VIP and histamine H_2 receptor activity in human fetal gastric glands

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Summary. Vasoactive intestinal peptide (VIP, $EC_{50} = 6.4 \times 10^{-10}$ M) and histamine ($EC_{50} = 3 \times 10^{-6}$ M) activated the cyclic AMP generating system in gastric glands isolated from two human fetuses at 23 weeks gestation. Histamine antagonism by the H_2 receptor blockers cimetidine ($Ki = 0.35 \times 10^{-6}$ M) and ranitidine ($ki = 0.51 \times 10^{-7}$ M) clearly characterized the histaminic activation as being of the H_2 type. It is suggested that these two vasoactive hormones may operate as neurocrine/paracrine regulators of the differentiation and/or function of the human gastric mucosa in utero.

Key words. VIP and histamine H_2 receptors; cimetidine; ranitidine; human fetal gastric glands; development; gastric secretion.

We previously characterized VIP and histamine H_2 receptors in cells and glands originating from mature and cancerous gastric mucosa in man[1-4]. The biological action of these two hormones in the stomach probably occurs via a neurocrine/paracrine mechanism. In human gastric mucosa, VIP was detected in nerve fibers surrounding the oxyntic and pyloric glands[5], whereas structures containing histamine were found in mucosal mast cells[6] and endocrine-like APUD cells capable of amine precursor uptake and decarboxylation[7]. As both VIP and histamine are present as local mediators during fetal life in gastrointestinal mucosa[8,9], this study was undertaken to identify and characterize pharmacologically functional VIP and histamine receptors, in gastric glands isolated from two human fetuses at 23 weeks of gestation. For this purpose, we measured cyclic AMP generation in these glands after treatment with VIP and histamine alone or in combinations with the H_2 receptor antagonists cimetidine and ranitidine.

Material and methods. Fetal human stomach was obtained just after legal or therapeutically-induced abortions, with the informed consent of the mothers. Stomachs from two fetuses at 23 weeks of gestational age were dissected out and placed in chilled Krebs Ringer phosphate buffer (KRP, pH 7.4) The tissue was then everted over a glass rod and washed free of luminal content. Next, gastric pouches were incubated for 30 min at 4 °C in a solution containing 2.5 mM EDTA and 0.25 M NaCl (pH 7.5), as previously described for the removal of gastric glands from rat fundus and antrum[10]. Gastric glands were isolated by gently shaking 10 times for 10 s each by hand. After each period of shaking, the stomach was placed in 30 ml of fresh medium. The resulting preparation was centrifuged (200 × g at 4 °C) for 2 min and washed three times at the same speed with 40 ml of the KRP buffer. As shown by phase contrast microscopy, the cell preparation almost exclusively contained gastric epithelial glands. VIP and histamine receptor activity reflected by cyclic AMP generation were measured in these human fetal gastric glands (2–4 µg cell DNA/ml) after their incubation for 30 min at 37 °C with 1 mM 3-isobutyl-1-methylxanthine (IBMX) as a phosphodiesterase inhibitor. The reaction was initiated by adding 100 µl of appropriate hormones or chemicals. It was stopped by adding 50 µl 11 N $HClO_4$, and cyclic AMP was then determined by the radioimmunoassay method already described in detail[11]. Neither VIP nor histamine and its H_2 antagonists interfered with the assay of cyclic AMP. Recovery of the tritiated cyclic AMP added to the cells before extraction was 83%. Absolute values are expressed as picomoles of cyclic AMP produced per µg cell DNA. VIP and histamine receptor activity were analyzed in relation to the efficacy and potency of the hormones on the cyclic AMP generating systems in human fetal gastric glands. The efficacy is expressed by the ratio of stimulated to basal cyclic AMP production (control stimulation). The potency, i.e., the apparent EC_{50} value, is the hormone concentration producing half-maximal stimulation. Antagonism against a fixed concentration of histamine (S) by different concentrations of cimetidine and ranitidine was calculated according to the following equation[12]:

