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Effects of gastric secretagogues on tissue glycerol in the isolated amphibian gastric mucosa[1]

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Summary. Histamine and theophylline, two gastric secretagogues, significantly increased tissue glycerol content by 121 and 66%, respectively, in the isolated toad gastric mucosa. This is new evidence in favor of the hypothesis that gastric segretagogues may act via lipid mobilization.
Key words. Gastric metabolism; acid secretion; histamine; theophylline; glycerol.

The process of acid secretion by the gastric mucosa is highly dependent on oxidative metabolism. Stimulation of H^+ secretion by gastric secretagogues, such as histamine and theophylline, is associated with an increase in oxygen consumption and substrate oxidation[3-9]. Some lines of evidence in the amphibian gastric mucosa have led to the hypothesis that gastric secretagogues may act via substrate mobilization and that the substrate mobilized is primarily lipid[10-13]. In the present work, we add further evidence in favor of the above hypothesis. If gastric secretagogues mobilize lipids (triglycerides), releasing fatty acids, an increase in the tissue concentration of free glycerol could be demonstrated. Therefore, the effects of gastric stimulants on mucosal glycerol concentration were investigated in the toad gastric mucosa in vitro.

Material and methods. Experiments were performed on gastric mucosa from fasted Venezuelan toads *(Bufo marinus)*. After the toad had been pithed, the stomach was removed and the muscularis layer was stripped from the gastric mucosa and discarded. Paired slices of gastric mucosa, previously weighed, were placed in screw capped 25-ml Erlenmeyer flasks with 5 ml of a Ringer solution containing 17 mM N-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) at pH 7.4 as a buffer and the testing compounds (10 mM theophylline or 0.1 mM histamine). After gassing with 100% O_2 for 10 min, the flasks were sealed and incubated for 2 h at 30 °C in a shaking water bath. At the end of incubation, the tissues were quickly frozen in liquid nitrogen and immediately pulverized with a stainless steel percussion mortar previously cooled in dry ice. Subsequently, tissue glycerol was extracted by perchloric acid and determined enzymatically with glycerokinase and α-glycerophosphate dehydrogenase, according to the method of Wieland[14]. The amount of reduced nicotinamide adenine dinucleotide (NADH) formed was measured in a Beckman spectrophotometer at 366 nm. Recovery of glycerol in tissue samples was above 95%. The statistical significance of the differences was calculated using Student's t-test.

Results and discussion. The effects of 10 mM theophylline and 0.1 mM histamine on tissue glycerol concentration are shown in the table. These doses of stimulants are known to produce maximal respiratory and secretory responses in the amphibian gastric mucosa in vitro. It can be observed that both gastric secretagogues significantly increased the tissue glycerol concentration above control values, the effect being more pronounced in the case of histamine. The percentile increments were 66 and 121% for theophylline and histamine, respectively.

The present results represent a new evidence in favor of the hypothesis that gastric secretagogues may act via substrate mobilization and that the substrate mobilized is primarily lipid in the isolated amphibian gastric mucosa. This is consistent with a number of previous observations[7,8,10-13]. Alonso et al.[10] have demonstrated a significant decrease in the triglyceride content of the mucosa in association with acid secretion. Assuming that the main sources of free glycerol in the tissue are

Effects of theophylline and histamine on glycerol concentration in the toad gastric mucosa

Condition (n)	Tissue glycerol (μmoles/g wet wt)	%Δ
Control (8)	0.32 ± 0.04	+ 66%*
10 mM theophylline (8)	0.53 ± 0.05	
Control (8)	0.38 ± 0.07	
0.1 mM histamine (8)	0.84 ± 0.09	+ 121%*

Experiments were performed as described in methods. %Δ is the change expressed as percentage of the control values. Values are means ± SE. Numbers in parentheses indicate the number of experiments. * $p < 0.05$ vs control.

triglycerides, the oxidation of the released fatty acids may account for an important fraction of the increase in respiration induced by gastric secretagogues. We[8] have shown in a previous study that the stimulatory effects of gastric secretagogues on respiration and acid secretion can be blocked by selective inhibition of fatty acid oxidation. Exogenous fatty acids significantly stimulate respiration and acid secretion, whereas glucose, pyruvate and lactate have little or no effect[10]. In addition, tracer studies have indicated a metabolic preference for fatty acid oxidation in the amphibian gastric mucosa[7,8]. The mechanism of lipid mobilization is not known at present. One possibility is that secretagogues, via cAMP, might activate a lipase in the gastric mucosa. Activation of a lipase by cAMP has been demonstrated in adipose tissue[15]. This point must await further investigation to be elucidated.

