flammation to the uveal tract. The results suggest that corneal lysosomes may participate in the pathogenesis of herpetic keratitis.

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In vitro ACTIVATION OF RAT GASTRIC MUCOSAL ADENYLATE CYCLASE BY TETRAGASTRIN

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Gastric ulcer is accompanied by hypersecretion of hydrochloric acid and increased adenylate cyclase (AC) activity [3]. Histamine is the principal pharmacological stimulus increasing hydrochloric acid secretion by the stomach [1, 5, 6, 10, 12]. According to the model suggested in [6] histamine is the essential stage in the stimulation of hydrochloric acid production by the stomach. An intermediate link in this chain is 3'5'-AMP. However, some workers were unable to find AC in the gastric mucosa of rats sensitive to histamine [6, 13].

C-terminal tetra- and pentapeptides of gastrin are known to stimulate hydrochloric acid secretion by the stomach in dogs [14]. The presence of gastrin receptors also has been shown in the parietal cells of the stomach [11] and AC has been found in cells of the gastric mucosa sensitive to pentagastrin [4, 9].

The object of this investigation was to test the action of tetragastrin and histamine on AC activity in the rat gastric mucosa and to study the effect of cimetidine (an antagonist of histamine H_2-receptors) on these effects.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 160-180 g. The total number of animals used was 46. Homogenate from the gastric mucosa (100 mg tissue to 1 ml 50 mM Tris-HCl buffer, pH 7.6) was prepared manually in the cold in a glass Potter's homogenizer with Teflon pestle (20 strokes). AC activity of the homogenate from gastric mucosa was determined by ion-exchange chromatography [2]. The main reaction mixture (total volume 60 µl) contained 50 mM Tris-HCl buffer, pH 7.6, 15 mM $MgCl_2$, 45 mM creatine phosphate, 1 mg/ml creatine phosphokinase, 3.0 mM 3',5'-AMP, 0.6 mM ^{3}H-ATP (specific activity 0.1 Ci/mmole), 50-150 µg protein. The biologically active substances to be tested (histamine, tetragastrin, cimetidine) were added to the reaction mixture immediately before the beginning of the reaction. The reaction was started by addition of protein to the reaction mixture and allowed to continue for 10 min at 32°C. The reaction was stopped by diluting the reaction mixture with 0.4 ml of a solution of unlabeled ATP (10 mM) and ^{14}C-3',5'-AMP (10,000 cpm), pH 6.0.

The ^{3}H-3',5'-AMP formed as the result of the reaction catalyzed by adenylate cyclase was separated from other labeled products and ATP by consecutive chromatography of the mixture on columns with Dowex 1 × 8, 100-200 mesh, Al_2O_3, and Dowex 1 × 8, 200-400 mesh. Addition of a known quantity of ^{14}C-3',5'-AMP to the reaction mixture enabled the actual yield of ^{3}H-3',5'-AMP to be determined after chromatography of each sample. Radioactivity was counted on an SL-40 liquid scintillation spectrometer (Intertechnique, France), using the program for double labeling with quenching. The yield of 3',5'-AMP after all stages of chromatography averaged 60-70%.

KEY WORDS: adenylate cyclase; gastric secretion; histamine; tetragastrin; cimetidine.

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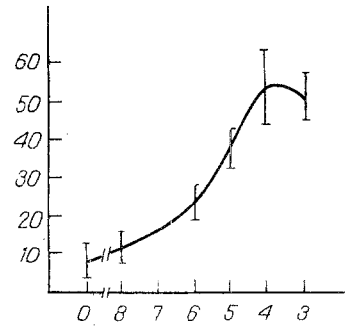

Fig. 1

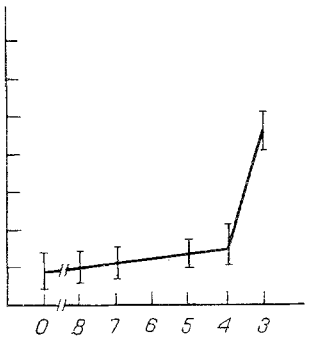

Fig. 2

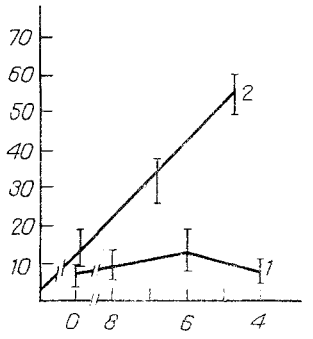

Fig. 3

Fig. 1. Responses of AC from rat gastric mucosal homogenate to different concentrations of histamine. Abscissa, $-\log_{10}$ of molar concentration of histamine; ordinate, AC activity of rat gastric mucosal homogenate (in pmoles 3',5'-AMP/mg protein/min). Each point is arithmetic mean of results for 12 animals.

Fig. 2. Responses of AC of rat gastric mucosal homogenate to different concentrations of tetragastrin. Abscissa, $-\log_{10}$ of molar concentration of tetragastrin. Remainder of legend as to Fig. 1.