$$Ki = IC_{50}/(1+S/EC_{50})$$

where Ki is the inhibition constant of the antagonist, IC_{50}, the concentration of antagonist required for half-maximal inhibition of the histamine-stimulated increase in cAMP, and S, the concentration of histamine. Cimetidine and ranitidine were generously donated by Dr R. Brimblecombe (Smith, Kline and French Laboratories Ltd., Welwyn Garden City, Herts, England) and Dr R.T. Brittain (Glaxo Group Research Ltd., Welwyn Garden City, Herts, England), respectively. Histamine dihydrochloride, cyclic AMP, IBMX were from Sigma Chemical Company (St. Louis, Mo, USA). Purified natural porcine VIP was purchased from Dr V. Mutt (GHI Laboratory, Stockholm, Sweden). ^3H cyclic AMP (20–30 Ci/mmol and ^{125}INa (IMS 300, 600–800 mCi/mmol) came from the Radiochemical Centre (Amersham, England). All the chemicals were the purest grade available.

Results. Both VIP and histamine considerably affected cell surface receptor activity in human fetal gastric glands, as shown by the increase they each caused in cyclic AMP generation, illustrated in the figure. The left panel of the figure shows that cyclic AMP generation was tripled by VIP concentrations in the 3×10^{-11}–10^{-7} M range, with an EC_{50} value of 7×10^{-10} M VIP. The potency of VIP in human fetal gastric glands compares well with the EC_{50} of 3×10^{-10} M measured in human gastric glands isolated from adult stomach[1]. Histamine significantly raised glandular cyclic AMP levels at a concentration of 3×10^{-7} M (right panel of the figure). This stimulation, which gradually increased in a monophasic manner with increasing histamine concentrations (S/B = 25-fold increase), was half-maximal and maximal at concentrations of 3×10^{-6} M and 10^{-4} M histamine respectively. The H_2 receptor antagonists cimetidine and ranitidine completely abolished the cyclic AMP production induced by 10^{-3} M histamine in human fetal gastric glands. With cimetidine, about 50% of the response was inhibited with an IC_{50} of 1.2×10^{-4} M, giving a Ki value of 0.35×10^{-6} M cimetidine. As regards the furane derivative ranitidine, the effect of this antagonist was about seven times more potent than the conventional H_2 receptor antagonist cimetidine ($IC_{50} = 1.7 \times 10^{-5}$ M, $Ki = 0.51 \times 10^{-7}$ M ranitidine).

Discussion. The present results provide the first demonstration of histamine H_2 receptor activity in human fetal gastric glands and therefore confirm that peptidergic and histaminergic receptors are linked to biological responses in gastrointestinal mucosa during fetal life in man and rat[13-23]. In stomach, we previously characterized specific secretin, glucagon and histamine H_2 receptors in gastric glands isolated from fetal rats at 20 days of gestation[14,17]. In intestine, VIP receptors were found to be functional in epithelial cells isolated from human fetuses at 18–23 weeks gestation[18,20,21], as well as in rat fetuses at 17 days gestation[18,19,21], before morphological differentiation of the intestinal mucosa[24].

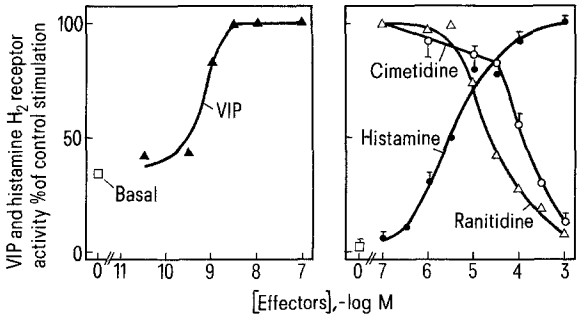
The inhibition curves and constants obtained here with the classical H_2 receptor antagonists cimetidine and ranitidine indicate that the pharmacological specificity of the histamine receptor linked to cyclic AMP generation in human fetal gastric glands is of the H_2 type. We previously showed that the furane derivative ranitidine was 7–9 times more potent than the imidazole deriva-

