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ROLE OF SEROTONIN IN THE PATHOGENESIS OF ULCER FORMATION

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KEY WORDS: ulcer formation; serotonin; proteolytic enzymes; lysosomal enzymes.

The largest quantity of serotonin in the body is produced in the stomach. However, the question whether this biogenic amine plays the role of a trigger mechanism for gastric secretion or whether its liberation is the result of the initiated process of digestion has not been completely elucidated. Meanwhile it is evident that a disturbance of serotonin synthesis and metabolism can lead to the development of pathological states of the gastrointestinal tract.

The results of investigations to determine serotonin in patients with peptic ulcer have proved fairly contradictory. According to some workers correlation is found between the acid-forming function of the stomach and the serotonin concentration in the gastric juice of such patients [2, 3]. According to other workers [14] serotonin regulates equilibrium between HCl and pepsin and also regulates the mucosal barrier and cell regeneration. There have been few investigations to determine serotonin in the gastric mucosa. In peptic ulcer an increase in the serotonin concentration has been found in the mucous membrane of the stomach and duodenum [6]. However, much remains to be explained regarding the role of serotonin in the genesis of ulcer formation.

The object of this investigation was to study the serotonin concentration in the mucous membrane of the rat stomach at various stages of experimental ulcer formation. At the same time the concentration of histamine and the proteolytic activity and spectrum of proteolytic enzymes were determined in the mucous membrane. Changes in the acid protease level in the gastric mucosa after administration of serotonin and histamine also were determined in intact animals.

EXPERIMENTAL METHOD

Experiments were carried out on 150 albino rats of both sexes, kept on the ordinary laboratory animal diet. The animals were deprived of food for 16-18 h before the investigations but were allowed water *ad lib*. The animals were divided into three groups: 1) intact, 2) control and undergoing laparotomy with application of physiological saline, and 3) experimental rats with application of acetic acid to the serous membrane of the anterior wall of the body of the stomach [12]. Some animals of group 1 were given a known ulcerogenic dose of serotonin (30 mg/kg, intraperitoneally) or histamine (50 mg/kg, intramuscularly). Pieces of mucosa for testing were taken from the floor and margin of the ulcer, and from the corresponding part of the stomach of the control rats.

Proteolytic activity was determined in extracts of gastric mucosa by the method in [8] and the pH-curve of proteolytic activity was plotted [1]. The concentrations of serotonin

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TABLE 1. Serotonin and Histamine Concentrations (in μg/g tissue) in Gastric Mucosa (M ± m)

Substance	Control (n = 16)	Time after application of acetic acid					
		1 min (n=6)	1 h (n=7)	3 h (n=6)	5 days (n=10)	10 days (n=10)	7 months (n=7)
Serotonin	1,7±0,2	4,5±0,4	2,3±0,2	0,95±0,09	4,1±0,3	4,1±0,5	2,5±0,2
P		<0,001	<0,05	<0,005	<0,001	<0,001	<0,02
Histamine	20,0±2,0	31,6±2,8	43,2±3,5	23,0±2,1	19,5±2,0	20,0±1,9	22,0±2,0
P		<0,005	<0,005	<0,4	<0,9	—	<0,5

