [30] in which antagonism is expressed by the dose ratios (DR) of histamine needed to produce half-maximal responses in the absence and presence of different concentrations of cimetidine:

$$\log (DR - 1) = n \log (\text{antagonist}) - \log K_b$$

For a simple competitive antagonism, the Schild plot yields a straight line with a slope of unity. The intercept with the abscissa (DR = 2) is the pA_2 value ($-\log K_b$), i.e. the negative log of the receptor–antagonist apparent dissociation constant. All calculations were performed on a HP-85 microcomputer (Hewlett-Packard).

RESULTS

Effect of time, phosphodiesterase inhibitor, histamine and its antagonists on cyclic AMP levels in HGT-1 cells

When HGT-1 cells were incubated in the absence of phosphodiesterase inhibitor, cellular cyclic AMP levels were 1.47 ± 0.22 pmoles/10^6 cells (N = 3) throughout the 10 min incubation at 20° (Fig. 2, left). After the addition of 0.1 mM histamine, basal cyclic AMP levels rose to a maximum of 2.55 ± 0.22 pmoles/10^6 cells (i.e. a 1.7-fold increase over the basal level), and remained constant for up to 10 min. These results are in agreement with our previous observations [12] indicating that in human fundic glands, histamine effectively stimulates cyclic AMP production in the absence of phosphodiesterase inhibitor, in contrast with the IBMX dependence of histamine stimulation in the rat stomach [10]. In cells incubated for 5 min in the absence of phosphodiesterase inhibitor, cyclic AMP

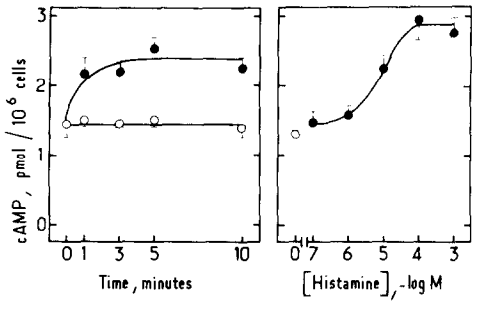

Fig. 2. Effects of time and histamine concentrations on cyclic AMP production in HGT-1 cells incubated in the absence of phosphodiesterase inhibitor. *Left*: Cells (0.6–1.2 × 10^6/ml) were preincubated at 20° for 10 min in KRP buffer containing 1% BSA and then incubated for the time indicated in the absence (basal cyclic AMP levels: ○) or presence of 0.1 mM histamine (●). *Right*: After 10 min preincubation, HGT-1 cells were incubated for 5 min with various concentrations of histamine (●). Data are means ± S.E.M. of the results of 3–4 experiments performed in duplicate or triplicate.

production was stimulated here by histamine concentrations ranging from 0.1 μM to 0.1 mM (Fig. 2, right). Half-maximal stimulation was observed at 8 μM histamine, and raising the histamine concentration to 1 mM did not produce more stimulation than that observed at 0.1 mM. In the presence of 1 mM IBMX as phosphodiesterase inhibitor (Fig. 3, left), basal and histamine-stimulated cyclic AMP levels were 1.95 ± 0.22 and 26.1 ± 2.8 pmoles/10^6 cells respectively (N = 9). Under these conditions, half-maximal and maximal cyclic AMP stimulation by 0.1 mM histamine were observed by 3 and 15 min, respectively, and a steady state was reached between 10 and 30 min incubation. The time required to obtain the maximal response to histamine is not dependent on its concentration, since cyclic AMP levels become maximal and constant 10–30 min after the addition of 1 μM, 10 μM or 0.1 mM histamine (data not shown). Simultaneous addition of histamine and the H_2 receptor antagonist cimetidine at 10 μM reduced histamine stimulation by 45% at 3 min, and by 65% during the remaining incubation time (Fig. 3, left). In contrast, when the H_2 receptor antagonist SKF 93479 (2 μM) was added simultaneously with 0.1 mM histamine, there was a 5 min lag phase during which no inhibition was observed, and the rises in cyclic AMP levels evoked by histamine were reduced by 46% at 10 min, 55% at 15 min and 72% at 30 min. At 60 min, basal cyclic AMP levels were only increased 2-fold, representing 85% inhibition by 2 μM SKF 93479 (Fig. 3, right). The relation between the SKF 93479 concentration (0.2–200 μM) and the rate and extent of inhibition is analyzed in Fig. 3, right. Four different inhibition curves were obtained; in the presence of 0.2 μM SKF 93479, inhibition occurred with a lag phase of 10 min and was increasing linearly with time from 15 to 60 min of incubation. In contrast, there was no lag phase when histamine was added together with 20 or 200 μM SKF 93479.

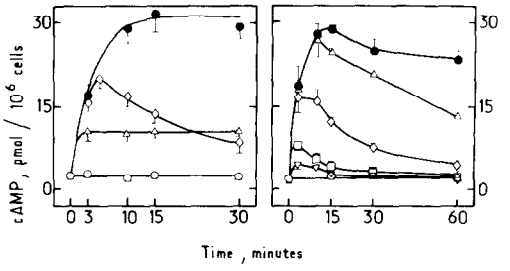

Fig. 3. Time course of control and histamine-stimulated cyclic AMP production in HGT-1 cells incubated in the absence or presence of the H_2 receptor antagonists cimetidine or SKF 93479. *Left*: Histamine (0.1 mM, ●) alone or together with cimetidine (10 μM, △) or SKF 93479 (2 μM, ◇) was added to HGT-1 cells incubated at 20° in the presence of 1 mM IBMX as a phosphodiesterase inhibitor. Cyclic AMP production in control HGT-1 cells (○). *Right*: Cells were suspended in standard incubation buffer containing 1 mM IBMX and incubated at 20° in the presence of histamine (0.1 mM, ●) alone or together with SKF 93479 at a concentration of 0.2 μM (△), 2 μM (◇), 20 μM (□) or 0.2 mM (▽). Cyclic AMP production in control HGT-1 cells (○). Data are means ± S.E.M. from 3 (right) to 9 experiments (left) performed in duplicate or triplicate.