Fig. 3. Effect of different concentrations of cimetidine on AC activity of rat gastric mucosal homogenate, stimulated by histamine (10^{-4} M) (1) and by tetragastrin (10^{-3} M) (2). Abscissa, $-\log_{10}$ of molar concentration of cimetidine; ordinate, AC activity of rat gastric mucosal homogenate (in pM 3',5'-AMP/mg protein/min). Each point is arithmetic mean of results for 14 animals.

The background in the control samples, to which protein was added after the reaction had stopped, did not exceed 100 cpm (^{3}H), within the limits of error of counting.

The protein concentration was determined by Lowry's method [8], with bovine serum albumin as the standard.

The following reagents were used: Phosphocreatine-Na and creatine phosphokinase (from Sigma, USA); histamine (from Fluka); tetragastrin N(t)-amyloxycarbonyl-l-tryptophyl-l-methionyl-l-aspartyl-l-phenylalanine-amide (from Sigma, USA); cimetidine; 8-^{3}H-ATP (from Izotop, USSR, or Radiochemical Centre, Amersham, England); ^{14}C-3',5'-AMP (from Radiochemical Centre).

EXPERIMENTAL RESULTS

Activity of AC in homogenate from rat gastric mucosa was found to be a linear function of incubation time (from 5 to 30 min) and of protein concentration (from 50 to 350 μg). Basal AC activity of the homogenate from rat gastric mucosa was 8.3 ± 0.8 picomoles/mg protein/min.

In the *in vitro* system histamine stimulated AC activity of the rat gastric mocusal homogenate. Responses of AC to the action of different concentrations (10^{-8}-10^{-3} M) of histamine are shown in Fig. 1. Maximal AC activity was obtained with histamine in a concentration of 10^{-4} M. Activation of AC by 50% of maximal was observed with histamine in a concentration of 10^{-6} M.

A study of the action of different concentrations (10^{-8}-10^{-3} M) of tetragastrin on AC activity of the rat gastric mucosal homogenate (Fig. 2) revealed a stimulating effect in a concentration of 10^{-3} M ($P < 0.01$).

The action of cimetidine (an antagonist of histamine H_2-receptors) in the *in vitro* system on stimulation produced by histamine (10^{-4} M) and tetragastrin (10^{-3} M) was tested. As Fig. 3 shows, cimetidine in concentrations of 10^{-8}, 10^{-6}, and 10^{-4} M inhibited the stimulating action of histamine (10^{-4} M) on AC activity of the rat gastric mucosa. By contrast to this, in the presence of the same concentrations of cimetidine, the action of tetragastrin (10^{-3} M) on the activity of this enzyme was potentiated.

These experiments thus revealed the presence of AC, sensitive to both histamine and tetragastrin, in cells of the rat gastric mucosa. Proof was obtained of the direct action of tetragastrin, not mediated through histamine, on AC of the gastric mucosa. Cimetidine, an antagonist of histamine H_2-receptors, inhibited the stimulating effect of histamine on AC but did not interfere with binding of tetragastrin with the receptors. Conversely, H_2-receptor block-

ade by cimetidine potentiated stimulation of AC activity of rat gastric mucosal cells by tetragastrin. Potentiation of the stimulating action of tetragastrin by cimetidine was not an experimental error, because cimetidine did not affect basal AC activity.

Histamine and tetragastrin probably act through different receptors to stimulate AC of rat gastric mucosal cells.

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COMPARATIVE STUDY OF INFRARED SPECTRA OF GLYCOSAMINOGLYCANS AND THEIR MONOMERS

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During the identification of certain absorption bands in the infrared (IR) spectra of glycosaminoglycans difficulties often arise because the oscillation frequencies of the different groups and bonds of these components of proteoglycans may overlap to some extent [1, 5-10, 12].

In the investigation described below, in an attempt to overcome these difficulties the IR spectra of hyaluronic acid (HUA), protein-chondroitin-keratan sulfate (PCKS), proteoglycan aggregates (PA), and monomers from which glycosaminoglycans of the proteoglycans most widely distributed in animals are composed [2], were compared.

EXPERIMENTAL METHODS

Glucuronic acid and its potassium salt, glucosamine, galactosamine (hydrochlorides of both), N-acetylglucosamine, N-acetylgalactosamine, and the ammonium salt of N-acetylneuraminic acid used in the experiments were from Serva, West Germany. Mixtures of monomers for spectroscopic investigations were composed of equivalent quantities of each of them. Potassium salts of HUA (from human umbilical cords), PCKS, and PA (from the bovine trachea) were isolated by methods described previously [1, 3, 4].

IR spectra were obtained by the use of dry preparations mixed with KBr in the ratio of 1:300 and pressed into tablets 13 mm in diameter under a pressure of 10 t. Spectra were recorded on a Perkin-Elmer model 577 spectrophotometer in the 4000-200 cm^{-1} region at room tem-

KEY WORDS: infrared spectra; glycosaminoglycans; glucuronic acid; N-acetylhexosamines; N-acetylneuraminic acid.

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