Desensitization by histamine of H_2 receptor-mediated adenylate cyclase activation in the human gastric cancer cell line HGT-1

Annie Prost+, Shahin Emami and Christian Gespach*

INSERM U.55, Hôpital Saint-Antoine, 184, rue du Fg. St. Antoine, 75571 Paris Cedex 12 and +*Service central d'hématologie (laboratoire d'Immunologie) Hôpital Tenon, 4, rue de la Chine, 75970 Paris Cedex 20, France*

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Short-term treatment of cultured HGT-1 cells with histamine produced a time-dependent (half-life: 20 min) and homologous desensitization of histamine H_2 receptor activity mediating cAMP generation in HGT-1 cells and gastric acid secretion in normal gastric mucosa. Histamine treatment resulted in loss of response of the adenylate cyclase to histamine in purified plasma membranes, but had no effect on basal, vasoactive intestinal peptide (VIP)- or NaF-stimulated enzyme activities. We propose that the desensitization of gastric histamine H_2 receptor by histamine evidenced in cellular or subcellular preparations from HGT-1 cells could be involved in the physiological regulation and pharmacological control of gastric cell function in man.

Desensitization *Histamine H_2 receptor* *Adenylate cyclase* *Acid secretion* *HGT-1 cell*

1. INTRODUCTION

Desensitization (the loss of drug or hormone response after exposure of cells to those agents) has been documented for a variety of ligands including glucagon, insulin, TSH, LH, FSH, prostaglandins, opiates, catecholamines, gastrin and carbachol. No data are yet available for such a mechanism after histamine interaction on tissues bearing H_2 receptors. The human gastric cancer cell line HGT-1 [1], which possesses peptide hormone and histamine H_2 receptors similar to those evidenced in gastric glands isolated from man or laboratory animals [2–8], is therefore a suitable model for this study.

We describe here results from experiments which indicate that the agonist-induced desensitization of the gastric histamine H_2 receptor mediating cAMP generation in HGT-1 cells (and acid secretion in normal gastric epithelia) is related to homologous modification of the histamine-sensitive adenylate cyclase in purified plasma membranes. This work has been presented in abstract form [9].

* To whom correspondence and reprint requests should be addressed

2. MATERIALS AND METHODS

2.1. *Chemicals*

Highly purified natural porcine vasoactive intestinal peptide (VIP) was purchased from Professor V. Mutt (GIH Laboratory, Stockholm). Cyclic AMP, NaF, 3-isobutyl-1-methylxanthine (IBMX), dithiothreitol, phenylmethylsulfonyl fluoride (PMSF), histamine dihydrochloride, GTP and pure ATP were from Sigma (St. Louis, MO). Creatine phosphate (A grade) and phosphocreatine kinase were from Calbiochem and Boehringer, respectively. $Na^{125}I$ was from the Radiochemical Centre (Amersham).

2.2. *HGT-1 cell line*

The HGT-1 cells, generously provided by Dr C. Laboisse (INSERM U.239, Faculté de Médecine Xavier Bichat, Paris) were routinely cultured in

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Dulbecco's modified Eagle medium (DMEM), as in [1]. HGT-1 cells between passages 89 and 95 were washed (3 times in DMEM) and incubated at 37°C for 20, 60 or 180 min in culture flasks with 10^{-3} M histamine. Control and histamine-treated HGT-1 cells were washed (3 times at 37°C) with phosphate-buffered saline (calcium-free) and removed from culture flasks after addition of 0.02% EDTA (37°C, 1 min). Isolated HGT-1 cells were then washed (3 times at 20°C) and centrifuged at 200 × *g* for 3 min in 40 ml Krebs-Ringer phosphate buffer (KRP, pH 7.4). Cell viability was about 90–95% in control or histamine-treated HGT-1 cells, before and after the incubations for cAMP determinations described below.