Effect of histamine H_2 receptor antagonists and agonists on histamine-induced cyclic AMP production

The stimulatory effect of 0.1 mM histamine was inhibited by the imidazole derivatives cimetidine, metiamide, SKF 92408 and SKF 91581 as well as by the isocytosine-furan analogue SKF 93479 (Fig. 4). The order of inhibitory potencies (IC_{50}) was: SKF 93479 = cimetidine (2.3 μM), metiamide (3.2 μM) > SKF 92408 (100 μM) > SKF 91581 (3.16 mM).

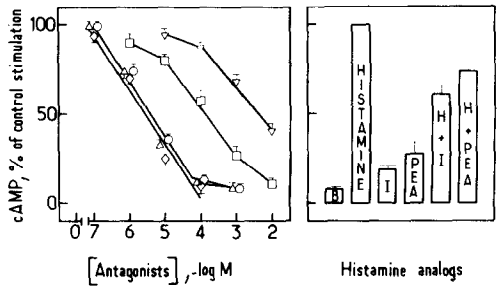

Fig. 4. Effect of H_1 or H_2 agonists and antagonists on H_2 receptor activity in HGT-1 cells. Cells were preincubated for 10 min at 20° in the presence of 1 mM IBMX and incubated for an additional 15 min after simultaneous addition of histamine with one of its H_2 antagonists (left), or its partial H_1 or H_2 agonists (right). *Left*: The K_i values [29] for the H_2 antagonists were : 0.2 μM SKF 93479 (◇) or cimetidine (△), 0.3 μM metiamide (○), 9.1 μM SKF 92408 (□) and 0.29 mM SKF 91581 (▽). The inhibition curves for SKF 93479, cimetidine, metiamide and SKF 92408 were parallel. *Right*: The H_1 agonist PEA and the H_2 agonist impromidine (I) were incubated alone or together with histamine at the following concentrations: 0.1 mM histamine, 10 mM PEA, 1 μM impromidine. Data are means ± S.E.M. of 3 (right) to 5 experiments (left), each performed in duplicate or triplicate. Values are expressed as the percentage of the control stimulation elicited by 0.1 mM histamine.

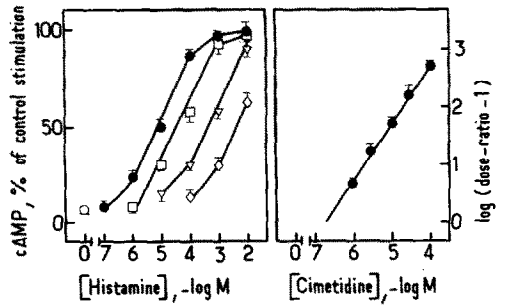

Fig. 5. Schild plot representation of the effect of cimetidine on H_2 receptor activity in HGT-1 cells. *Left*: HGT-1 cells were incubated for 15 min at 20° in the presence of histamine alone (●) or together with cimetidine, at the concentrations indicated below. Data are means ± S.E.M. from a single experiment performed in triplicate. Comparable changes in cyclic AMP production were observed in 5 other experiments. The following K_i values [29] were obtained for cimetidine in the presence of 3 μM (□, K_i = 0.46 μM), 10 μM (▽, K_i = 0.15 μM) and 30 μM cimetidine (◇, K_i = 0.1 μM). Cyclic AMP production in control HGT-1 cells (○). *Right*: Cyclic AMP values were obtained with different concentrations of histamine in the absence or presence of cimetidine at the 5 different concentrations indicated. Dose ratios [30] were estimated graphically from the parallel displacement of straight portions of five concentration–response curves similar to the data in Fig. 5 (left). HGT-1 cells were incubated in the standard KRP buffer containing 1 mM IBMX.

The effect of histamine was completely inhibited by 0.1 mM SKF 93479, cimetidine or metiamide or by 10 mM SKF 92408. As shown in Fig. 4 (right), stimulation of cyclic AMP production by histamine was also inhibited by 1 μM of the partial histamine H_2 agonist impromidine (38% inhibition) and by 10 mM of the partial histamine H_1 agonist PEA (26% inhibition), both tested at maximally effective concentrations in this system [13]. The inhibitory action of PEA was consistent with the partial agonistic action of this H_1 receptor agonist acting at pharmacological doses on the H_2 receptor in HGT-1 cells and other tissues bearing H_2 receptors [9].

The dose–response curves for histamine (potency = 10 μM) exhibited parallel shifts to the right following treatment of HGT-1 cells with three different concentrations of cimetidine (3 μM, 10 μM, 30 μM), but no change in the maximal response to histamine (Fig. 5, left). The mean K_i value for cimetidine (0.24 μM) was calculated from the values obtained in Fig. 5 (left). The data from 2–5 other similar experiments were plotted in Fig. 5 (right) as:

log (DR-1) against − (log concentration of cimetidine).

The dose-ratio was obtained from 3–6 point dose–response curves. The Schild plot was linear for cimetidine ($P < 0.001$), with a regression coefficient r = 0.917. The slope of the regression line was 0.97 and did not differ significantly from unity (95% confidence limit = 0.86 −1), indicating a simple mechanism of competition between histamine and cimetidine. The affinity of cimetidine for the H_2 receptor, expressed as the pA_2 value (the point of intersection of the regression line and abscissa) was estimated at 6.72 ± 0.12 (K_i = 0.18 μM). This inhibition constant was in agreement with the mean K_i values determined from the experimental design of Fig. 4 (left) and Fig. 5 (left). It is noteworthy that half-maximal activation of the H_2 receptor by histamine was observed at 0.8 and 1 μM in the absence and presence of IBMX, respectively, indicating that this phosphodiesterase inhibitor does not change the potency of histamine in HGT-1 cells, as observed earlier for H_2 receptors characterized in human leukemic HL-60 cells [31].

