DISCOVERY OF AN HOURLY RHYTHM OF PROTEIN SYNTHESIS IN HUMAN GASTRIC BIOPSY MATERIAL

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Rhythms with a period of about 1 h are found when many functions of cells and organs are studied, including protein synthesis and secretion and activity of various enzymes [1]. Hourly rhythms are widespread in the digestive organs. Synthesis of salivary proteins [5], secretion of saliva [7], peristalsis of the stomach and intestine [8], activity of the principal digestive enzymes [9], total protein synthesis in the pancreas [2], and the secretion of bile [3] are all manifestations of digestive functions in mammals that have a period which varies around 20-70 min.

The aim of this investigation was to determine changes in the rate of protein synthesis in slices of human stomach incubated in culture medium.

EXPERIMENTAL METHOD

Biopsy specimens from the stomach of patients with duodenal ulcer in different stages of the disease (exacerbation and remission) were studied. Among the patients studied was one case with no observable pathology of the gastric and duodenal mucosa, and another with signs of chronic gastroduodenitis.

Biopsy material was taken from the lower third of the lesser curvature above the angle of the stomach. The investigation lasted from September 23, 1982 until January 31, 1983. Two gastric biopsy specimens each about 4 mm^2 in area, taken from one patient, were cut into 5-10 slices, which were arranged perpendicularly to the surface of the mucosa. Before incubation in medium with isotope the slices were incubated for 25-30 min at 37°C in nonradioactive medium 199 with the addition of bovine serum. The results of preliminary series of experiments on some mammalian glandular tissues showed that preliminary incubation for 25-30 min is essential for stabilization of some parameters of tissue protein metabolism. These same experiments showed no decrease in the average rate of protein synthesis during short-term incubation of the tissues for 100-120 min [4].

The dynamics of protein synthesis in slices of the human stomach was investigated for 30-90 min. The sections were transferred in succession, one after the other at 10-min intervals, to culture medium containing [^{3}H]leucine or [^{3}H]lysine in a concentration of 74 × 10^7 Bq/ml and incubated at 37°C for 10 min. The slices were then washed with cold (4°C) medium with an excess of one of the unlabeled amino acids used and treated with cold (4°C) $HClO_4$ for 1.5 h. The supernatant in a volume of 0.5 ml was immersed in Bray's scintillator. The residue was hydrolyzed with 5% $HClO_4$ at 90°C for 20 min, washed with 96% ethanol, dissolved in hyamine, and transferred to toluene scintillator. Radioactivity of the acid-soluble and protein fractions was measured on the SL-30 scintillation counter.

The rate of protein synthesis was determined by calculating the ratio of effective incorporation of amino acid into proteins to the amino acid pool. The total of the values of radioactivity of protein and the acid-soluble fraction, characterizing the total amino acid pool in the slice (or permeability of the slice for amino acids) was taken as unity for the first time point. Depending on the values of the total pool for the other time points studied a correction was introduced into the corresponding values of protein radioactivity [6].

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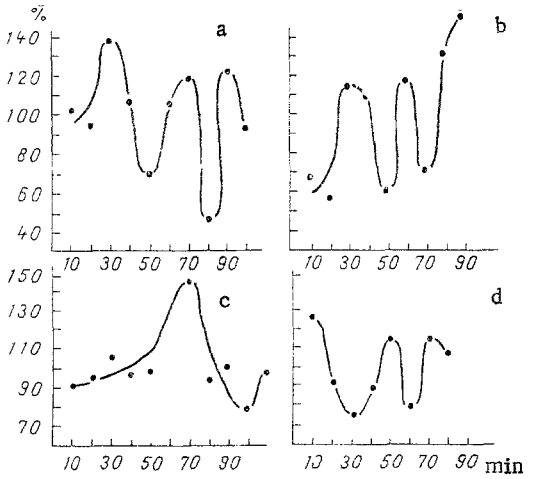

Fig. 1. Examples of fluctuations of rate of protein synthesis in human gastric biopsy material. Abscissa, time after taking samples (in min); ordinate, rate of protein synthesis (in % of mean level). a, b, c, d) Results of measurements of parameters in gastric biopsy specimens from one patient.

