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IRREVERSIBLE AND SPECIFIC INACTIVATION BY AH 22216 OF HISTAMINE H_2 RECEPTORS IN THE HUMAN GASTRIC CANCER CELL LINE HGT-1

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Summary. We compared the interaction of AH 22216 (a new histamine H_2 receptor antagonist) and cimetidine on the receptor-cAMP systems sensitive to histamine and to Vasoactive Intestinal Peptide (VIP) in the human gastric cancer cell line HGT-1. When added simultaneously with histamine (10^{-4} M), the potency of AH 22216 is similar to that of cimetidine (IC_{50}= 4-6.6x10^{-6} M, respectively). Schild plot analysis indicated a non-competitive inhibition by AH 22216 (pA_2=6.22, slope= 1.4±0.03). Preincubations of AH 22216 (10^{-5} M) with HGT-1 cells (even after a washout period) resulted in a complete and persistent (60 min) inactivation of the subsequent histamine effect, without changing the kinetics of the VIP-induced stimulation in the system. Under these conditions, the potency of AH 22216 increased from 6.6 to 0.7 x 10^{-6} M. This inactivation was not observed with cimetidine. The data indicate that AH 22216 is an irreversible and specific inhibitor of the gastric histamine H_2 receptor.

It is now well accepted that cAMP is involved as a second messenger in the mechanisms of gastric acid secretion stimulated by histamine (1). These biochemical and physiological effects of histamine could be blocked by a variety of histamine H_2 receptors antagonists such as cimetidine and ranitidine (2-4) used clinically during the treatment of gastric ulcer in man (5-7). The compound AH 22216 (a triazole derivative) is a new H_2 antihistamine which differs in structure and pharmacological properties from cimetidine (an imidazole derivative) and ranitidine (bearing a furane ring) (8). In the conscious Heidenhain pouch dog, AH 22216 was 20-30 times and 5 times more potent than cimetidine and ranitidine, respectively, and had also a very persistent antisecretory action *in vivo* (8).

Our current report concerns the direct and initial interaction of AH 22216, *in vitro*, on the gastric H_2 receptor. We therefore measure cAMP production after histamine or AH 22216 added simultaneously or separately on HGT-1 cells. This cell line originated from a human gastric cancer localized in fundus (9), possesses histamine H_2 receptors (9-11) having pharmacological properties remarkably similar to those we

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have established in human fundic glands (2). As proposed (10, 11), these cultured cells represent a suitable model to study the interaction of H_2 blockers on histamine receptors in human gastric cells. The specificity of the AH 22216 action on gastric receptors is determined in regard to the effect of the drug on the receptor-cAMP system highly sensitive to VIP in HGT-1 cells (11) that we have previously characterized in human (12) and guinea pig (13-15) gastric glands. Results were compared to those obtained with the classical antihistamine cimetidine.

EXPERIMENTAL

Chemicals and drugs. The compound AH 22216 was a gift from Dr R.T. Brittain (Glaxo Group Research Ltd, Ware, Hertforshire, England) and cimetidine was kindly donated by Dr R. Brimblecombe (Smith, Kline and French Laboratories, Ltd, Welwyn Garden City, Hertforshire, England). Purified natural porcine VIP (lot 79.4.25) was a generous gift from Pr V. Mutt (GHI Laboratory, Stockholm, Sweden). All other chemicals used were of the highest purity available.

HGT-1 cells. The HGT-1 cell line (9), generously provided by Dr C. Laboisse (INSERM U.239, Faculté de Médecine Xavier Bichat, Paris, France) was routinely cultured in DME (Dulbecco's modified Eagle medium, Gibco) supplemented with 10% heat inactivated foetal bovine serum (Grand Island Biological Co). Adherent cells between passages 66 and 93 were removed from culture flasks by 2 min incubation at 37°C with 0.02% EDTA. Then, the cells were washed (3 times, 200 x g, 3 min) in Krebs ringer phosphate buffer (pH 7.4). Cell viability (trypan blue exclusion test) was about 90-95% and additions of histamine (10^{-4} M) alone or in combination with AH 22216 (10^{-5} M) or cimetidine (10^{-5} M) did not change this percentage during 30 min incubation (vide infra).

