EFFECT OF CYNGAL ON GASTRIC SECRETION IN RATS

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The effect of the gallate of the alkaloid cynoglossophine-heliosupine (cyngal), isolated from the plant Cynoglossum officinale L., of the Boraginaceae family, on gastric secretion was studied in rats. Cyngal was shown to stimulate gastric secretion in rats starting from a dose of 0.25 mg/kg. This action is explained by the ability of the alkaloid to liberate histamine from labile depots.

KEY WORDS: gastric secretion; cyngal

A previous investigation [4] showed that the gallate of the alkaloid cynoglossophine-heliosupine (cyngal), isolated from Cynoglossum officinale L., family Boraginaceae, starting from the threshold dose of 0.5 mg/kg, stimulates motor activity in all parts of the gastrointestinal tract, with a predominant effect on activity of the small intestine. It was interesting to discover whether cyngal affects the secretory and acid-forming functions of the stomach, and should the result be positive, to study the mechanism of action of the alkaloid.

This paper describes the results of an investigation of the effect of cyngal* on gastric secretion in rats under normal conditions and after blocking of histaminergic and cholinergic receptors by metiamide and atropine respectively. Data are given on the action of cyngal on the histamine concentration in the blood, gastric juice, and walls of the stomach and small intestine.

EXPERIMENTAL METHOD

The action of cyngal on gastric secretion was studied in 150 male rats weighing 160-180 g by the method of Shay et al. [13]. The rats were deprived of food for 20 h but allowed water ad lib. Under ether anesthesia laparotomy was performed and a ligature applied to the pyloric part of the stomach. The wound was sutured and the drugs at once injected subcutaneously. Cyngal was injected in doses of 0.25, 0.5, 1, 5, and 10 mg/kg. The activity of the alkaloid was compared with the activity of histamine dihydrochloride in doses of 0.01, 0.016, and 0.024 mg/kg [3].

A dose of 0.01 mg/kg is used in clinical practice for the simple histamine test, 0.016 mg/kg is the mean dose capable of producing a prolonged secretion plateau, and 0.024 mg/kg is the dose which has been shown to realize to the full the secretory powers of the parietal cells [11]. Metiamide, an inhibitor of H-2 histamine receptors [6], was injected in a dose of 10 mg/kg and the muscarinic cholinolytic atropine sulfate was injected in a dose of 0.5 mg/kg. The rats were killed 3 h after application of the ligature, the stomach was removed, the volume of the gastric contents was measured, and the concentration of free acids in them was determined.

The histamine concentration in the blood, in the wall of the antrum of the stomach, in the gastric juice, and in the wall of the small intestine (10 cm caudally to the pylorus) of guinea pigs was determined by the fluorometric probe method of Shore [14]. Cyngal was injected subcutaneously in doses of 1 and 10 mg/kg. The animals were killed 10 and 40 min after injection of the alkaloid.

EXPERIMENTAL RESULTS

The experiments showed that cyngal stimulates the formation of gastric juice (Table 1). The maximal effect was observed after injection of the alkaloid in a dose of 1 mg/kg, when not only the volume of gastric secretion was increased, but also hydrochloric acid production per unit time.

*The akaloid cynoglossophine-heliosupine was isolated from C. officinale and the gallate prepared by Assistant Professor I. V. Man'ko in the Department of Technology of Galenicals, Leningrad Pharmaceutical Chemical Institute.

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TABLE 1. Comparison of Secretory Responses of Rat Stomach to Injection of Histamine and Cyngal

Experimental conditions	Number of observations	Volume of gastric juice, ml/3 h	Total acidity, ml 0.1 N NaOH/100 ml juice	Hydrochloric acid production, meq/h
Isotonic solution (control)				
Histamine, mg/kg	20	1,4±0,41	51	0,24
0,01	5	2,6±0,53*	55	0,47
0,016	5	3,4±0,45*	57	0,62
0,024	5	4,4±0,56*	56	0,73
Cyngal, mg/kg				
0,25	5	1,71±0,28	50	0,28
0,5	7	1,6±0,51	57	0,3
1	10	3,2±0,31*	49	0,52
5	5	2,5±0,35*	35,5	0,28
10	5	2,3±0,42	26	0,2

*Difference from control statistically significant ($P \leq 0.05$).