Effect of pre-exposure of HGT-1 cells to cimetidine, ranitidine, oxmetidine and SKF 93479 on subsequent histamine or VIP stimulation

When 2 μM SKF 93479 (a concentration giving half-maximal inhibition of the histamine effect, 10 min after its addition together with 0.1 mM histamine) was preincubated for 10 min with HGT-1 cells, basal cyclic AMP levels remained unchanged throughout the subsequent 30 min period of incubation with 0.1 mM histamine (Fig. 6, left). This inactivation after SKF 93479 preincubation was specific for the H_2 receptor, since the drug did not modify the time course of VIP-induced cyclic AMP production in HGT-1 cells. The same observation was made 10 min after simultaneous addition of SKF 93479 with VIP (Table 1). Preincubation of HGT-1 cells with cimetidine (10 μM) did not suppress H_2 receptor activity, since this antagonist only produced partial inhibition. The time course obtained under these conditions was comparable to that depicted in Fig. 3 (right), when cimetidine and histamine were added simultaneously. As shown in Fig. 6 (right), elimination of 2 μM SKF 93479 from

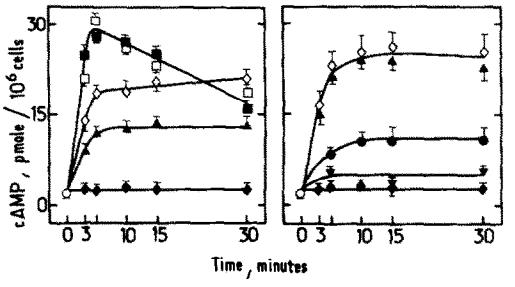

Fig. 6. Effect of cimetidine, oxmetidine, ranitidine and SKF 93479 preincubated for 10 min with HGT-1 cells on the subsequent histamine or VIP receptor activity. *Left*: Histamine (0.1 mM; ◇, ▲, ◆) or VIP (0.1 μM: □, ■) were added to HGT-1 cells previously incubated for 10 min at 20° in the presence of 1 mM IBMX, without antagonist (KRP buffer; ◇, □) or with either 10 μM cimetidine (▲) or 2 μM SKF 93479 (◆, ■). Data are means ± S.E.M. from a single experiment done in triplicate. Comparable cyclic AMP changes were observed in 3 other similar experiments. *Right*: HGT-1 cells were preincubated for 10 min in the absence (KRP buffer: (◇) or presence of 10 μM cimetidine (▲) or 2 μM ranitidine (●), oxmetidine (▼) or SKF 93479 (◆) and then washed 4 times at 20° with KRP buffer before addition of 10 mM histamine for the standard cyclic AMP assay, as indicated in Methods. Cyclic AMP production in control HGT-1 cells (○). Data are means ± S.E.M. from 3–7 experiments done in duplicate or triplicate.

Table 1. Effect of the H_2 receptor antagonists cimetidine and SKF 93479 on VIP receptor activity in HGT-1 cells

Substance	Concentration	Cyclic AMP (pmol/10^6 cells)
None		2.9 ± 0.24
VIP	0.1 μM	19.5 ± 2
VIP + cimetidine	0.1 mM	19.4 ± 2.3
VIP + SKF 93479	0.1 mM	19 ± 2.4

HGT-1 cells were incubated at 20° for 10 min in Krebs Ringer phosphate buffer containing 1 mM IBMX and 1% BSA, plus the indicated additions.

Results are expressed as means ± S.E.M. of 4 experiments performed in duplicate.

the medium by serial washings after 10 min preincubation with HGT-1 cells did not restore H_2 receptor sensitivity to histamine. Such persistent inactivation is also peculiar to SKF 93479, since the time course of histamine-stimulated cyclic AMP production was unchanged when 10 μM cimetidine, preincubated for 10 min with HGT-1 cells, was removed from the incubation medium under the same conditions. Similarly, pretreatment of HGT-1 cells with 2 μM oxmetidine and ranitidine for 10 min before washing the cells resulted respectively in 90 and 65% inhibition of H_2 receptor activity during the 5–30 min incubation period with 0.1 mM histamine (Fig. 6, right). As shown in Fig. 7, daily treatment of HGT-1 cells for 6 days with 2 μM SKF 93479 resulted in a complete and homologous disappearance of histamine H_2 receptor activity in the system since the potency and the efficacy of the cyclic AMP-inducing hormones VIP (EC_{50} = 1.9 ± 0.7–2.3 ± 0.6 nM), glucagon (EC_{50} = 13 ± 3 nM) and GIP (EC_{50} = 13 ± 7–23 ± 4 nM) remained unaffected after this treatment. In contrast, chronic treatment of HGT-1 cells with 10 μM cimetidine does not change histamine H_2 receptor activity (EC_{50} = 13 ± 4–15 ± 5 μM) as well as the activation of cell surface receptors by VIP (EC_{50} = 1.7 ± 0.3–2.3 ± 0.6 nM), glucagon (8 ± 2–13 ± 3 nM) and GIP (EC_{50} = 13 ± 7–23 ± 4 nM).

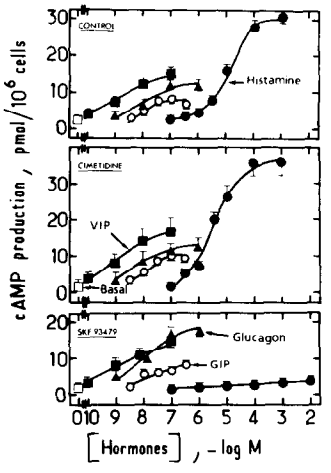

Fig. 7. Effect of cimetidine and SKF 93479 cultured for 6 days with HGT-1 cells on the subsequent histaminergic and peptidergic receptor activity. Control or HGT-1 cells treated by 10 μM cimetidine or 2 μM SKF 93479 were compared for their cyclic AMP formation capacity after VIP (■), glucagon (▲), GIP (○) or histamine (●). Data are means ± S.E.M. from 3–4 experiments performed in duplicate or triplicate.