tive cimetidine in inhibiting histamine H_2 receptor activity in human fundic glands isolated from normal adult stomach[2] as well as in HGT-1 human gastric cancer cells[3]. These two compounds are widely used as antiacids and histamine H_2 receptor blocking drugs in the therapy of the Zollinger-Ellison syndrome and other hypersecretory states[25]. The relative potencies and inhibition constants reported here for cimetidine and ranitidine ($Ki = 0.35 \times 10^{-6}$ M and 0.5×10^{-7} M respectively) are consistent with their antagonism towards typical H_2 receptors expressed in gastric cells[2,3,11,25-28] and other tissues[29-31]. In human and guinea pig gastric cells, VIP and histamine have been shown to interact with two distinct sets of recognition sites, which are activated in two discrete cellular populations. The first evidence was sustained by the higher potency of VIP ($EC_{50} = 6.4 \times 10^{-10}$ M) as compared to the EC_{50} of 3×10^{-6} M histamine measured in the figure and in other reports originating from this laboratory[1,3,11,32]. The relative potencies of these two vasoactive hormones are in line with their secretory characteristics in the vicinity of the gastric cells, since VIP is a neurotransmitter peptide and histamine a paracrine/endocrine bioamine[5-7]. The tissular and cellular localization of hormonal effect revealed that the histamine action was preferentially localized in the acid-secreting fundic part of the stomach, while VIP action was preferentially localized in the antral mucosa, which is devoid of parietal cells[1,4,11,32].

As we proposed earlier, these biochemical regulations by VIP and histamine (i.e adenylate cyclase and cyclic AMP – dependent protein kinase activation) are respectively involved as second messengers in pepsinogen and acid secretion by the stomach[1-4,11,27,28]. Accordingly, VIP and histamine H_2 receptor activations were additive[1,11,32], selectively inhibited by somatostatin[11], and VIP was unable to mimic the ultrastructural transformation induced by histamine during the onset of acid secretion by the parietal cells[33]. Pharmacological analysis of gastric receptor activation by VIP and histamine in normal and cancerous gastric cells in mammals indicated that H_2 receptor desensitization by histamine and its antagonists after short-term or chronic treatment was highly specific since GIP, glucagon and VIP receptor activities remained unaffected[28,34-36]. Similarly, VIP treatment does not modify H_2 receptor activity[37]. Furthermore, we have previously shown that the H_2 receptor antagonists AH 22216, cimetidine and SKF 93479 did not modify the kinetics of VIP-induced cyclic AMP generation after their addition simultaneously with the peptide[38,39].

The presence of VIP and histamine H_2 receptors in human gastric glands during fetal life and their persistence during the malignant transformation of the human gastric mucosa[3] raise the possibility that these two vasoactive agents might play a role in both the normal and tumoral development of the stomach. Similarly, we demonstrated the presence of typical histamine H_2 receptors in the human monocytic and promyelocytic precursors U-937 and HL-60 leukemic cells[30,31] and suggested a possible role for histamine in the proliferation and differentiation of human myeloid progenitor cells[30]. In agreement with this concept, Kalinyak et al. recently showed[40] that the histamine H_2 receptor agonist dimaprit induces differentiation of HL-60 cells into more mature myeloid cells, as revealed by morphology, NBT reduction test and lysozyme enzyme production.

The results presented here reinforce the view[17,18,21] that cell surface receptors mobilized during various types of regulation of digestive functions in the mature gastrointestinal tract might be implicated in the complex biochemical processes of proliferation and differentiation of the gastric and intestinal mucosa during fetal life in man and rat.



Effect of VIP, histamine and its H_2 receptor antagonists cimetidine and ranitidine on cyclic AMP generation in gastric glands isolated from two human fetuses at 23 weeks gestational age. Results are expressed as the percentage of control stimulation, i.e. the maximal cyclic AMP generation evoked by VIP (left) and histamine (right). Data are means for one experiment performed in duplicate for VIP (▲) or ranitidine (△), and the means ± half range for two experiments performed in duplicate for histamine (●) and cimetidine (○). No addition (□).

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Reduced tuberoinfundibular dopaminergic neuronal function in rats after long-term withdrawal of estrogen treatment[1]

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Summary. Hypothalamic fragments from female rats treated repeatedly with estradiol valerate (EV) and bearing prolactin (PRL)-secreting tumors contained, seven months after the last EV injection, lower concentrations of dopamine (DA) than age-matched controls. Depolarizing concentrations of K^+ (35 mM) and amphetamine (50 μM) evoked in PRL-secreting tumor bearing rats an endogenous DA release significantly lower than in controls.
Key words. Tuberoinfundibular dopaminergic system; prolactin; estrogen-treated rats; prolactin secreting adenoma.