Cyclic AMP assay. In a standard assay, 150 µl from the HGT-1 cells were preincubated at 20°C in absence of in presence (50 µl) of the H_2 antagonists AH 22216 and cimetidine in 250 µl of Krebs ringer phosphate buffer (pH 7.5) containing 1% BSA and 2 mM IBMX. In order to eliminate the compound AH 22216 preincubated 10 min with HGT-1 cells, we washed the cell pellets with 40 ml of KRP and serial (4 times) centrifugations (200xg, 3 min). Control cells (preincubated with KRP) were treated under similar conditions. The reaction was initiated by the addition of appropriate hormones or drugs (100 or 50 µl). The reaction was stopped at the time indicated by adding 50 µl 11 N $HClO_4$ and cAMP was determined (11) by the radioimmunoassay method already described (16). None of the agents used in the present study interfered with the assay of cAMP.

Processing of the data and statistical analyses. Data are given as pmoles of cAMP produced per 10^6 cells. Each value is expressed as the mean ± standard error of the mean. Regression lines were fitted to the linear portions of the concentration-response curves from individual experiments and the apparent EC_{50} and IC_{50} values, i.e., the dose required to produce, respectively, 50% of the maximal stimulation or inhibition produced by the effectors, were calculated by the method of the least squares. Antagonism by different concentrations of AH 22216 or cimetidine against a fixed concentration of histamine (S) was analyzed according to the following equations (18): $Ki = IC_{50}/(1 + S/EC_{50})$ and $Ki = I/(K'a/Ka)-1$, where Ka and K'a are the concentrations of histamine required to produce half-maximal cAMP stimulation in absence and presence of AH 22216, respectively, and I is the concentration of antagonist. Histamine inhibition by AH 22216 was analyzed by Schild plot (17) in which antagonism is expressed by the dose ratios (DR) of histamine needed to produce half-maximal responses in absence and presence of different concentrations of AH 22216: $\log (DR - 1) = n \log (\text{antagonist}) - \log K_b$.

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. Student's t-test was used to test the significance.

RESULTS

As shown in Figure 1, basal cAMP levels in HGT-1 cells incubated at 20°C averaged 2 $\pm$ 0.1 pmol/10^6 cells during the 30 min incubation period considered. Half-maximal cAMP stimulation by 10^{-4} M histamine was observed at 3 min and maximal effect (about 15 times basal levels) was obtained between 5 and 30 min. When AH 22216 (10^{-5} M) was added simultaneously with histamine, the rises in cAMP were reduced by 38% at 5 min, 43% at 10 min, 53% at 15 min and 74% at 30 min. At 60 min, basal cAMP levels were only increased 2-fold (85% inhibition) by histamine (data not shown). In the presence of cimetidine (10^{-5} M), cAMP production caused by histamine became maximal and constant within 3 and 30 min and was reduced by 68% between 10 and 30 min.

Cyclic AMP response to a fixed concentration of histamine (10^{-4} M) was completely inhibited by the H_2 antagonists AH 22216 and cimetidine in increasing concentrations from 10^{-7} to 10^{-4} M (Fig.2). Approximately 50% of the response was inhibited with an IC_{50} of 6.6 x 10^{-6} M AH 22216 (Ki = 0.6 x 10^{-6} M) or 4 x 10^{-6} M cimetidine (Ki = 0.36 x 10^{-6} M). Comparable Ki values (0.3 - 0.4 x 10^{-6} M cimetidine) were measured for cimetidine on H_2 receptors in other gastric or non-gastric tissues in man or laboratory animals (2-4, 13, 19-22).

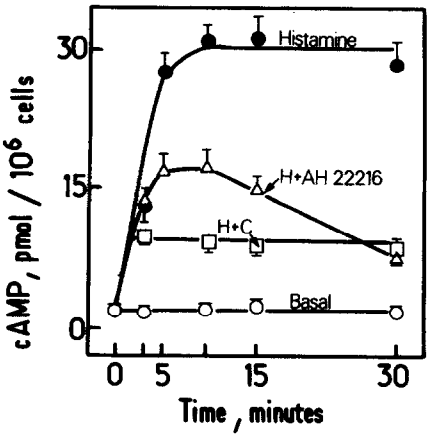

Figure 1: Effect of time and AH 22216 or cimetidine on histamine-induced cAMP production in HGT-1 cells. Histamine 10^{-4} M and AH 22216 10^{-5} M (Δ) or cimetidine 10^{-5} M ($\square$) were added simultaneously. Basal levels in HGT-1 cells ($\circ$). Results are mean $\pm$ SEM from three (cimetidine) to seven (AH 22216) experiments performed in duplicate.