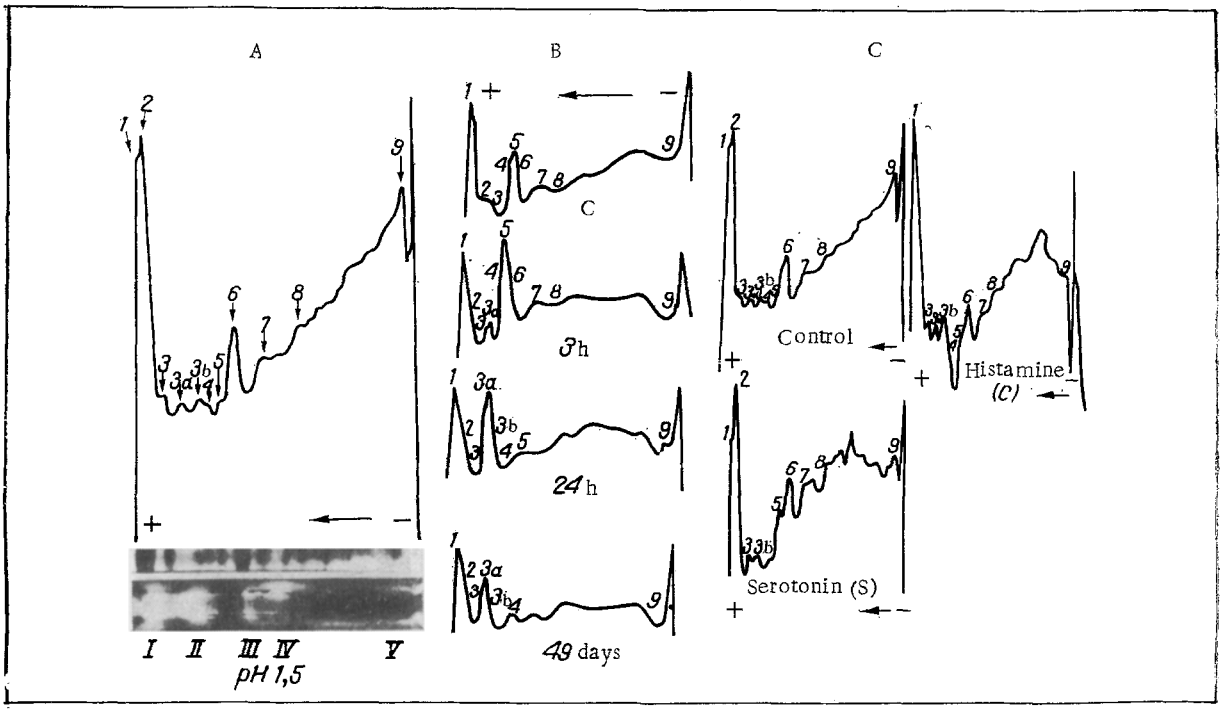

Fig. 1. Densitograms of electrophoretic protein fractions of extracts from rat gastric mucosa. A) Comparison of densitogram and electrophoretic profiles showing protein fractions and zones of proteolysis at pH 1.5; B) densitograms of protein fractions at different stages of "acetate" gastric ulcer (C — control, 3 h, 24 h, and 49 days); C) densitograms of protein fractions after administration of serotonin (30 mg/kg) and histamine (50 mg/kg) to animals.

TABLE 2. Histamine and Serotonin Concentrations (in μg/g tissue) in Gastric Mucosa after Administration of Biologically Active Substances (M ± m)

Substance	Control (n = 16)	Experiment	
		histamine 50 mg/kg (n = 5)	serotonin, 30 mg/kg (n = 5)
Serotonin	20,0±2,0	41,0±3,5	32,0±3,0
P		<0,001	<0,02
Histamine	1,7±0,2	2,1±0,2	2,5±0.3
P		<0,2	<0,05

and histamine were determined by a modified fluorometric method [2, 13]. Proteins in extracts of rat gastric mucosa were separated by disc electrophoresis on polyacrylamide gel [9]. To detect proteolytic enzymes some of the gel columns after electrophoresis were covered with agar containing hemoglobin and incubated at 37°C. The agar disks were then fixed and stained with Amido black 10B. The zones of proteolysis did not stain and were clearly distinguishable against the general blue background. Other gel columns after electrophoresis

