Biochemical Pharmacology, Vol. 26, pp. 979–981. Pergamon Press, 1977. Printed in Great Britain.

INHIBITORY EFFECT OF CYCLOHEXIMIDE ON GASTRIC SECRETION IN RATS

LIBUŠE KORBOVÁ, JIŘÍ KOHOUT, JIŘINA ČÍŽKOVÁ and ALOIS ČIHÁK

Institute of Pathological Physiology, First Surgery Clinic, Charles University, Prague 2, and Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6, Czechoslovakia

(*Received* 25 *June* 1976; *accepted* 24 *November* 1976)

Abstract—Cycloheximide administered to rats in non-toxic doses immediately after pyloric ligation results in a lower incidence of experimental gastric ulcers. The effect is paralleled by an impaired secretion of hydrochloric acid (more than 90 per cent inhibition). Simultaneously the activity of pepsin in gastric juice and in homogenates of gastric mucosa was reduced by 60 per cent and mucoproteins were lowered by 30 per cent. The basal and pentagastrin-stimulated secretion were inhibited by cycloheximide to the same extent.

Cycloheximide was used by Young and Dowling [1] to produce defervescence in patients with Hodgkins' disease. Low toxicity of the drug facilitated its successful clinical use. Nausea and vomiting were, however, side effects and their severity and duration were dosage related. Cycloheximide inhibits protein synthesis [2] and affects the synthesis of RNA in various systems [3, 4]. An increased uptake of orotic acid into liver RNA in rats pretreated with the drug was observed recently [5]. Since 5-azacytidine-mediated decrease of gastric secretion [6] resulting in digestion disturbances [7, 8] was found to be connected with the enhanced incorporation of orotic acid into liver RNA we followed in this study the effect of cycloheximide on gastric secretion.

MATERIALS AND METHODS

Chemicals. Cycloheximide was obtained from Calbiochem, hemoglobin was product of Difco, galactose, mannose and pentagastrin were obtained from Merck.

Animals. Groups of 8–16 male rats (270–300 g) kept under standard conditions were fasted 22 hr before starting the experiments. Pyloric ligation [9] was performed at 2 p.m., the animals received cycloheximide and were killed 22 hr later. In the case of stimulated secretion ligation was performed at 8 a.m., pentagastrin was administered subcutaneously immediately after the operation and then four times at 20 min intervals. The animals were killed 40 min after the last dose of the drug. Cycloheximide was administered 30 min before pyloric ligation.

Analysis of gastric juice. Gastric juice was diluted twenty times. The content of hydrochloric acid was assayed by titration with NaOH, 0.1 M and expressed in milli equivalents of $H^+ \pm$ S.E. Proteolytic activity was measured with 2.5% hemoglobin at pH 1.6 and expressed [10] as milliequivalents of released tyrosine ± S.E. Total mucoproteins were assayed by the orcin method [11] and expressed as mg of hexoses ± S.E. All the results are expressed for the totality of the excreted gastric juice. The classification system applied for the evaluation of the number and size of experimental gastric ulcers was the same as already described [6]. The data were analyzed for statistical significance by the Student's t test.

RESULTS AND DISCUSSION

The changes in gastric secretion in rats receiving increasing amounts of cycloheximide in a single dose immediately after pyloric ligation are expressed in Figs 1 and 2. The secretion of gastric juice was significantly lowered after the administration of the drug at the dose of 1.0 to 2.5 mg per kg body wt. Similar depression was observed measuring gastric acidity and proteolytic activity. A significant decrease of total hexoses was found only after the highest dose of cycloheximide. The most pronounced changes were observed in the number and size of experimental gastric ulcers (Fig. 2). Their decrease was observed already after the administration 0.2 mg of cycloheximide per kg body wt. Also total hexoses and, especially, the amount of pepsinogen in gastric mucosa were lowered in the treated animals.

Cycloheximide inhibits basal as well as stimulated gastric secretion. However, following pentagastrin administration only the output of hydrochloric acid was significantly stimulated. There were no differences in the secretion following 50 or 100 μg of pentagastrin administered per kg body wt (Table 1). Cycloheximide (0.25–2.5 mg per kg) injected 30 min before pentagastrin decreased markedly the vol. of gastric juice and the output of hydrochloric acid. The secretion of pepsin was significantly lowered only using higher doses of the drug. The changes in hexose levels were not significant (Table 2).