2.3. *Cellular cAMP generation*

In a standard assay, 150 μl from the HGT-1 cell suspension (1–2 × 10^6 cells/ml) were preincubated at 20°C for 10 min in 250 μl KRP buffer containing 1% bovine serum albumin (BSA) and 2 mM IBMX. The reaction was initiated by addition of histamine at various concentrations (100 μl) and stopped after 15 min incubation by adding 50 μl of 11 N $HClO_4$. Cyclic AMP was determined by the radioimmunoassay method [5]. Absolute values are expressed as pmol cAMP produced per 10^6 HGT-1 cells.

2.4. *Membrane-bound adenylate cyclase preparation and assay*

Control or histamine-treated HGT-1 cells were resuspended (20 × 10^6 cells per ml) in 10 mM Tris–HCl buffer (pH 7.5) containing 1 mM EDTA, 30 mM NaCl, 1 mM dithiothreitol, 5 μM PMSF, and disrupted with a Polytron homogenizer (Kinematica, Luzern, Switzerland) using 3 bursts of 5 s. The homogenate was layered over 10 ml of a 41% solution of sucrose in the homogenization buffer and centrifuged (95000 × *g*, 1 h, 4°C) in a Beckman SW27 swinging bucket rotor [10]. The white interfacial band of membranes was collected and diluted 4 times in homogenization buffer. The membranes were collected by centrifugation at 4°C (40000 × *g*, 30 min) in a Sorvall centrifuge RC-5B (Newton, CT). The membranes were then stored frozen at −80°C for 1 week. Adenylate cyclase activity was measured by a modification of the method in [11]. The standard incubation mixture (final volume, 250 μl) contained 1 mM ATP, 5 mM $MgCl_2$, an 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, 200 μM GTP and test substances in 25 mM Tris–HCl (pH 7.5). Reaction was initiated by addition of the enzyme (10 μg membrane protein per tube) and the mixture was incubated at 30°C for the indicated times. Data are expressed as nmol cAMP produced per μg membrane protein.

3. RESULTS

Fig.1 shows that short-term treatment of cultured HGT-1 cells with histamine produced a progressive decline in the subsequent efficacy of histamine on H_2 receptor activity. After a 3 h preincubation with the hormone (10^{-3} M), there was a remarkable reduction of the cAMP response to a second challenge with histamine (10^{-7}–10^{-3} M). After only 20 min, the response was reduced to 50% of the control response. Basal levels of cAMP were not affected by exposure of cultured HGT-1 cells with histamine. As illustrated in fig.2, the histamine-induced decreases in hormonal responses observed in the experiment shown in fig.1 were associated with the desensitization of adenylate cyclase to histamine. NaF (10–17-fold) and histamine (4–6-fold) increased adenylate cyclase activity in purified plasma membranes (fig.2,left) as in crude plasma membranes prepared from HGT-1 cells [3]. Reaction rates were linear

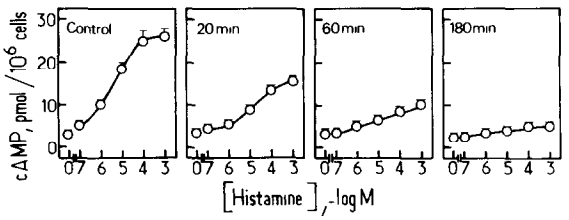

Fig.1. Time course of desensitization of histamine H_2 receptor activity in cultured HGT-1 cells. HGT-1 cells were preincubated at 37°C with 10^{-3} M histamine in culture flasks for the indicated time, then washed, isolated and incubated for 15 min in the absence (basal cAMP production) or presence of different histamine concentrations before the assay of cAMP. Data are means ± SE from 3 separate experiments performed in duplicate or triplicate.