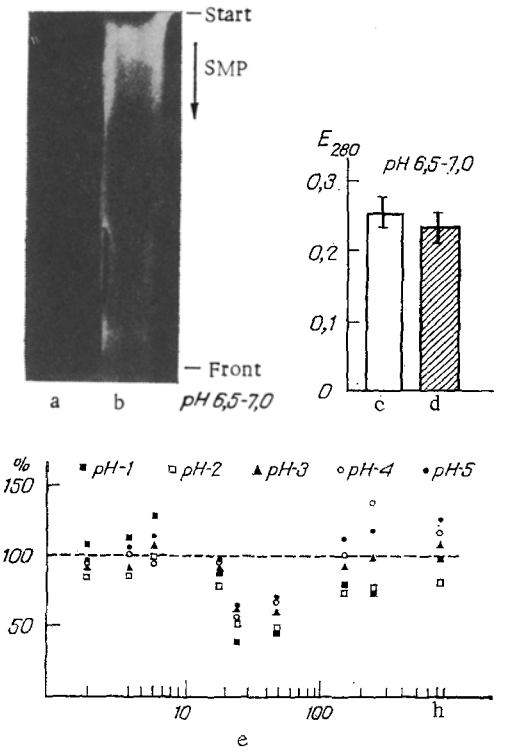

Fig. 2. Proteolytic activity of extracts of gastric mucosa: a, b) electrophoretic profiles after determination of proteolytic activity at pH 6.5-7.0: a) control, b) 1-h stage of acetate ulcer; c, d) proteolytic activity in extracts of gastric mucosa at pH 6.5-7.0; c) 1-h stage of acetate ulcer, d) after administration of serotonin (30 mg/kg); e) proteolytic activity at different stages of acetate ulcer at pH from 1.0 to 5.0. Broken line indicates control level of proteolytic activity in intact rats, conventionally taken as 100%.

were fixed in 10% TCA solution and stained with Coomassie to reveal the protein fractions. The gel columns were subjected to densitometry, after which the protein fractions were compared with the zones of proteolysis. Protein fractions with proteolytic activity were conventionally numbered from the front to the start.

EXPERIMENTAL RESULTS

The process of ulcer formation in the stomach by means of acetic acid was divided into two periods: the first — an acute gastric ulcer (from the 1st minute to the 10th day), the second — a gastric ulcer with signs of becoming chronic (starting from the 10th day for a period of several months).

To rule out any possible effect of the operation on changes in the level of biologically active substances (BAS) in the gastric mucosa a special series of experiments was undertaken. In intact rats the serotonin concentration was 1.7 ± 0.2 μg/g tissue and the histamine concentration 20.0 ± 2.0 μg/g. In rats with laparotomy and application of physiological saline no appreciable changes in the BAS content in the gastric mucosa could be found after 1 min and 1, 3, and 5 days (five rats at each time). The serotonin concentration at the above times was 2.0 ± 0.2, 1.8 ± 0.2, 2.0 ± 0.2, and 1.8 ± 0.2 μg/g, and the histamine concentration 25.0 ± 2.6, 24.5 ± 2.6, 21.6 ± 2.2, and 20.0 ± 2.0 μg/g respectively. Since all changes in rats of the control group compared with intact animals at the above periods were not significant, later intact animals were used as the control for the experimental rats.

A marked increase in the serotonin concentration in the gastric mucosa was observed 1 min after application of acetic acid (Table 1), but later it fell below the control level. By the 5th day after injury the serotonin concentration increased considerably and for a long time. As long as 7 months after application of acetic acid the serotonin level was significantly higher than the control. The histamine concentration was increased 1 min after application (Table 1), but its highest level was observed after 1 h. By 3 h the hista-

amine concentration had fallen to the control level, and later it did not differ significantly from it.

Comparison of the zones of proteolysis and of the protein fractions of the electrophoretic profiles of extracts of gastric mucosa revealed that the proteolytic enzymes were mainly distributed in the zone of fast-migrating proteins (Fig. 1a). The spectrum of proteolytic enzymes of the gastric mucosa was changed with effect from the first hour after application (Fig. 1B). The changes took place mainly in the zone of fast-migrating proteins including fractions 3, 3a, and 3b. The change in structure of the spectrum of proteolytic enzymes continued for a long time. Acid gastric proteases exhibited greater or lesser proteolytic activity at different pH values, depending on the stage of ulcer formation. As will be clear from Fig. 2e, during the first hours proteolytic activity of extracts of gastric mucosa increased at pH 1.0, 3.0, and 5.0, but after 24 h it fell sharply at all pH values. By the 5th day this index had risen to its original level, and at later stages it was found to be higher at pH 4.0-5.0.

In the acute period of ulcer formation proteolytic activity also was observed to appear at pH 6.5-7.0, which was not observed in the control animals. It was seen most clearly 1 h after application of acetic acid, but at subsequent stages it was practically nonexistent. Proteolytic activity at pH 6.5-7.0 was stronger in the zone of "slowly migrating proteases" (SMP), i.e., in the start zone (Fig. 2a, b).