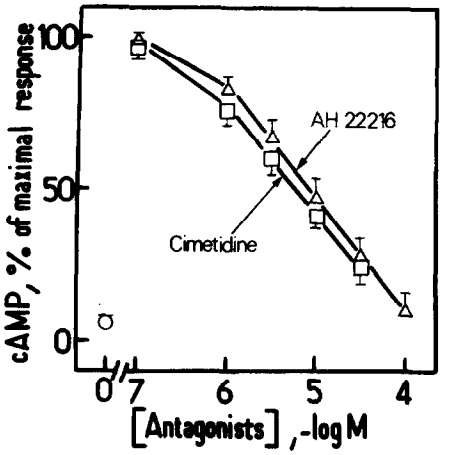

Figure 2: Effect of different concentrations of AH 22216 and cimetidine on cAMP production evoked by histamine in HGT-1 cells. Cells were incubated for 10 min after addition of a fixed concentration of histamine (10^{-4} M) in combinations with AH 22216 (△) or cimetidine (□). Results were expressed as the percentage of cAMP production elicited by histamine at 10^{-4} M. All values represent mean ± SEM of 5-6 experiments performed in duplicate.

The dose-response curves for histamine (potency = 10^{-5} M histamine) exhibited a parallel shift to the right following treatment with three different concentrations of AH 22216 (Fig.3, left), without change of

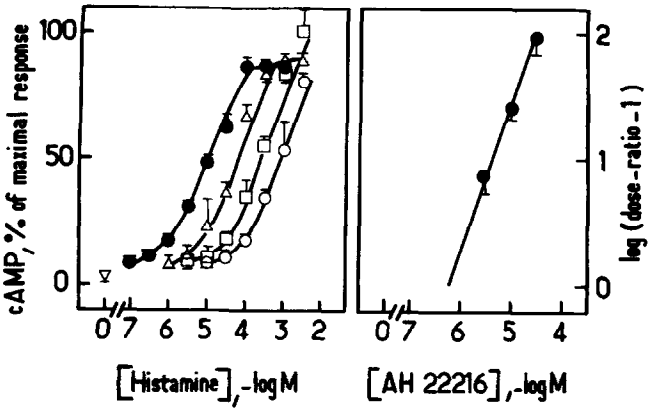

Figure 3: Inhibition by the H_2 antagonist AH 22216 of cAMP production induced by various concentrations of histamine in HGT-1 cells.
Left: Cells were incubated for 10 min in the presence of histamine alone (●) or in combinations with AH 22216 at the concentrations indicated below. Data are mean ± SEM of triplicates from a single experiment. Comparable cAMP changes were observed in three other experiments. The Ki values were obtained for AH 22216 in the presence 3×10^{-6} M (△ , Ki = 0.6×10^{-6} M), 10^{-5} M (□ , Ki= 0.42×10^{-6} M), 3×10^{-5} M AH 22216 (○ , Ki = 0.35×10^{-6} M).
Right: Schild representation of inhibition by AH 22216 of histamine-stimulated cAMP production by HGT-1 cells. Cyclic AMP values were obtained with different concentrations of histamine in the absence and in the presence of AH 22216 at the concentrations indicated. Dose ratios were estimated graphically from the parallel displacement of straight portions of four concentrations responses curves similar to data in Fig.3, left.

the maximal response to histamine. The mean Ki value ($0.45 \pm 0.06 \times 10^{-6}$ M) was calculated for AH 22216 from the values obtained in Fig. 3, left. The data from three other similar experiments were plotted in Fig.3, right as log (DR - 1) against - (log concentration of AH 22216). The dose ratio was obtained from 6-9 points dose-response curves. The Schild plot was linear for AH 22216 ($p < 0.001$), with a regression coefficient $r = 0.978$. AH 22216 did not fulfil the criteria of competitive antagonism since the slope of the Schild plot was significantly different from unity ($n = 1.4 \pm 0.031$, $p < 0.001$). The affinity of the antagonist, expressed as pA_2 value was estimated to be 6.22 for AH 22216 ($Ki = 0.6 \times 10^{-6}$ M). This inhibition constant obtained for AH 22216 is in agreement with the mean Ki values determined from the experimental design of Fig. 2 and 3, left.

When AH 22216 (10^{-5} M) was preincubated during 10 min with HGT-1 cells (Fig. 4, left), basal cAMP levels became unchanged during the subsequent 0-60 min incubation period with histamine (10^{-4} M). The inactivation of the cAMP generating system by AH 22216 is specific of the histamine H_2 receptors since the drug does not modify the time course of VIP-induced cAMP production in HGT-1 cells. Elimination of AH 22216 (10^{-5} M) from the medium by washing HGT-1 cells does not restore the sensitivity of the H_2 receptor to histamine (data not shown). Such inactivation is also peculiar to AH 22216 since the time course of inhibition of histamine-stimulated cAMP production in HGT-1 cells was unchanged when cimetidine was added