Considerable evidence suggests that hypothalamic infundibular dopaminergic (TIDA) neurons are involved in the control of prolactin (PRL) secretion from the anterior pituitary gland. Dopamine released from the medial palisade zone reaches the anterior pituitary, via portal vessels, activates dopaminergic receptors on mammotrophs and inhibits PRL secretion[1]. An increased PRL secretion, in turn, exerts a positive feedback on DA terminals in the median eminence by increasing dopamine synthesis[2-4] and release[5,6] from TIDA nerve endings. Although dopamine is not the sole prolactin-inhibiting factor, it is probably the most physiologically important[7]. Among the factors that influence the activity of TIDA neurons, estrogens exert a prominent role. Short-term (3–5 days) estrogen treatment increases the activity of TIDA neurons, as revealed by increases in the rate of synthesis[8] and turnover[9,10] of dopamine in the median eminence and in the concentration of dopamine in hypophyseal portal blood[11]. Long-term treatment with estradiol (many weeks), on the other hand, results in a decrease in the concentration and synthesis of dopamine in the median eminence[12,13]. We have shown in previous work[14] that multiple injection of 2 mg estradiol valerate at 3-week intervals into mature cycling rats is accompanied by a striking rise in plasma PRL concentration, development of pituitary-secreting tumors (prolactinoma), a progressive decrease in median eminence dopamine concentration and unresponsiveness of PRL secretion to nomifensine, a drug which promotes release of dopamine and inhibits its reuptake from nerve terminals[15]. The neurochemical mechanisms by which long term estrogen treatment cause these alterations are unclear. The changes may represent an irreversible decrease in the number of TIDA neurons, in view of the cytopathological changes present in the arcuate nucleus of long-term estradiol treated rats[14,16-18] or, alternatively, may be related to a transient decrease in the activity and function of TIDA neurons.

In the present study, we investigated the activity of TIDA neurons of long-term estradiol valerate treated rats bearing pituitary prolactinomas, seven months after discontinuation of estrogen treatment. As a direct index of TIDA function, we evaluated in vitro the release of endogenous dopamine from arcuate-periventricular nucleus-median eminence complexes.

Experimental procedure. Female Sprague-Dawley rats (Nossan Corezzana Inc., Milano, Italy) weighing 160–200 g were housed (5 per cage) in an air conditioned (24 °C) and light controlled (light on 06.00–20.00 h) room with free access to chow and water. Normal vaginal cyclicity (4 days) was established for at least two weeks before initiating the experiments. Each rat was then injected with 2 mg s.c. of 17-β-estradiol valerate (Progynon Depot, Schering, Berlin, West Germany), 5 times at 3-week intervals. Seven months after the last injection, estradiol valerate-treated rats and age-matched vehicle-injected controls were decapitated and trunk blood collected for serum PRL determination by a double antibody RIA[19] with reagents provided by the National Pituitary Agency (NIAMDD). Plasma samples were assayed in duplicate: the volume used was 25 μl undiluted or diluted 1:10. PRL values were expressed in terms of NIADDK rat PRL-RP-3 with a biological potency of 30 IU/mg. The intra-and interassay coefficients of variation were 8.7% and 9.8%, respectively. The sensitivity of the assay was 0.75 ng/ml. Arcuate-periventricular nucleus-median eminence fragments were obtained by dissecting with fine scissors under a stereomicroscope a triangular shaped area (1 mm × 1 mm, about 2 mg in weight) according to Cuello et al.[20]. After a 30 min preincubation, pools of three fragments were incubated in flasks containing 1 ml Krebs-Ringer Bicarbonate (KRB) buffer (118 nM NaCl, 5mM KCl, 1.15 mM $MgSO_4$, 1.15 mM NaH_2PO_4, 2.5 mM $Ca Cl_2$, 25 mM $NaHCO_3$, 11 mM glucose, 10 μM tyrosine and 1

Endogenous dopamine release from TIDA neurons expressed as percent of dopamine total content (dopamine release during the whole experiment plus dopamine content measured in the fragments at the end of the experiment) in control and prolactinoma-bearing rats

	Control rats	Prolactinoma-bearing rats
5 mM K^+ KRB medium	1.07 ± 0.2	0.99 ± 0.2
35 mM K^+ KRB medium	7.96 ± 1.0	6.48 ± 0.6
5 mM K^+ KRB medium + d-amphetamine	3.90 ± 0.6	3. 3 ± 0.6

For experimental details see legend of the figure.