In rats with experimental gastric ulcer the serotonin concentration in the gastric mucosa thus undergoes considerable changes, accompanied by qualitative and quantitative changes in proteolytic enzymes. With these data in mind, the effect of exogenous serotonin and histamine on proteolytic enzymes was investigated in intact rats. Injection of serotonin and histamine caused no significant changes in the pH curve of proteolytic activity of gastric mucosal extracts between pH values of 1.0 and 5.0. Meanwhile the spectrum of proteolytic enzymes was considerably modified, especially in the groups of fractions 3 (Fig. 1C). Injection of serotonin also increased the histamine concentration in the gastric mucosa (Table 2). Even a single injection of serotonin in a dose of 30 mg/kg was sufficient to cause injury to the gastric mucosa, in the form of extensive hemorrhages and erosions, accompanied by a change in the proteolytic enzyme spectrum and the appearance of proteolytic activity at pH 6.5-7.0 (Fig. 2c, d).

During the first minutes of ulcer formation, changes were thus observed in the BAS content in the stomach wall. The changes in the serotonin and histamine levels took place in a clearly defined order and were interconnected, as was observed also in clinical studies by Grokhovskii [4]. These substances evidently play a direct part in the development and maintenance of the pathological process in the stomach.

It is well known that pepsin exhibits its activity only at pH 5.4 or below. At pH 6.5-7.0 or above it is inactivated. However, it has also been shown that in patients with reduced acidity, with a pH of their gastric juice of 5.0 or above, proteolysis still takes place in the stomach through the action of secreted proteolytic enzymes [7]. In the present writers' view, the proteolytic activity observed at pH 6.5-7.0 is exhibited by intracellular cathepsins [5], which appear in the tissues as a result of a disturbance of metabolism of cell membranes. It is also worth noting that the appearance of high proteolytic activity, observed in the present experiments in the SMP zone, is associated by some workers with cathepsins [10, 11].

As a result of the production of an experimental ulcer in the gastric mucosa, the serotonin concentration was thus increased, and this was followed by changes in the histamine level. A change in BAS content evidently disturbs the circulation in the mucosa and leads to the appearance of proteolytic activity at pH 6.5-7.0, which is most probably due to lysosomal enzymes. It is these enzymes which are responsible for proteolysis within the tissues themselves and for ulcer formation. Subsequent stabilization and maintenance of this process are dependent on changes in the spectrum of acid gastric proteases and the increased serotonin concentration in the stomach wall.

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EFFECT OF ALCOHOL ON THE SERUM TSH LEVEL IN RATS PREDISPOSED AND NOT PREDISPOSED TO ALCOHOL

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KEY WORDS: thyroid-stimulating hormone; alcohol; blood serum.

An important aspect of the search for substances for the prevention and treatment of alcoholism is the discovery of the mechanisms of predisposition and nonpredisposition to alcohol. The results of investigations in this direction have shown that alcohol induces aggressiveness in unpredisposed animals and a tranquilizing effect in animals predisposed to alcohol [1], and it activates the positive reinforcement system in predisposed animals but does not change the activity of this system in animals rejecting alcohol [2]. Increased tyrosine hydroxylase activity has been found in the thalamus [3] and an increased serotonin concentration in various brain structures [5] of rats predisposed to taking alcohol.

Evidence has also been obtained of differences in the reactivity of the hypothalamic-hypophyseal neuroscretory system (HHNS) of animals predisposed to and rejecting alcohol [4], and also of changes in the activity of this system depending on the duration and stage of alcohol intoxication [7, 8, 14]. Changes in reactivity of the HHNS may be the cause of the different modulating effects on regulation of production and secretion of thyroid hormones in animals predisposed and not predisposed to taking alcohol.

This paper describes a study of the effect of chronic administration of alcohol on the blood serum levels of thyroid-stimulating hormone (TSH) in rats predisposed and not predisposed to alcohol, kept in a state of physical dependence on and abstinence from alcohol.

EXPERIMENTAL METHOD

Experiments were carried out on 156 female Wistar rats weighing 150-220 g. In the course of the experiment the rats were kept on a standard diet and were allowed water *ad lib*.

In previous investigations negative correlation was found between the length of sleep of the rats following administration of alcohol in a narcotic dose and the quantity of alcohol consumed under conditions of free choice between alcohol and water. Accordingly, in the present investigation the animals were divided into those predisposed and not predisposed to alcohol, by determining the duration of sleep after intraperitoneal injection of a narcotic dose (4.5 ml/kg body weight) of 25% ethanol solution. Rats sleeping a short time (less than 60 min) were described as predisposed, and those sleeping a long time (over 2 h) as not pre-

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