Time dependency relationship between the potency and degree of inhibition of SKF 93479 and its binding to HGT-1 cells

This study was designated to compare the interaction of 2 μM [^{3}H] SKF 93479 with HGT-1 cells and the kinetics of the histamine inhibition obtained with the same concentration of this H_2 antagonist, either added simultaneously with histamine (Figs. 3 and 4, left), or preincubated with cells before histamine addition (Figs. 6 and 8). As shown in Fig. 8, preincubation of HGT-1 cells with SKF 93479 concentrations ranging from 3.16 nM to 3 μM increased the inhibitory potency of the drug, from 2.3 μM after simultaneous addition (see Fig. 4) to 0.5 μM after 10 min preincubation, to 0.2 μM after 30 min, to 0.1 μM after 60 min, and to 45 nM after 120 and 180 min preincubation. This is in agreement with the kinetics of the [^{3}H] SKF 93479 radioactivity associated with HGT-1 cells (Fig. 9, left). Half-maximal and maximal binding of [^{3}H] SKF 93479 to HGT-1

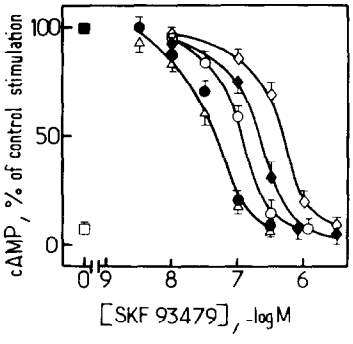

Fig. 8. Effect of various concentrations of SKF 93479 preincubated for 10 min (◇), 30 min (◆), 60 min (○), 120 min (●) and 180 min (△) with HGT-1 cells on the subsequent H_2 receptor activity. Control (□) and histamine-stimulated cyclic AMP levels (■) in HGT-1 cells incubated at 20° in the presence of 1 mM IBMX. Results are expressed as the percentage of cyclic AMP production evoked by 0.1 mM histamine. Data are means ± S.E.M. from 4–7 experiments performed in duplicate.

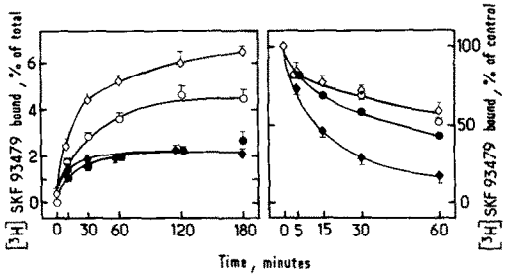

Fig. 9. Association and dissociation of [^{3}H] SKF 93479 from HGT-1 cells. *Left*: Time course of [^{3}H] SKF 93479 association with HGT-1 cells. Cells (2–2.5 × 10^6 cells) were incubated at 20° with 2 μM [^{3}H] SKF 93479 in KRP buffer. Total (◇), specific (○) and nonspecific binding were evaluated at the times indicated, in the absence or presence of 2 mM SKF 93479 (◆) or 10 mM histamine (●). Each point represents the mean ± S.E.M. of 4 determinations of bound/total [^{3}H] SKF 93479 radioactivity at the different times indicated. *Right:* Time course of [^{3}H] SKF 93479 dissociation from HGT-1 cells. HGT-1 cells (20–30 × 10^6 cells/ml) were preincubated with 2 μM [^{3}H] SKF 93479 in KRP buffer for 1 hr at 20°, washed 4 times in the same buffer (centrifugations at 200 *g*, 3 min, 4°) and resuspended in fresh standard solution. HGT-1 cells were then incubated at 20° in the absence (KRP buffer: ◇) or presence of 2 mM SKF 93479 (◆), or of 0.1 mM (○) or 10 mM (●) histamine. Results are expressed as the percentage of the radioactivity present at the beginning of the incubation. Results are means ± S.E.M. of one (10 mM histamine) or 4 experiments performed in duplicate.

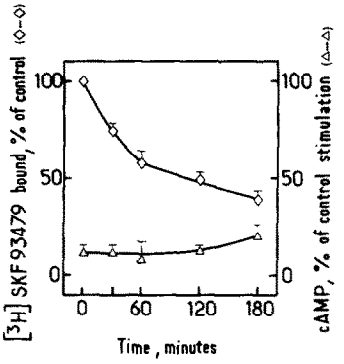

Fig. 10. Relationship between dissociation of bound [^{3}H] SKF 93479 from HGT-1 cells and recovery of H_2 receptor activity. HGT-1 cells were preincubated for 1 hr at 20° in the absence (control HGT-1 cells) or presence of either unlabelled or tritiated SKF 93479 at 2 μM. The 3 batches of cells were then washed and incubated for 3 hr, as described in Fig. 8, right. At each time considered, [^{3}H] SKF 93479 radioactivity (◇) and H_2 receptor activity (△) were measured in control and SKF 93479-treated HGT-1 cells. Cells were incubated for 15 min in the absence (control) or presence of 0.1 mM histamine for the standard cyclic AMP assay described in Materials and Methods. Data are means ± S.E.M. of results from 3 experiments performed in triplicate.

cells (27 pmoles per 10^6 cells) occurred within 10 and 30 min, respectively. Cell-associated radioactivity remained constant up to 120 min. Nonspecific binding represented 2.12 ± 0.18% of the total radioactivity added, and 34 ± 4% of the maximal binding observed at 60 min. Specific binding of [^{3}H] SKF 93479 to HGT-1 cells was maximal at 60 min (14 pmoles/10^6 cells), and stayed constant until 180 min. As shown in Fig. 9 (right), spontaneous dissociation of [^{3}H] SKF 93479 from HGT-1 cells was relatively slow and did not follow a first-order process. At 20°, 30% of the radioactivity was dissociated after 30 min incubation and 40% after 60 min. In agreement with the kinetic data in Figs. 3 and 6, addition of 0.1 mM histamine did not increase this spontaneous dissociation rate. In contrast, 10 mM histamine slightly increased dissociation rates. Only the compound SKF 93479 at 2 mM significantly accelerated [^{3}H] SKF 93479 dissociation rates from HGT-1 cells, half-maximal dissociation was observed at 15 min and 85% dissociation at 60 min.