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Demonstration of retinoic acid isomers in human urine under physiological conditions

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Summary. Untransformed retinoic acid has never been demonstrated in human excreta under normal physiological conditions. We have developed a two-step liquid chromatographic system for the demonstration of subnanogram amounts of this compound in human urine without administration of any precursor.
Key words. Retinoic acid metabolites; human urine; high performance liquid chromatography; retinoic acid isomers; UV detection.

The metabolism and excretion of vitamin A has been the subject of numerous investigations. Retinol is primarily metabolized to a number of compounds with an intact side chain. Some of these metabolites were identified as retinoic acid[3], epoxyretinoic acid[4], 4-hydroxy- and 4-ketoretinoic acid[5-7], retinoyl-beta-glucuronides[8,9] and some cyclisized derivatives[10]. Other minor metabolites lack either the terminal carbon or carbons 14 and 15[11,12]. However, in all these experiments large and nonphysiological amounts of retinoic acid itself, retinyl acetate or other structurally related compounds had been administered. Hence the possibility remains that these metabolites do not occur under normal physiological conditions[13]. Retinoic acid is formed by oxidation of the terminal alcoholic group of retinol. The concentration of vitamin A acid in human serum is in the low nanogram range[14,15]. In view of the high biological activity of this compound[16,17], the aim of this study was to look for the presence of retinoic acid in human urine under normal physiological conditions. Due to its nondestructive character and to its high separating capacity, high performance liquid chromatography is the method of choice for this purpose. We describe the isolation and purification of retinoic acid from crude urine extracts.

Materials and methods. All-trans-retinoic acid was purchased from Fluka AG (Buchs, Switzerland). 9-Cis- and 13-cis-retinoic acid were supplied by Hoffmann-La Roche Inc., Nutley (NJ, USA). Reagents and solvents were of analytical grade and were purchased from Merck AG (Darmstadt, West Germany). Isomerization and/or photodegradation of the retinoic acid was avoided by working in a darkened room under yellow light. Whenever possible, low actinic (amberized) glassware was used. Extraction and storage of the organic layer before evaporation were carried out at 4 °C.

Urine samples were from healthy fasting adults (male or female) and the samples were analyzed directly after collection. A pre-extraction followed by a double-phase extraction at different pH values was applied to 200 ml of human urine. At an alkaline pH (10 ml of 2N NaOH), 200 ml of urine together with 100 ml of ethanol were extracted with 400 ml of n-hexane at 4 °C. The n-hexane layer was discarded and a second extraction with another 400 ml n-hexane was performed in acidic medium (15 ml of 2N HCl). The organic phase was concentrated on a Büchler Evapo-Mix (Büchler Instruments Inc., Fort Lee, NJ, USA) or alternatively, under a stream of nitrogen. The residue of an extract of 200 ml of urine was dissolved in 2.2 ml of the chromatographic solvent and two 1-ml aliquots were injected in the chromatographic system. Each sample was centrifuged prior to HPLC analysis to avoid the injection of particulate matter.

The analytical high performance liquid chromatographic system consisted of a Pye Unicam LC_3-XP (Cambridge, England) pump, a sampling valve (Model CV-6-UHPa-N60, Valco Instruments Co., Houston, TX, USA) with a 50-µl loop and a Pye Unicam LC_3-UV variable wavelength detector set at 350 nm and used at the maximum sensitivity (0.005 AUFS).

The analytical column (15 × 0.32 cm) was packed in our laboratory with silica RSIL 5 µm (RSL; St. Martens-Latem, Belgium). Elution was performed with a mixture of n-hexane:acetonitrile:acetic acid (99.5:0.2:0.3, v/v) at a flow rate of 0.75 ml/min.

Extracts of a higher sample volume (200 ml) were subjected to a clean-up step on a semipreparative reversed-phase liquid chromatographic column. A RSIL C18 10 µm column (50 × 1 cm ID) (Alltech Europe, Eke, Belgium) was eluted with methanol:water:acetic acid (89.7:10:0.3, v/v) at a flow rate of 4.5