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EFFECT OF ACETAZOLAMIDE ON CARBONIC ANHYDRASE ACTIVITY IN THE BLOOD AND GASTRIC MUCOSA AND ON THE PEPSINOGEN CONTENT IN THE GASTRIC MUCOSA OF RATS

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An important role in the mechanism of hydrochloric acid biosynthesis by the parietal cells of the gastric mucosa is ascribed to carbonic anhydrase [5], activation of which by pentagastrin, histamine, carbachol, theophylline, or cyclic 3,5-AMP increases hydrochloric acid secretion [6, 9-11, 13]. Liberation of HCl is stimulated by electrical stimulation of the gastric mucosa [7] and by protein kinase [12]. Acetazolamide and atropine abolish the activating action of pentagastrin and carbachol on carbonic anhydrase [10].

However, there is nothing in the literature on the subject of dependence of the level of pepsin secretion on carbonic anhydrase activity.

The object of this investigation was to study the effect of acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) on carbonic anhydrase activity in the blood and gastric mucosa and on the pepsinogen content in the gastric mucosa.

EXPERIMENTAL METHOD

Experiments were carried out on 70 male albino rats weighing 120-150 g. Blood for testing was taken from a cervical vein. To obtain a homogenate of the mucosa the stomach was opened, washed with cold distilled water, dried with filter paper, and the mucosa was separated, and a weighed sample of the mucosa was homogenized in water (ratio 1:100). Carbonic anhydrase activity in the blood and homogenate was determined by a micromethod [4]. The pepsinogen concentration in the gastric mucosa was studied after its conversion into the active form — pepsin. For this purpose the homogenate was treated with 0.1 N HCl (ratio 10:1)

TABLE 1. Carbonic Anhydrase Activity in Blood (in units/μl) and Gastric Mucosa (in units/mg tissue) and Pepsin Activity in Gastric Mucosa (in units/g tissue) after a Single Dose of Milk, Histamine, and Acetazolamide in Various Combinations (M ± m)

Group of animals	Stimulus of gastric secretion	Carbonic anhydrase		Pepsin in gastric mucosa
		in blood	in gastric mucosa	
1 (n=10)	Fasting state	67,6±9,42	4,22±0,46	34,9±2,72
2 (n=10)	Milk	88,2±12,58 (+33,4%)	5,02±0,43 (+19%)	42,4±3,25 (+21%)
P_{1-2}		<0,01	<0,01	<0,01
3 (n=10)	Histamine	105,5±8,94 (+55,8%)	6,47±0,61 (+53%)	48,5±3,81 (+39%)
P_{1-3}		<0,01	<0,01	<0,01
4 (n=10)	Acetazolamide + milk	36,1±5,47 (—59%)	3,21±0,45 (—36%)	33,3±1,77 (—21,5%)
P_{2-4}		<0,01	<0,01	<0,01
5 (n=10)	Acetazolamide + histamine	36,6±4,81 (—65%)	3,09±0,50 (—52%)	39,0±2,98 (—19,6%)
P_{3-5}		<0,01	<0,01	<0,01

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TABLE 2. Carbonic Anhydrase Activity in Blood (in units/μl) and Gastric Mucosa (in units/mg tissue) and Pepsin Activity in Gastric Mucosa (in units/g tissue) 1 h after Administration of Food Stimulus to Intact Rats and Rats Receiving Acetazolamide for 5 Days (M ± m)

Parameter	Group of animals		P
	6 (control, n= 10)	7 (experiment, n= 10)	
Carbonic anhydrase in blood	80,9±9,05 —	19,2±2,07 (—76%)	<0 01
Carbonic anhydrase in gastric mucosa	4,01±0,22 —	2,34±0,24 (—42%)	<0 01
Pepsin in gastric mucosa	43,1±0,73 —	30,0±1,42 (31%)	<0 01

and the resulting mixture (pH 1.5) was incubated in a water thermostat at 37°C for 1 h. Pepsin activity was determined by a method based on its proteolytic activity [8].

Enzyme activity in the rats of group I were determined in the fasting state, in the rats of group 2, 1 h after introduction of 1.5 ml milk into the stomach, and in the rats of group 3, 1 h after subcutaneous injection of histamine (200 μg/100 g body weight). The rats of groups 4 and 5 received acetazolamide (3.5 mg of the Soviet preparation diacarb/100 g body weight). Milk (1.5 ml) was introduced into the stomach of the animals of group 4, and animals of group 5 were given a subcutaneous injection of histamine (200 μg/100 g body weight) 2 h later. The animals were killed after another hour and enzyme activity was determined. The rats of group 6 received a standard diet for 5 days, and animals of group 7 additionally were given 4-5 mg acetazolamide on those days. On the 6th day milk (1.5 ml) was introduced into the stomach of all the animals, which were killed 1 h later, after which the enzyme activity was determined. The results were subjected to statistical analysis by the difference method [2].

EXPERIMENTAL RESULTS

Introduction of the food stimulus (milk) into the stomach caused an increase in carbonic anhydrase activity in the blood and gastric mucosa by 30 and 19% respectively (Table 1). Activity of the enzyme increased by 56 and 53% respectively after subcutaneous injection of histamine. This was accompanied by a simultaneous rise in the pepsinogen concentration in the gastric mucosa. For instance, in response to introduction of milk into the stomach the pepsinogen content increased by 21%, whereas after subcutaneous injection of histamine it increased by 39%.

Since correlation was found between carbonic anhydrase activity in the blood and gastric mucosa, on the one hand, and the pepsinogen content in the gastric mucosa on the other hand, it was decided to study the action of acetazolamide on pepsin biosynthesis and activity. Acetazolamide is widely used in medical practice as a diuretic [1, 3] acting indirectly through carbonic anhydrase. As Table 1 shows, after administration of acetazolamide the blood carbonic anhydrase activity fell in response both to introduction of the food stimulus (—59%) and in response to injection of histamine (—65%). Carbonic anhydrase activity in the gastric mucosa fell by 36 and 52% and pepsin activity by 21 and 20% respectively.

Experiments *in vitro* showed that acetazolamide inhibits not only carbonic anhydrase, but also pepsin. Accordingly, the effect of prolonged administration of acetazolamide on the pepsinogen content in the gastric mucosa had to be studied in experiments *in vivo*. Animals were given acetazolamide for 5 days, and on the day of sacrifice they received the food stimulus only. Carbonic anhydrase activity in the experimental group was 76% below the control in the blood and 42% below in the gastric mucosa. After administration of acetazolamide the pepsin content in homogenates of the mucosa fell by 31% (Table 2).

In response to stimulation of gastric secretion by a food stimulus and by histamine, carbonic anhydrase activity was thus increased not only in the blood but also in the gastric mucosa. Meanwhile the pepsinogen content in homogenates of the mucosa also increased.

Inhibition of carbonic anhydrase in the blood and mucosa following injection of acetazolamide was accompanied by a decrease in the pepsinogen content in homogenates of the gastric mucosa. Repeated administration of acetazolamide caused more profound inhibition of carbonic anhydrase in the blood and mucosa and also a more marked decline in the pepsinogen content.

Correlation was thus found between the level of carbonic anhydrase activity and the intensity of pepsin biosynthesis in the gastric mucosa. Acetazolamide, which inhibits carbonic anhydrase, is evidently not only an inhibitor of pepsin, but also a factor inhibiting its biosynthesis.

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