Following the administration of cycloheximide both the depression and the stimulation of various metabolic processes were observed in different biological systems. Sundler *et al.* [12] have demonstrated that the addition of cycloheximide to isolated hepatocytes leads to inhibition of lipoprotein secretion. The drug blocks the development of experimental acute pancreatitis in rats by a process associated with the

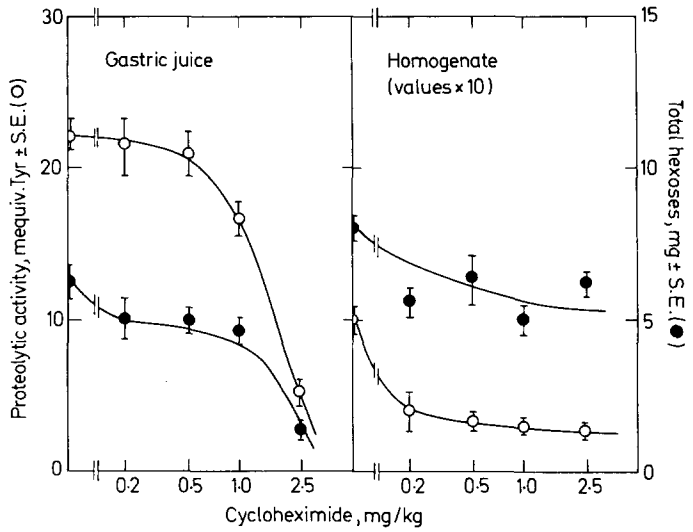

Fig. 1. Decreased activity of proteolytic enzymes and lower level of mucoproteins in gastric juice and the stomach of rats following cycloheximide treatment (mg/kg). Groups of 10–16 male rats received the drug immediately after pyloric ligation 22 hr before killing.

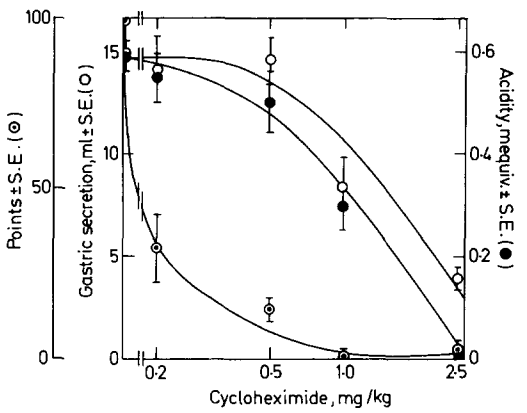

Fig. 2. Lower gastric secretion and acid output associated with the decreased incidence of experimental gastric ulcers in rats treated with cycloheximide. Groups of 12 male rats received the drug (mg/kg) immediately after pyloric ligation 22 hr before killing. Gastric ulcers are expressed [6] as the number of points ± S.E.M.

lowering of lipase and amylase activities in abdominal fluid [13]. On the contrary, cycloheximide causes an increase of sucrase and maltase activities in the jejunum of suckling rats [14]. Also hepatic uridine kinase [5] and renal ornithine decarboxylase [15] are known to be elevated in cycloheximide-treated animals

A single injection of cycloheximide given to animals with ligated pylorus causes the depression of gastric secretion and prevents the development of gastric lesions (Figs 1 and 2). While there was only a slight effect of the drug on mucoproteins, proteolytic activity and secretion of hydrochloric acid were markedly affected. The almost complete block of hydrochloric acid secretion caused by a single dose of cycloheximide represents a way of depressing gastric acidity for a relatively long period of time.

The development of gastric ulcers in animals with ligated pylorus depends on the metabolic and neurovegetative changes. The inhibition by cycloheximide of pentagastrin-stimulated gastric secretion (Table 2) indicates the possible role of cyclic 3′, 5′-AMP in this process [16]. The lowering of carbonic anhydrase

Table 1. Gastric secretion in rats stimulated by pentagastrin*

Measured	Expressed in	Controls	Pentagastrin-stimulated 50 μg/kg	(%)	100 μg/kg	(%)
Secretion	ml	3.23 ± 0.52	4.00 ± 0.50	124	4.10 ± 0.33	127
Acid output	mequiv. H^+	0.116 ± 0.063†	0.241 ± 0.040†	208	0.206 ± 0.020	178
Total hexoses	mg	2.30 ± 0.39	2.54 ± 0.35	110	2.08 ± 0.42	91
Proteolytic activity	mequiv. Tyr	2.82 ± 0.56	3.51 ± 0.55	124	3.87 ± 0.73	137

* Pentagastrin or saline were administered s.c. to groups of 10 male rats immediately after pyloric ligation and then 4 times at 20 min intervals. The animals were killed 40 min later.
† $P < 0.01$.