TABLE 2. Secretory Response of Rat Stomach to Injection of Cyngal with or without Preliminary Injection of Atropine and Metiamide

Experimental conditions	Number of observations	Volume of gastric juice, ml/3 h	Total acidity, ml 0.1 N NaOH/100 ml juice	Hydrochloric acid production, meq/h
Cyngal 1 mg/kg	10	3,2±0,31*	49	0,52
Metiamide 10 mg/kg	20	1,4±0,57	14	0,066
Metiamide + cyngal (in above doses)	10	1,8±0,51	42	0,25
Atropine 0.5 mg/kg	10	1,2±0,48	36	0,14
Atropine + cyngal (in above doses)	10	2,2±0,5	47	0,33

*Difference from control statistically significant ($P \leq 0.05$).

Activation of the secretory function of the stomach under the influence of cyngal began with a dose of 0.25 mg/kg. An increase in the dose of the alkaloid above 1 mg/kg reduced both the volume of gastric secretion and the hydrochloric acid production. The last parameter fell below the initial level.

Comparison of the magnitude of the secretory responses and acid formation by the stomach after injection of cyngal and histamine showed that the activity of the alkaloid in a dose of 1 mg/kg is equal to the activity of histamine in doses of between 0.01 and 0.016 mg/kg.

The activity of the glandular apparatus of the stomach, including the function of the acid-forming parietal cells, is known to be under neurohumoral control. It might be supposed that cyngal stimulates gastric juice formation by virtue of certain cholinomimetic properties [5], and also by interference with the metabolism of histamine, one of the principal humoral stimulators of parietal cell activity [10, 12].

Preliminary blocking of the H-2 histamine receptors prevented the development of the stimulant effect of cyngal both on the volume of gastric secretion and on hydrochloric acid production (Table 2). Antagonistic relations between cyngal and metiamide led to interference with the effect of both substances, as a result of which the normal secretory function of the stomach was restored.

Preliminary atropinization of the rats reduced the stimulant effect of cyngal on gastric secretory activity only a little. The volume of gastric secretion remained more than 70% higher than in the control, and hydrochloric acid production was 37% above normal. These facts are evidence that cyngal stimulates the glandular apparatus of the stomach through its effect on both the nervous (partial reduction of the effect by atropine) [1] and the humoral (total abolition of this action by metiamide) components of the mechanism of regulation of secretion, with a predominant effect on the humoral component.

The study of the ability of cyngal to affect the histamine concentration in the blood, gastric juice, and the mucosa of the stomach and small intestine showed that 10 min after its injection in doses of 1 and 10 mg/kg the alkaloid increased the histamine concentration in all organs and physiological fluids tested (Fig. 1). The histamine concentration in all these objects except gastric juice fell below normal 40 min after a single injection of the alkaloid, possibly on account of its diffusion into the tissues and, to a lesser degree in rodents, on account of the action of enzymes destroying histamine [2, 7].

An increase in the histamine concentration in the blood is the result of its liberation from granules in leukocytes and platelets [8, 9]. The present experiments confirmed this view. An increase in the histamine concentration in the blood plasma of 51 ± 8.3% 10 min after injection of the alkaloid was accompanied by a simultaneous fall in the histamine concentration by 50.5% below its initial level in the residue after centrifugation, consisting of blood cells.

The decrease in the free histamine concentration in the test objects 40 min after injection of cyngal was due to the development of a phase of exhaustion of the labile histamine depots. This can also explain why an increase in the dose of cyngal to 5 and 10 mg/kg led ultimately to a reduction in the 3-hourly gastric secretion (Table 1).

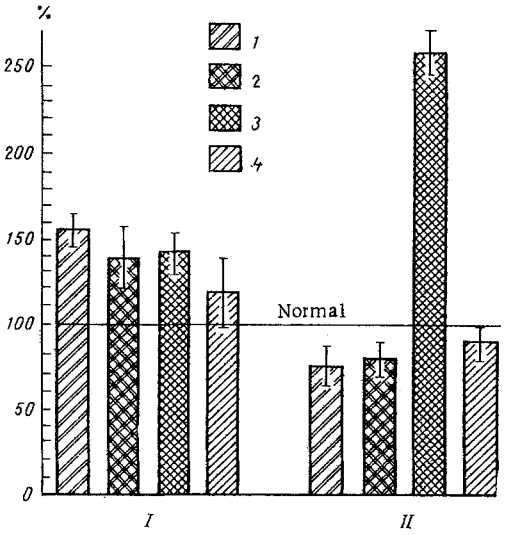

Fig. 1. Effect of cyngal (10 mg/kg) on histamine concentration in organs and biological fluids of guinea pigs 10 (I) and 40 min (II) after injection. Ordinate, histamine concentration (in % of normal); abscissa, object in which histamine was determined; 1) in blood, 2) in gastric mucosa, 3) in gastric juice, 4) in mucosa of small intestine.

