Action of histamine and vasoactive intestinal peptide (VIP) on cyclic AMP in gastric glands isolated from human fetal stomach

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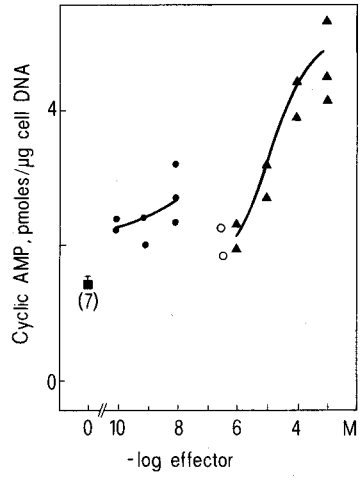
Summary. Histamine and VIP produce an elevation of cAMP production in gastric glands isolated from the human fetal stomach at 15 weeks of gestation. These effects were attributed to the activation of 2 distinct receptor-cAMP systems, one being sensitive to histamine in parietal cells, and the other being sensitive to VIP in muco-peptic cell populations. The results suggest that histamine and VIP may play a role in inducing gastric secretion during fetal life in man.

The identification and the morphological development of the parietal, zymogen and mucoid cells has been studied by electron microscope in man[1], rat[2,3], hamster[4] and rabbit[5] fetuses. In man, most investigators considered that both parietal and mucoid cells differentiate from the gastric epithelial cells in 12 weeks of gestation[1,6]. We have recently demonstrated that histamine and vasoactive intestinal peptide (VIP) stimulate the production of cyclic AMP in gastric epithelial glands isolated from the human fundus and antrum[7]. The effects we have observed with histamine and VIP or secretin are similar to the dual effects of these agents on rat[8] or canine[9] gastric mucosa. Thus, histamine exerts its effects on parietal cells[9,10] through an H_2-receptor cyclic AMP system[11,12] implicated in gastric acid secretion[13] while VIP or secretin act selectively on pepsinogen and/or mucous-secreting cells[7-9]. We have therefore examined the interaction of histamine and VIP on gastric glands isolated from the human fetal stomach to determine whether histamine- and VIP-receptors could be present and functional during the intrauterine life in man. The results presented here are preliminary data obtained in a human fetus at 15 weeks of gestation.

Materials and methods. The stomach was removed from a human fetus at 15 weeks of gestational age obtained just after legal abortion. The stomach was incised along the greater curvature and the luminal contents were washed out with tap water. Gastric glands were isolated and harvested from the whole mucosa of the stomach using EDTA[14], as described for the isolation of human colonic epithelial glands[15]. The preparation was centrifuged at $200 \times g$ for 2 min followed by 3 washings at the same speed with Krebs-Ringer phosphate, pH 7.5. As shown by phase contrast microscopy, the cell preparation contained gastric epithelial glands; indeed, in the human fetus of 12-14 weeks of gestational age, the gastric epithelium contains gastric glands consisting of parietal, mucoid and undifferentiated cells facing a glandular lumen[1]. Cyclic AMP production was measured in gastric glands (2 µg DNA/ml) incubated for 30 min at 37 °C in 0.5 ml Krebs-Ringer phosphate containing 1.4% bovine serum albumin and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) as phosphodiesterase inhibitor[7]. The reaction was started by the addition of 100 µl of the agents after 10 min preincubation of the other reaction constituents. The incubation was stopped by the addition of 50 µl 11 N $HClO_4$. Cyclic AMP was extracted and determined using a radioimmunoassay technique[16]. DNA was measured in the homogenate of gastric glands as in Kissane et al.[17]. Data were expressed in pmoles cyclic AMP/µg cell DNA.

Results and discussion. In the presence of 0.5 mM IBMX, the basal level of cyclic AMP in gastric glands isolated from the fetal human stomach was 1.42 ± 0.162 pmol cyclic AMP/µg DNA at 37 °C ($n = 7$). This value compared well with adult levels found in human gastric mucosa[18] and in preparations of human gastric glands[7]. As shown in the figure, both VIP and histamine increased basal cyclic AMP levels in human fetal gastric glands. At concentrations ranging from 10^{-10} M to 10^{-8} M, VIP produced a dose-dependant stimulation of cyclic AMP production. VIP (10^{-8} M) increased the basal cyclic AMP level 1.9-fold ($p < 0.01$) while secretin at high doses (3×10^{-7} M) produced a slight effect. This was consistent with our previous studies showing that secretin was 10^4 times less potent than VIP in stimulating cyclic AMP formation in human gastric epithelium[7]. On the other hand, the concentration-response relationship of histamine stimulation was in the range of 10^{-6}-10^{-3} M. The maximal stimulation was achieved with 10^{-4}-10^{-3} M histamine and represented a 3.3-fold increase above the control ($p < 0.001$). The approximate ED_{50} was observed with 10^{-5} M histamine. The potency and the efficacy of histamine in human fetal gastric glands were comparable to the action of histamine on cellular and subcellular preparations from the adult human stomach. Indeed, histamine 10^{-6}-10^{-3} M produced a 4.5-fold stimulation of cyclic AMP production in fundic epithelial glands[7] and half-maximal effects were observed with 5×10^{-5} M histamine both on mucosal adenylate cyclase[19,20] and in epithelial glands[7].