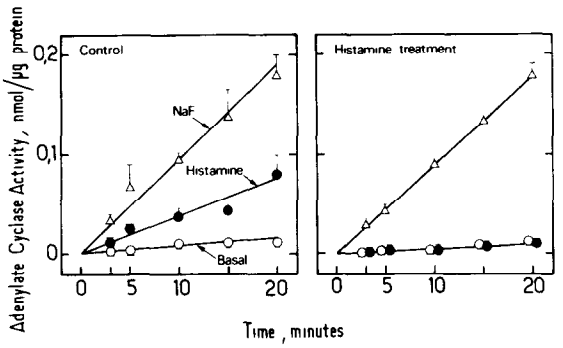

Fig.2. Effect of culturing HGT-1 cells with histamine on the kinetics of histamine- and NaF-stimulated adenylate cyclase activity in purified plasma membranes. Control or histamine-treated HGT-1 cells (10^{-3} M histamine, 3 h at 37°C in culture flasks, as in fig.1) were compared for their adenylate cyclase activities in the absence (basal activity, ○) or presence of NaF (10^{-2} M, △) or histamine (10^{-3} M, ●). We have verified that adenylate cyclase activities under basal or stimulated conditions were linear with protein concentrations (3–15 µg membrane protein/tube). Purified plasma membranes (about 10 µg membrane protein/tube) were incubated at 30°C for the indicated time. Data are means ± SE from 4 (control HGT-1 cells) to 6 experiments (histamine-treated cells) performed in duplicate or triplicate.

with time (0–20 min) in the absence or presence of 10^{-3} M histamine or 10^{-2} M NaF. In HGT-1 cells cultured for 3 h at 37°C in the presence of 10^{-3} M histamine, adenylate cyclase was insensitive to histamine during the period considered (fig.2,right). In contrast, the ubiquitous activator of the enzyme (NaF) was still able to produce a comparable increase in adenylate cyclase activity, measured in control HGT-1 cells. Similarly, the VIP-induced adenylate cyclase activation remained unchanged after histamine treatment (not shown).

4. DISCUSSION

Our experiments conducted on the human gastric cancer cell line HGT-1 are the first example of the homologous desensitization of the gastric histamine H_2 receptor.

Decreased histamine H_2 receptor response in human granulocytes [12] has been reported in vivo, in asthmatic patients [13], and desensitization by histamine of the histamine H_1 receptor-mediated glycogen hydrolysis has been characterized in vitro, in rat brain slices [14].

Pretreatment of HGT-1 cells with histamine produced a rapid (half-life, 20 min) and remarkable suppression of H_2 receptor activity (cellular cAMP generation and adenylate cyclase activation) in cultured HGT-1 cells. This desensitization process is observed in intact HGT-1 cells after serial washings or after membrane-bound adenylate cyclase preparation. It is of interest to note that the membrane desensitization does not seem to alter the regulatory feature of the adenylyl cyclase system, i.e., the fluoride stimulation of the enzyme, known to exert a direct effect on the N component. Similarly, we demonstrated that short-term or chronic treatment of HGT-1 cells with histamine [15] or with the H_2 receptor antagonist SKF 93479 [15,16] resulted in the homologous disappearance of histamine H_2 receptor activity, since receptor activation by peptide hormones (VIP, pancreatic glucagon, gastric inhibitory peptide) remained unchanged in the system. However, in this case, the loss of the cAMP response to histamine in HGT-1 cells was due to a slow onset of dissociation of SKF 93479 from the H_2 receptor, and was not observed after the classic H_2 antihistamine cimetidine [15–17].

Desensitization may be produced by a number of different mechanisms, including uncoupling of the receptor to the adenylate cyclase or biological responses, a decrease in the concentration of receptors (internalization, receptor degradation), or progressive alteration of adenylate cyclase or phosphodiesterase activity. Further characterization of the desensitization of the gastric H_2 receptor in normal and cancerous gastric cells will be presented elsewhere (in preparation).

Occupancy of the H_2 receptor by histamine results in a cascade of biochemical events, including adenylate cyclase and cAMP-dependent protein kinase activation [18], leading to gastric acid secretion (review [19]). In the stomach, histamine-containing cells occur in the lamina propria, in close proximity to the acid-secreting parietal cells [20]. It is therefore conceivable that histamine H_2 receptors are exposed to histamine released by histaminocytes in vivo or in vitro during gastric cell preparation or separation, producing alteration (desensitization) in H_2 receptor activity. The homologous desensitization of H_2

receptor-mediated adenylyl cyclase activity by histamine presented here could therefore be involved in the physiological regulation and pharmacological control of gastric cell function in man.

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