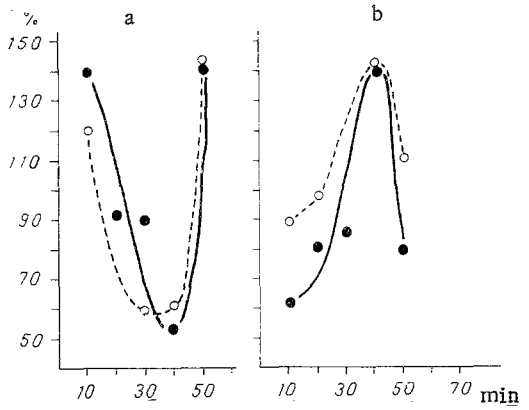

Fig. 2. Variation of fluctuations in rate of protein synthesis in slices during simultaneous investigation of two biopsy specimens from the same patient. Abscissa, time after taking samples (in min); ordinate, rate of protein synthesis (in % of mean level). Each two curves (continuous and broken lines) give result of measurement of parameters in biopsy specimens from the same patient.

In each case the dynamics of protein synthesis was studied in slices obtained from biopsy material from one patient.

EXPERIMENTAL RESULTS

In 7 of the 22 cases the mean rate of incorporation of amino acid into protein (protein synthesis) in the biopsy specimens was high, namely 900-2500 cpm. A level of incorporation of average intensity (450-600 cpm) was observed in five cases and a low level (200-350 cpm) in 10 cases.

In 8 of 22 cases a study of the dynamics of protein synthesis revealed no change in the rate of synthesis, or the changes were indistinct. In most cases (14 of 22) distinct changes in the rate of protein synthesis were found. If the investigations were of short duration (from 30 to 50 min) a marked decrease or increase in the original level of synthesis was observed, followed by a change in the opposite direction. During long investigations fluctuations in the rate of protein synthesis were observed (Fig. 1). The periods varied from 20 to

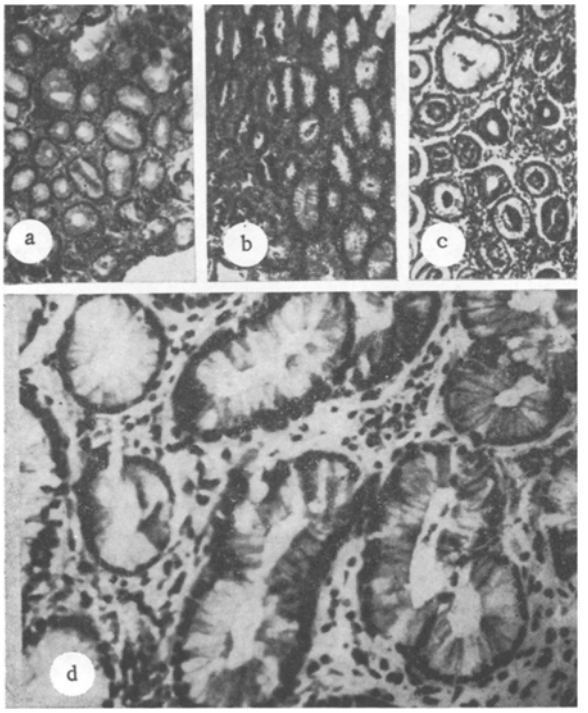

Fig. 3. Histological sections from biopsy specimens of human stomach. a, b, c, d) Different patients, biopsy specimens from whom were used in the experiments. Stained with **Caracci's hematoxylin. Magnification: a, b, c)** 6.3 × , d) 160 ×.