Time dependency relationship between the spontaneous dissociation of bound [^{3}H] *SKF 93479 and the recovery of* H_2 *receptor activity*

Figure 10 shows that over a 120 min incubation period, the spontaneous dissociation of [^{3}H] SKF 93479 (half-life: 120 min) from HGT-1 cells preexposed to 2 μM SKF 93479 for 60 min, was biphasic and was not associated with the recovery of H_2 receptor sensitivity to histamine until 120 min. After 180 min, basal cyclic AMP levels in HGT-1 cells were only doubled by 0.1 mM histamine, when 60% of [^{3}H] SKF 93479 initially bound was dissociated from HGT-1 cells.

Kinetics and potencies of cimetidine and SKF 93479 inhibition against histamine-induced adenylate cyclase activation in HGT-1 cells

As shown in Fig. 11, half-maximal linear inhibition of histamine-induced adenylate cyclase activation was already produced at 3 min by 0.1 μM SKF 93479

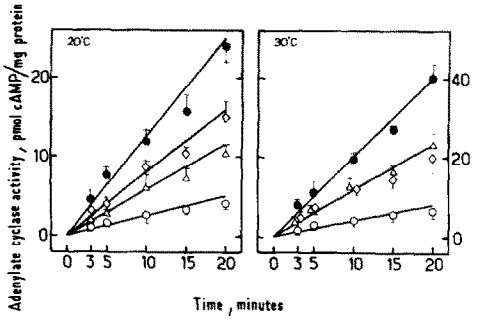

Fig. 11. Effect of time and temperature on basal and histamine-induced adenylate cyclase activation in purified plasma membranes from HGT-1 cells, incubated in the absence or presence of the H_2 receptor antagonists cimetidine or SKF 93479. Membranes were incubated at 20° (left), or 30° (right) in the absence (basal activity: ○) or presence of 0.1 mM histamine alone (●) or together with 3 μM cimetidine (△) or 0.1 μM SKF 93479 (◇). Data are means ± S.E.M. from 3 experiments performed in duplicate.

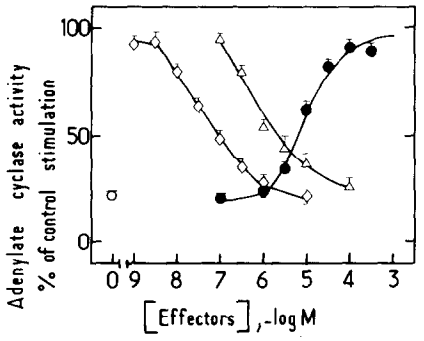

Fig. 12. Inhibition by cimetidine and SKF 93479 of histamine-stimulated adenylate cyclase activity in HGT-1 cells. Purified plasma membranes were incubated for 15 min at 30° in the presence of various concentrations of histamine (●) or in the presence of 0.1 mM histamine together with various concentrations of cimetidine (△) or SKF 93479 (◇). We verified that adenylate cyclase activities under basal or stimulated conditions (1 mM histamine or 10 mM sodium fluoride) were linear with time (0–3–5–10–15–30 min) and protein concentrations (3–15 μg membrane protein per tube). Neither 0.1 mM cimetidine nor 10 μM SKF 93479 inhibited adenylate cyclase activation by 0.1 μM VIP or 10 mM NaF. Basal, sodium fluoride- and histamine-stimulated adenylate cyclase activities were respectively 455 ± 76; 4400 ± 619 and 1960 ± 237 pmol cyclic AMP produced per min per mg membrane protein. Data are means ± S.E.M. from 4–6 experiments performed in duplicate.

or 3 μM cimetidine in purified plasma membranes incubated for 20 min at 20° (left) or at 30° (right). In membranes incubated at 30° for 15 min, stimulation of adenylate cyclase by histamine (Fig. 12) was observed for concentrations ranging from 1 μM to 0.1 mM (K_a = 8 μM histamine). This potency of histamine on adenylate cyclase was consistent with the EC_{50} value of 8–10 μM histamine measured on intact HGT-1 cells (see Figs. 2 and 5). Histamine stimulation of adenylate cyclase activity was gradually and completely inhibited by increased concentrations of cimetidine and SKF 93479. The two inhibition curves were parallel. When added simultaneously with 0.1 mM histamine to membranes incubated at 30°, SKF 93479 (IC_{50} = 40 nM) was 25 times more potent than cimetidine (IC_{50} = 1 μM). In one experiment conducted at 20°, we observed that the inhibitory potency of SKF 93479 was 100 nM, in the presence of the same concentration of histamine.

DISCUSSION

In this paper, we compared the ability of the four imidazole cimetidine analogs, metiamide, SKF 92408, SKF 91581 and the isocytosine-furan derivatives ranitidine, oxmetidine and SKF 93479 to antagonize the action of histamine in gastric cancer HGT-1 cells originating from the human fundus. This is therefore the first investigation of pharmacological control by H_2 antagonists of the H_2 receptor mediating acid secretion in the human stomach.