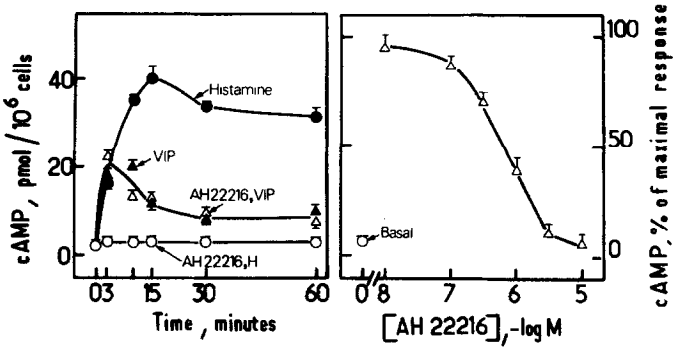

Figure 4: Effect of AH 22216 preincubated 10 min with HGT-1 cells on the subsequent effect of histamine or VIP on cAMP production in the system. Left: Histamine (10^{-4} M:●,○) or VIP (10^{-7} M:▲, △) were added after a 10 min exposition of HGT-1 cells without (KRP buffer:●, ▲) or with 10^{-5} M AH 22216 (○, △). Data are mean ± SEM of a representative experiment performed in triplicate. Two other experiments gave similar results. Right: Effect of various concentrations of AH 22216 (△) preincubated 10 min with HGT-1 cells on the subsequent effect of histamine (10^{-4} M) incubated 10 min with HGT-1 cells. Basal levels in HGT-1 cells(○). Results are expressed as the percentage of cAMP production evoked by histamine at 10^{-4} M. Data are mean ± SEM from three experiments performed in duplicate or triplicate.

simultaneously with histamine (Fig. 1) or preincubated 10 min with HGT-1 cells, before histamine addition (Emami, S. and Gespach, C. manuscript in preparation).

Preincubation of various concentrations (10^{-8} - 10^{-5} M) of AH 22216 during 10 min (or 30 min, data not shown) increased the inhibitory potency (IC_{50} = 7 x 10^{-7} M AH 22216) of the drug on histamine-stimulated cAMP production in HGT-1 cells (Fig.4, right).

DISCUSSION

These studies indicate that the compound AH 22216 belongs to a new class of H_2-receptor blocking drugs, according to its irreversible and specific inactivation of the histamine H_2-receptors in the human gastric cancer cell line HGT-1. The irreversible blockade by AH 22216 could be evidenced: 1) only 10 min after the simultaneous addition of the drug with histamine since the efficacy of the inhibitor increased with time until 60 min; 2) when AH 22216 is preincubated with HGT-1 cells before histamine addition. In this case, the inactivation is rapid (10 min exposition), persistent (during at least 60 min), specific for H_2-receptor (no interaction on the receptor-cAMP system highly sensitive to VIP) and is observed even after the elimination of the H_2 blocker from the medium by the washout period. The irreversibility of the interaction of AH 22216 on the gastric histamine H_2-receptor is consistent with our finding that the drug did not fulfil the criteria for competitive antagonism, as shown by the Schild plot in Fig.3. All these characteristics are peculiar to AH 22216 since cimetidine is a reversible and competitive antagonist of the histamine action in gastric mucosa (3-5, 19) as well as in HGT-1 cells (Emami, S. and C. Gespach, manuscript in preparation). It is likely that AH 22216 does not stimulate the cAMP-dependent phosphodiesterase (cAMP-PDE) in HGT-1 cells (11) since experiments were done in the presence of IBMX as a potent cAMP-PDE inhibitor and because preincubations with AH 22216 did not affect the VIP-stimulated cAMP production in the system. The physico-chemical nature of the interaction of AH 22216 with the histamine H_2-receptor remains thus to be elucidated. This matter is currently under investigation in our laboratory.

The pharmacological properties of AH 22216 and cimetidine in HGT-1 cells are in agreement with the time course of the inhibitory action of oral doses of the antagonists in inhibiting histamine-induced gastric acid secretion in the conscious Heidenhain pouch dog (8). Indeed, AH 22216 had a slower and a more prolonged action than cimetidine *in vivo* (8). Therefore, the long acting effect of AH 22216 observed *in vivo* could be entirely explained by its direct and apparent irreversible interaction on the gastric histamine H_2-receptor and does not result on the disposal

of the orally administered drug. This drug could be thus administered with benefit in patients with gastric ulcer, especially during the resting phase of the parietal cells (23-25) before the endogenous secretion of histamine (25). This compound can be also used *in vivo* or *in vitro* as a ligand for binding studies, cellular localization, turn-over (degradation-regeneration) and isolation of histamine H_2-receptor.

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