100 min, but most of them were 30-40 min. The amplitude of the waves, which amounted to 20-40% of the mean level, was characteristic of hourly rhythms of protein synthesis studied in various mammalian tissues [1]. A rhythm of the rate of protein synthesis was found in the biopsy specimens with a high level of synthesis (average level of incorporation 1850 **cpm;** Fig. 1c, d) and with a low level (average level of incorporation 340 cpm; Fig. 1a, b). Of 14 patients in whom a rhythm was found, eight were aged between 40 and 56 years and five between 23 and 30 years. Among patients in whom no rhythm was found, fivewere between 41 and 48 years old and one was 22 years old. The level of protein synthesis could be determined both as actual incorporation of amino acid into protein and as the size of the pool and permeability of the cells for amino acid. Both these values varied in the material studied, and in different cases a high value of synthesis was determined either by high incorporation or by a low pool. For each patient, however, changes in the rate of protein synthesis in gastric slices, if they were observed, were detected throughout the period of investigation, either by incorporation of amino acid into protein or only by the pool. Among patients with a high rate of synthesis, five were observed in January and two at the end of September and middle of October. Cases with a low level of synthesis were observed both in January and in October. However, a low level of the free amino acid pool was observed in winter; in one biopsy specimen in December and in seven in January, of the 12 studied in these months, out of a total number of 22 biopsies. In patients with an unhealed, sometimes chronic ulcer, both fluctuations in the rate of protein synthesis (in six cases) and a stable level of synthesis (in two cases) were observed. After cicatrization of the ulcer and when the pattern of the rhythm was relatively normal, fluctuations in the rate of protein synthesis could either be observed (more often) or not.

Variation of the parameters during the study of biopsy specimens from the same stomach was not significant (Fig. 2). Histological investigation showed that the material taken at biopsy consists entirely of the surface layer of the gastric mucosa: the covering epithelium and, chiefly (by weight), the fundal glands (Fig. 3).

The main result of this investigation was the discovery of a rhythm of protein synthesis in the glandular tissue of the stomach. A feature which distinguishes the human stomach from

other glandular tissues which have been studied is the relatively high frequency of discovery of a stable rate of protein synthesis. Whether this difference is due to pathological changes in the stomach tissue will be made clear by future research.

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ACTION OF KETOTIFEN ON MITOGEN-INDUCED PROLIFERATIVE RESPONSE OF HUMAN MONONUCLEAR CELLS

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The present writers [1, 2] and other investigators [8, 10, 12] have shown that one point of application of the action of the antiallergic agent ketotifen, which gives multiple pharmacological effects [7], is the target cells of allergy, namely mast cells and basophils. The stabilizing action of ketotifen on the target cells of allergy is mediated through its membranotropic action [2], possibly by blocking membrane calcium channels [8]. The above remarks suggest that the action of ketotifen may also extend to other cell systems involved in the allergic response and, in particular, to immunocompetent cells, with which certain particular features of the therapeutic action of the drug may be linked. Accordingly the aim of the present investigation was to study the action of ketotifen on the mitogen-induced proliferative response of human peripheral blood lymphocytes.

EXPERIMENTAL METHOD

Mononuclear cells (MNC) were isolated from heparinized blood from clinically healthy persons (six men aged 25-35 years) by centrifugation on a Ficoll-Verografin density gradient (density 1.080 g/cm^3). The isolated MNC were suspended in medium No. 199 containing 10% inactivated embryonic calf serum, HEPES (5 mM), glutamine (20 mM), and monomycin (100 U/ml) and cultured in flat-bottomed 96-well plates (Falcon Plastics, USA). The mitogens used were phytohemagglutinin (PHA; from Difco), concanavalin A (con A; from Sigma, USA), and pokeweed mitogen (PM; Sigma, USA). In the case of preliminary treatment of the MNC with ketotifen (Sandos, Switzerland) the cells (2×10^6) were preincubated in the presence of the drug in 2 ml of medium in glass flasks with bottom area of 4.1 cm^2. The viability of the cells (detected by uptake of trypan blue) cultured in the presence of the concentrations of ketotifen used was indistinguishable from that in the corresponding control. The proliferative response of MNC was estimated by the method described previously [3] based on incorporation of [^{3}H]thymidine, added in a dose of 1 μCi per well 6 h before the end of 72 h of culture. Protein

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