When the above H_2 receptor antagonists were added to intact HGT-1 cells or plasma membrane preparations either before histamine or together with it, they exhibited remarkable differences in relative inhibitory potencies, duration of action, reversibility and type of antagonism. Increasing concentrations of cimetidine caused a parallel shift of the histamine dose–response curve and the Schild plot fulfilled the criteria of competitive antagonism with respect to histamine (slope = 0.97). The pA_2 value of 6.7 (K_i = 0.18 μM) calculated for cimetidine, was in good agreement with the estimations derived from inhibition curves for a fixed concentration of histamine (K_i = 0.2–0.24 μM cimetidine). These results suggest that the affinity of cimetidine for the histamine receptor in HGT-1 cells is higher than has been reported (K_i = 0.8–1 μM) for H_2 receptor-mediated stimulation of cyclic AMP formation in the gastric mucosa in man or guinea pig [3, 12]. In contrast, comparable values for cimetidine (pA_2 = 6.4–6.9) were found on H_2 receptors mediating cyclic AMP production, aminopyrine accumulation and oxygen consumption in gastric parietal cells [6, 7, 10, 11, 32, 33], cyclic AMP generation in human neutrophils [34] and the chronotropic action of histamine on the guinea pig right atrium [35]. The difference noticed for the potency of cimetidine on H_2 receptors might be related (1) to the origin of the tissues; (2) to the gastric cell types bearing histamine H_2 receptors [33]; (3) to the method [9, 11, 33] used for gastric gland or cell preparation (EDTA or enzyme treatment of the mucosa); (4) to the absence or presence of endogenous histamine [36] during cell isolation or incubation, leading to H_2 receptor desensitization [9, 28]; and (5) to the activity of the enzymes forming or degrading histamine [36], since some H_2 receptor antagonists such as burimamide are potent inhibitors of histamine-*N*-methyl transferase, HMT [37], the principal enzyme for histamine degradation in tissues. In this connection it is noteworthy that HGT-1 cells are devoid of endogenous histamine and of histidine decarboxylase, HMT and monoamine oxydase activities (S. Emami *et al.*, manuscript in preparation).

When added simultaneously with histamine, the relative potencies of the antagonists were, if we assign a value of 100 to cimetidine inhibition: SKF 93479 = cimetidine = 100 > metiamide = 62 > SKF 92408 = 2 > SKF 91581 = 0.07. These differences in potency were comparable to those observed for gastric acid secretion in the rat or for the contraction of atrial smooth muscle in the guinea pig: cimetidine = 100 > metiamide = 85–87 > SKF 92408 = 5–11. They were also comparable to the inhibitory potencies noted for adenylate cyclase activation in guinea pig heart ventricule or hippocampus: cimetidine = 100 > metiamide = 66–80 > SKF 91581 = 0.1. In contrast, the isocytosine-furan derivative SKF 93479 was found to be up to 20 times more potent than cimetidine in reducing gastric acid secretion stimulated by histamine in Heidenhain pouch of conscious dog [18, 19]. This apparent discrepancy was due to several striking differences in the kinetics and characteristics of the interaction of SKF 93479 and cimetidine on intact HGT-1 cells: (1) When 2 μM SKF 93479 was added simultaneously with histamine, the onset of its action was slow with a lag phase of 3–5 min and its inhibitory effect

increased with time, reaching complete blockade at 60 min. Under the same conditions, 10 μM cimetidine produced rapid inhibition which was apparent at 3 min, but remained constant and partial up to 60 min. At these above concentrations, SKF 93479 and cimetidine respectively produced 50% and 65% inhibition, 10 min after their simultaneous addition with histamine (see Figs. 3 and 4). (2) After simultaneous addition of 0.2–200 μM SKF 93479, there was no equilibrium time for its inhibition (Fig. 3, right). Transformation of the inhibition curves indicated that the initial rate of inhibition was not linear with the SKF 93479 concentration, but was saturated by increasing concentrations of the H_2 antagonist. This suggests the involvement of an association-dissociation reaction prior to an irreversible-slowly dissociable-attachment to the receptor. Progressive occupancy of the inhibitory site by SKF 93479 resulted in an obvious increase of blockade of H_2 receptor activity, leading to the progressive disappearance of cyclic AMP in HGT-1 cells. This process can be ascribed to the cyclic AMP-dependent phosphodiesterase activities previously determined in this gastric cancer cell line [13]. Indeed, the equilibrium conditions obtained after addition of 0.1 mM histamine, either alone or together with 10 μM cimetidine are due to the equivalence between cyclic AMP generation and degradation (Fig. 3, left). (3) Preincubation of 2 μM SKF 93479 with HGT-1 cells for 10 min prior to the addition of 0.1 mM histamine caused a complete and persistent inactivation of H_2 receptor activity lasting at least 60 min. (4) Elimination of SKF 93479 from the medium after 10 min preincubation or chronic treatment of HGT-1 cells for 6 days resulted in a complete and persistent inactivation of H_2 receptor activity. This effect, which was not observed after cimetidine is specific for the H_2 receptor (Figs. 6 and 7). In agreement with the above observations, the time-dependent interaction of [^{3}H] SKF 93479 with HGT-1 cells showed a slow association process (half-life: about 10 min) with a slow dissociation rate (half-life: 120 min). The fact that the [^{3}H] SKF 93479 disappearance curve was not linear (Fig. 9, right) and was not related to the recovery of H_2 receptor sensitivity to histamine until 120 min (Fig. 10), suggested the involvement of two dissociating components respectively corresponding to binding sites with low affinity (rapid extracellular component) and high affinity for the drug (slowly dissociating or intracellular component). In agreement with the presence of a transport-uptake system for histamine in tissues bearing histamine H_2 receptors [38, 39], we observed that [^{3}H] SKF 93479 binding to HGT-1 cells did not reach saturation over a concentration range of 0.2 μM–0.1 mM. However, the data shown in Fig. 12 indicate that 10 nM–1 μM SKF 93479 completely inhibited H_2 receptor activation induced by 0.1 mM histamine, a dose giving the maximal stimulation. It is therefore likely that the binding sites involved in the pharmacological control of the H_2 receptor activated by physiological doses of histamine are completely saturated at SKF 93479 concentrations above 1 μM. (5) It is noteworthy that SKF 93479 was 15–25 times more potent than cimetidine in inhibiting H_2 receptor activity after 120 min preincubation with intact HGT-1 cells and also in inhibiting adenylate cyclase activity after its simultaneous addition with histamine to purified plasma membranes. In the latter case, 0.1 μM SKF 93479 or 3 μM cimetidine produced a rapid onset of inhibition at 3 min which remained constant and persisted for at least 20 min. Therefore, there was a good correlation between the relative potencies of those drugs as regards gastric acid secretion *in vivo* [18, 19], membrane-bound adenylate cyclase, and intact HGT-1 cells preincubated for 120–180 min with SKF 93479, before histamine addition. The present results raise the possibility that human gastric HGT-1 cells possess a receptor-mediated histamine-sensitive system for the inhibition of H_2 receptor activity by the isocytosine-furan analogue SKF 93479. The differences in the kinetics and inhibitory potency of SKF 93479 in intact HGT-1 cells and in purified plasma membranes might be due to differences in the accessibility of the inhibitory site to the drug, which may be intracellular. If true, this might explain why the inhibition by SKF 93479 has a faster onset (no lag phase), and increased potency in cell-free preparations.