The stimulation of cyclic AMP production by histamine and VIP clearly indicates the apparent activation of 2 functional receptor-cyclic AMP systems in gastric glands isolated from the human fetus at 15 weeks of gestation. This observation raises the possibility of biological actions by histamine or VIP on acid- and non-acid-secreting cells[7] during intrauterine life in man. Indeed, the secretion of acid could be detected during fetal development in man[6,21], rat[22] or rabbit[23] and was stimulated by histamine and pentagastrin in the cannulated ovine fetus[24]. Furthermore,



Effect of various concentrations of VIP (●), secretin (○) and histamine (▲) on basal cyclic AMP production (■) in gastric glands isolated from the human fetal stomach. Individual determinations are represented for the effect of different agents.

the acid secretory mechanisms are apparently operative in the late embryonic stages in man since premature babies secrete acid during the first 6 h of life[25].

In conclusion, the occurrence of the histamine- and VIP-sensitive cyclic AMP systems in a human fetus at 15 weeks of age is temporally related to the respective appearence of parietal and mucoid cells in fetuses at 12 weeks of gestation[1]. Thus, these 2 cyclic AMP systems would seem to be appropriate tools for studying the early differentiation and the functional development of the gastric mucosa in man.

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Evaporative resistance of pulmonary surfactant films[1]

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Summary. Films of surfactant from the lungs of rabbits, tortoises *(Testudo hermanni)* and frogs *(Rana pipiens)* offer resistance to the evaporation of water.

Pulmonary surfactant forms thin films at the air-fluid interfaces within the distal respiratory passages of vertebrate lungs and it is thought to be involved in the maintenance of a stable pulmonary architecture[2-4]. Surfactant films contribute to the bulk of the air-blood barrier and obviously offer some resistance to the diffusion of respiratory gases, water and anaesthetic agents. Direct measurement of the film resistance is rendered difficult by the inaccessibility of pulmonary alveoli. Consequently, as a first step towards quantifying this resistance, it was decided to determine what effect surfactant films have on the rate of evaporation from aqueous surfaces in vitro.

Methods. Surfactant was extracted from the lungs of 12 rabbits, 15 tortoises *(Testudo hermanni)* and 15 frogs *(Rana pipiens)* by saline lavage[5]. The lavage fluid obtained from each animal was cooled to 4 °C and centrifuged at 800 × g for 10 min. The supernatant was then adjusted to a density of 1.10 with sodium chloride and recentrifuged at 100,000 × g for 1 h. The surfactant was recovered in visible bands at the top of the centrifuge tubes. The protein content of a sample of each extract was determined using the method of Lowry et al.[6].

The evaporative resistance of surfactant films was measured using an evaporator of the type described by Archer and La Mer[7]. Essentially, the method determines the mass of water vapour absorbed by a desiccant (anhydrous lithium chloride) positioned just above an aqueous surface which bears a thin film. Buffered saline (0.145 M NaCl in 0.01 M Na_2HPO_4/NaH_2PO_4 buffer, pH 7.4) was used as the aqueous subphase and a measured quantity of surfactant extract was added to its surface using an Agla microsyringe. The quantity added was sufficient to form a complete surface film; an interval of 10 min was allowed for spreading. A correction was made by a method previously described[7] to compensate for the mass of water vapour absorbed from the surrounding air.

Results. Interfacial films of rabbit surfactant offered significant resistance to evaporation (see table). At 37 °C, for example, the presence of a surface film caused a 5% decrease in the overall mass of water lost by evaporation. Increasing the subphase temperature stepwise over the range 35–41 °C resulted in a modest decrease in the value of the evaporative resistance. In contrast, the evaporative

Evaporative resistances of surfactant films at different temperatures

	Temperature (°C)	Evaporative resistance (sec $cm^{-1} \times 10^2$)*	Overall decrease in water loss due to presence of film (%)
Rabbit surfactant	25	87.9 ± 4.6	7.7
	35	42.1 ± 2.4	5.4
	37	37.2 ± 2.2	5.1
	39	13.9 ± 0.8	3.5
	41	5.1 ± 0.5	1.4
Tortoise surfactant	20	187.5 ± 9.3	16.3
	25	137.8 ± 8.2	12.0
	30	51.5 ± 3.6	8.4
Frog surfactant	15	276.5 ± 19.4	24.1
	20	188.3 ± 15.0	16.5
	25	144.3 ± 7.0	12.6

* Mean ± SEM of measurements on 8 films. All films had a surface coverage of 8 $cm^2/\mu g$ surfactant protein.