Table 2. Inhibitory effect of cycloheximide on gastric secretion in rats stimulated by pentagastrin*

Measured	Expressed in	Pentagastrin alone	+Cycloheximide 0.25 mg/kg	(%)	1.0 mg/kg	(%)	2.5 mg/kg	(%)
Secretion	ml	4.00 ± 0.50†	2.00 ± 0.28†	50.0	2.40 ± 0.60	60.0	1.38 ± 0.20†	34.5
Acid output	mequiv. H^+	0.24 ± 0.04†	0.048 ± 0.010†	20.0	0.048 ± 0.022†	20.0	0.008 ± 0.004†	3.3
Total hexoses	mg	2.54 ± 0.35‡	2.17 ± 0.51	85.4	1.56 ± 0.34	61.4	1.41 ± 0.16‡	55.5
Proteolytic activity	mequiv. Tyr	3.51 ± 0.55†	2.88 ± 0.48	82.1	1.40 ± 0.34†	39.9	1.22 ± 0.14†	34.8

* Cycloheximide was administered i.p. to groups of 10 male rats 30 min before pyloric ligation. Pentagastrin (50 μg/kg) was injected s.c. immediately after operation and then four times at 20 min intervals. The animals were killed 40 min later.

† $P < 0.005$. ‡ $P < 0.01$.

activated following pentagastrin administration by 3′,5′-AMP dependent protein kinase was observed recently in animals treated with cycloheximide [17]. 5-Azacytidine blocks the evacuation of the stomach and causes a similar depression of gastric secretion and the lower incidence of experimental gastric ulcers [6] as cycloheximide. However, while the pyrimidine analogue is rather toxic [18] cycloheximide is non-toxic at low doses. Experiments are in progress to show the possible use of cycloheximide as an antiulcer agent.

REFERENCES

1. C. W. Young and M. D. Dowling, *Cancer Res.* **35**, 1218 (1975).
2. L. I. Rothblum, T. M. Devlin and J. J. Chih, *Biochem. J.* **156**, 151 (1976).
3. J. L. Farber and R. Farmar, *Biochem. biophys. Res. Commun.* **51**, 626 (1973).
4. P. J. Goldblatt, J. Archer and C. Eastwood, *Lab. Invest.* **33**, 117 (1975).
5. A. Čihák, *Eur. J. Biochem.* **58**, 3 (1975).
6. L. Korbová, J. Kohout and A. Čihák, *Arch. int. Pharmacodyn. Thér.* **209**, 332 (1974).
7. M. Karon, L. Sieger, S. Leimbrock, J. Z. Finklestein, M. E. Nesbit and J. J. Swaney, *Blood* **42**, 359 (1973).
8. W. R. Vogler and S. N. Arkun, *Proc. Am. Assoc. Cancer Res.* **14**, 235 (1973).
9. H. Shay, S. A. Komarov, S. S. Fels, D. Meranze, M. Gruenstein and H. Sipplet, *Gastroenterology* **5**, 43 (1945).
10. M. L. Anson and A. E. Mirsky, *J. gen. Physiol.* **16**, 59 (1932).
11. M. Sørensen and G. Haugart, *Biochem. Z.* **260**, 247 (1933).
12. R. Sundler, B. Akesson and A. Nilsson, *Biochem. biophys. Res. Commun.* **55**, 961 (1973).
13. L. Korbová, J. Kohout, F. Mališ, J. Čížková, J. Marek and A. Čihák, *Gut*, in press.
14. O. Koldovský, J. Jumawan, J. J. Herbst and M. Palmieri, *Biochim. biophys. Acta* **309**, 370 (1973).
15. J. H. Levine, W. E. Nicholson and D. N. Orth, *Proc. Natn. Acad. Sci. U.S.A.* **72**, 2279 (1975).
16. W. Domschke, S. Domschke, M. Classen and L. Demling, *Nature, Lond.* **241**, 454 (1973).
17. R. I. Salganik, R. I. Bersimbaev, S. V. Argutinskaya, E. V. Kiseleva, N. B. Khristolyubova and V. I. Deribas, *Molec. Cell. Biochem.* **12**, 181 (1976).
18. A. Čihák, *Oncology* **30**, 405 (1975).