The pharmacological properties of the imidazole and isocytosine-furan analogues studied as H_2 receptor antagonists on HGT-1 cells were analyzed in relation to their chemical structure (Fig. 1). Thus, in the case of SKF 91581, extending the side chain by introduction of a thioether sulphur atom and introducing a methyl substituent into the imidazole ring in position 4 to form metiamide increases the potency of the drug 900-fold possibly because these substitutions favor the 1–4 tautomerism of the imidazole ring in metiamide [40]. For SKF 92408, on the contrary, replacement of the thiourea sulphur atom of metiamide by the iminonitrogen provides a very basic guanidine derivative ($pK_a > 13$), but also reduced this antagonist's inhibitory potency 30-fold. The guanidine derivative impromidine which contains the entire structure of SKF 92408 plus an imidazole ethyl substituent, possesses mixed agonistic-antagonistic properties in HGT-1 cells. On H_2 receptors, impromidine has been found to act as a partial histamine agonist [2, 6, 12, 13] and to exhibit 30–50 times more potency than histamine both *in vivo* [41] and *in vitro* [2, 12, 13, 31, 34]. Accordingly, impromidine behaved like a competitive antagonist on the action of histamine ($K_i = 0.21$–0.25 μM) on H_2 receptors in gastric [2] and nongastric cells [31]. Replacement of one of the hydrogen atoms of the guanidine group in SKF 92408 by the powerful electron withdrawing cyano group to form cimetidine considerably reduces the basicity of the drug, avoids the side-effects of the thiourea group in metiamide [40], and increases 30- to 50-fold the potency of cimetidine and metiamide. The compound SKF 93479, which possesses an isocytosine ring (as oxmetidine, with a different substituent in the position 5), a dimethylaminomethyl furan group (as ranitidine) and the CH_2—S—CH_2—CH_2 side chain (as oxmetidine, ranitidine and cimetidine), was proved here to be equipotent to cimetidine when added to HGT-1 cells simultaneously with histamine. Under the same conditions, oxmetidine and ranitidine were 9 times more potent than cimetidine or SKF 93479 in HGT-1 cells [13]. The conjunction of the above 3

radicals with the pyridyl group conferred particular originality to the action of SKF 93479, both in HGT-1 cells and *in vivo*. Intravenous or oral administration of SKF 93479 to the Heidenhain pouch of conscious dog has been reported to produce much more lasting inhibition than cimetidine [18, 19], and by both routes, SKF 93479 was 16–20 times more potent.

The present studies indicate that SKF 93479 belongs to a new class of H_2 receptor-blocking drugs which include AH 22216 [7, 18, 19] and L-643, 441 [16]. These three drugs possess greater inhibitory potency than cimetidine and more prolonged action *in vivo* and *in vitro*. In the case SKF 93479, this is due to its relatively slow rate of dissociation from the inhibitory site located in the plasma membrane of HGT-1 cells. Comparison of the action of cimetidine, ranitidine, oxmetidine and SKF 93479 on H_2 receptor activity after preincubation or culture with HGT-1 cells, suggests that the isocytosine ring in SKF 93479 and oxmetidine is the major constituent of the molecules responsible for their apparent long lasting, irreversible actions. The molecular and the pharmacological properties of the histamine-dependent [^{3}H] SKF 93479 binding site identified in these cells are not known. Despite the intensive investigation on histamine H_2 receptors over the past 10 years, little is known about their structure and subcellular localization [38, 42, 43]. The search of suitable agonist or antagonist reproducing all the pharmacological and biological properties of histamine stimulation or blockade would be expected for ligand binding study at physiological H_2 receptors. Further studies with radiolabeled SKF 93479 should help to establish the connection between this inhibitory site and the histamine H_2 receptor.

Acknowledgements—We thank Dr R. W. Brimblecombe and Dr M. E. Parsons of Smith, Kline and French Laboratories for gifts of histamine receptor agonists and antagonists, Miss H. Bodéré for skilful technical assistance, Mr Y. Issoulié for illustrations; Mrs D. Lhenry and Miss M. Le Hein for excellent and patient secretarial assistance.