In a dose of 1 mg/kg cyngal thus stimulates gastric secretion and the acid-forming function of the parietal cells to the same degree as these functions are stimulated by histamine in doses of 0.01 to 0.016 mg/kg.

The action of cyngal in stimulating gastric secretion can be explained primarily by its ability to liberate histamine from labile reserves, thereby stimulating the parietal cells, and also by certain cholinomimetic properties [5].

The properties of cyngal are of undoubted interest and may prove useful during the study of gastric parietal cell function in cases when intolerance to exogenous histamine makes the use of the histamine test impossible.

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INJURY TO THE ISOLATED HEART BY ADRENALIN DURING SALINE PERFUSION

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Perfusion of the isolated heart with Krebs' solution containing adrenalin in concentrations of 5 and 20 μg/ml induced micronecroses of the cardiocytes. Perfusion with adrenalin in a concentration of 0.5 μg/ml did not cause micronecroses. Dispersion analysis revealed a statistically significant effect of high concentrations of adrenalin on the intensity of the cardionecrotic effect. The fact that micronecroses develop in the isolated heart when perfused with saline raises doubts about the leading role of the blood factor in the realization of the cardionecrotic effect of catecholamines. The appearance of necroses during exposure to adrenalin in concentrations activating the mechanism of amine uptake by the cardiac muscle cells is evidence of a causal connection between the accumulation of biogenic amines by the myocytes and the development of necrosis in them.

KEY WORDS: isolated heart; adrenalin injury to myocardium; micronecroses of the myocardium

The mechanism of the cardionecrotic action of high concentrations of catecholamines remains unclear. Their damaging effect on the myocardium is exerted against the background of the numerous effects of these agents both in the heart itself and at the level of the whole body [3, 9, 10]. The aim of the present investigation was to study the possible direct harmful action of adrenalin on the myocardium during perfusion of the isolated heart.

EXPERIMENTAL METHOD

Experiments were carried out on 34 noninbred male albino rats weighing 180-200 g. The animals were anesthetized with urethane (1 g/kg, intraperitoneally) with the addition of heparin (5,000 units/kg) and the heart was removed and perfused by Langendorf's method with Krebs' solution in the modification of Neely et al. [13], under a pressure of 70 mm Hg and at 37°C and pH 7.4. The composition of the perfusion solution was as follows (in mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 2.5, $NaHCO_3$ 25, Na_2-EDTA 0.5, and glucose 5.6. Ascorbic acid was added to the solution (20 mg/liter) which was aerated with a mixture of oxygen (95%) and CO_2 (5%). The acting concentrations of adrenalin were chosen after calculating concentrations activating the neuronal (0.5 μg/ml) and extraneuronal (5 and 20 μg/ml) uptake of catecholamines [11]. The last two concentrations also correspond to those found in the blood of experimental animals after injection of the cardiotoxic doses of adrenalin usually used (1-100 mg/kg body weight) [2, 4, 7, 9].

The heart was perfused for 5 min with Krebs' solution and then for 10 min with solutions containing adrenalin hydrochloride in final concentrations of 0.5, 5, and 20 μg/ml (calculated as the base), after which it was again perfused with the original solution for 1.5, 3, or 6 h. To all experiments there was a parallel control in which the heart was perfused without adrenalin. At the end of the experiment the heart was stopped in cold (4°C) Krebs' solution, cut into halves in the frontal plane, and frozen in liquid nitrogen. To detect damage to the myocardium, the reaction with nitro-BT for succinate dehydrogenase activity, after Nachlas et al., was carried out on frozen sections 10 μ thick. Lipids were stained with Oil Red 0 in one of the serial sections.

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