REFERENCES

1. J. W. Black, W. A. M. Duncan, G. J. Durant, C. R. Ganellin and M. E. Parsons, *Nature*, Lond. **236**, 385 (1972).
2. S. Batzri, J. W. Harmon and W. F. Thompson, *Molec. Pharmac.* **22**, 33 (1982).
3. S. Batzri, J. W. Harmon, J. Dyer and W. F. Thompson, *Molec. Pharmac.* **22**, 41 (1982).
4. C. V. Perrier and L. Laser, *J. clin. Invest.* **49**, 73a (1970).
5. P. Mangeat, C. Gespach, G. Marchis-Mouren and G. Rosselin, *Regul. Peptides* **3**, 155 (1982).
6. C. S. Chew, S. J. Hersey, G. Sachs and T. Berglindh, *Am. J. Physiol.* **238**, G 312 (1980).
7. C. Gespach, I. Menez and S. Emami, *Biosci. Rep.* **3**, 871 (1983).
8. A. Anteunis, C. Gespach, A. Astesano, S. Emami, R. Robineaux and G. Rosselin, *Peptides* **5**, 277 (1984).
9. C. Gespach and S. Emami, in *Advances in the Biosciences*, Vol. 51, (Eds. C. R. Ganellin and J. C. Schwartz), p. 265. Pergamon Press, Oxford (1985).
10. C. Gespach, C. Dupont, D. Bataille and G. Rosselin, *FEBS Lett.* **114**, 247 (1980).
11. C. Gespach, D. Hui Bon Hoa and G. Rosselin, *Endocrinology* **112**, 1597 (1983).
12. C. Gespach, S. Emami, N. Boige and G. Rosselin, in *Gut Peptides and Ulcer* (Ed. Miyoshi A.), p. 56. Biomedical Research Foundation, Tokyo (1983).
13. S. Emami, C. Gespach, M.-E. Forgue-Lafitte, Y. Broer and G. Rosselin, *Life Sci.* **33**, 415 (1983).
14. I. Menez, C. Gespach, S. Emami and G. Rosselin, *Biochem. biophys. Res. Commun.* **116**, 251 (1983).
15. T. O. Yellin, S. H. Buck, D. J. Gilman, D. F. Jones and J. M. Wardleworth, *Life Sci.* **25**, 2001 (1979).
16. M. L. Torchiana, R. G. Pendleton, P. G. Cook, C. A. Hanson and B. V. Clineschmidt, *J. Pharmac. exp. Ther.* **224**, 514 (1982).
17. M. Harada, M. Terai and H. Maeno, *Biochem. Pharmac.* **32**, 1635 (1983).
18. R. C. Blakemore, R. H. Brown, G. J. Durant, C. R. Ganellin, M. E. Parsons, A. C. Rasmussen and D. A. Rawling, *Br. J. Pharmac.* **74**, 200P (1981).
19. R. Stables, M. J. Daly and J. M. Humphray, *Agents Actions* **13**, 166 (1983).
20. C. L. Laboisse, C. Augeron, M.-H. Couturier-Turpin, C. Gespach, A.-M. Cheret and F. Potet, *Cancer Res.* **42**, 1541 (1982).
21. C. Gespach, S. Emami and G. Rosselin, *Biochem. biophys. Res. Comm.* **120**, 641 (1984).
22. C. Dupont, C. Gespach, B. Chenut and G. Rosselin, *FEBS Lett.* **113**, 25 (1980).
23. C. Gespach, D. Bataille, M.-C. Dutrillaux and G. Rosselin, *Biochim. biophys. Acta* **720**, 7 (1982).
24. D. Bataille, C. Gespach, A.-M. Coudray and G. Rosselin, *Biosci. Reports* **1**, 151 (1981).
25. M. Dubrasquet, D. Bataille and C. Gespach, *Biosci. Reports* **2**, 391 (1982).
26. S. Emami, C. Gespach and H. Bodéré, European Histamine Research Society 13th Meeting, Florence, 16–19 May (1984).
27. S. Emami and C. Gespach, Frontiers in Histamine Research. International Symposium, Jouy-en-Josas, 25–27 July, p. 52 (1982).
28. A. Prost, S. Emami and C. Gespach, *FEBS Lett.* **177**, 227 (1984).
29. Y. C. Chen, and W. H. Prusoff, *Biochem. Pharmac.* **22**, 3099 (1973).
30. O. Arunlakshana and H. O. Schild, *Br. J. Pharmac.* **14**, 48 (1959).
31. C. Gespach, F. Saal, H. Cost and J.-P. Abita, *Molec. Pharmac.* **22**, 547 (1982).
32. G. C. Rosenfeld, S. J. Strada, E. J. Dial, C. F. Bearer and W. J. Thompson, *Adv. Cyclic Nucleotide Res.* **12**, 255 (1980).
33. C. Gespach, D. Bouhours, J.-F. Bouhours and G. Rosselin, *FEBS Lett.* **149**, 85 (1982).
34. C. Gespach and J.-P. Abita, *Molec. Pharmac.* **21**, 78 (1982).
35. M. E. Parsons, in *Cimetidine* (Eds. W. L. Burland and M. Aliqon Simkins), p. 13. Excerpta Medica, Amsterdam (1977).
36. M. A. Beaven, A. H. Soll and K. J. Lewin, *Gastroenterology* **82**, 254 (1982).
37. K. M. Taylor, *Biochem. Pharmac.* **22**, 2775 (1973).
38. T. Berglindh and K. J. Obrink, in *Histamine Receptors* (Ed. T. Yellin), p. 35. New York Spectrum (1979).
39. C. Gespach, N. Marrec and N. Balitrand, *Agents Actions* **16**, 279 (1985).
40. C. R. Ganellin in *Pharmacology of Histamine Receptors* (Eds C. R. Ganellin and M. E. Parsons), p. 10. John Wright, Bristol (1982).
41. G. J. Durant, W. A. M. Duncan, C. R. Ganellin, M. E. Parsons, R. C. Blackemore and A. C. Ramussen, *Nature, Lond.* **276**, 403 (1978).
42. M. Osband and R. McCaffrey, *J. biol. Chem.* **259**, 9970 (1979).
43. C. Heitianu, M. Simionescu and N. Simionescu, *J. Cell. Biol.* **93**, 357 (1982).