DETERMINATION OF CHOLESTEROL ESTERASE ACTIVITY IN HUMAN DUODENAL CONTENTS

Academician A. S. Loginov*, L. O. Shibaeva, K. S. Planutis, and V. M. Sadokov

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Cholesterol esters are known to be hydrolyzed in the small intestine to cholesterol and fatty acids [4, 5]. This process is catalyzed by cholesterol esterase (ChE), which is synthesized in the acinar cells of the pancreas and is secreted as a component of the pancreatic juice into the lumen of the duodenum [2, 3]. ChE is present in the pancreatic juice in considerable quantities, and may account for up to 5% of the total protein of the pancreatic juice [1]. The presence of ChE in the duodenal contents has been demonstrated by gel-filtration [2], but its activity in man has not been determined quantitatively. The study of this problem is interesting because of the possibility of evaluating the external secretory function of the pancreas with respect to pancreatic enzyme activity in the duodenal contents. The aim of this investigation was to determine whether pancreatic juice is the main source of the ChE activity of the duodenal contents. For this purpose ChE activity was determined in the duodenal contents and also in the saliva, gastric juice, and bile, which may also be components of the duodenal contents.

EXPERIMENTAL METHOD

Cholesterol esterase activity was determined fluorometrically. The cholesterol ester of o-coumaric acid, dispersed by Triton X-100, was used as the substrate. On hydrolysis of this substance by ChE, o-coumaric acid, which gives fluorescence in an alkaline medium, is formed. The increase of fluoroscence is proportional to the quantity of o-coumaric acid formed. Activity of the enzyme was determined by measuring the increase of fluorescence. Cholesteryl-o-coumarate (1 mg) was dissolved in 1 ml of diethyl ether and 40 µl of Triton X-100 was added. The sample was mixed and the diethyl ether evaporated. The residue was treated with 20 ml of 300 mM K-phosphate buffer, pH 6.6, and 5 mM sodium cholate, and mixed. To 200 µmoles of the resulting substrate mixture 200 µl of 50 mM K-phosphate buffer, pH 6.6, and 6 mM percaptoethanol were added. The sample was heated to 37°C and the reaction started by the addition of 20 µl of the biological test fluid. Viscous fractions of material for analysis were diluted (1:10) beforehand and filtered if necessary. The enzyme–substrate mixture was incubated for 15 min at 37°C. Measurements were made on the "Bian-130" fluorometer, with excitation and emission wavelengths of 365 and 470 nm respectively. To calibrate the fluorometer scale, various quantities of o-coumaric acid were added. ChE activity was calculated from the increase in fluorescence during incubation of the reaction mixture for 15 min, and expressed in µmoles o-coumaric acid formed during incubation for 1 min.

The duodenal contents were obtained separately from the gastric contents by means of a double-barreled tube. ChE activity was studied under basal conditions and after intravenous stimulation by pancreozymin (2 U/kg body weight) and secretin (1 U/kg; both were from Boots, England).

EXPERIMENTAL RESULTS

The results of investigation of ChE in saliva, gastric juice, and duodenal contents before and after stimulation of enzyme secretin for 20 clinically healthy subjects aged 18-53 years are given in Table 1. Bile was obtained after cholesystectomy and by drainage of the common bile duct. No ChE activity could be found in the saliva, gastric juice, or bile.

*Academy of Medical Sciences of the USSR.

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TABLE 1. ChE Activity (in μmoles substrate/min/ml) × 10^{-3} in Human Duodenal Contents and Other Biological Fluids (M ± m)

Material tested	Concentration	Absolute output
Duodenal contents:		
before stimulation (20)	5,02±0,82	97,56±16,36
after stimulation (20)	7,38±0,59	325,20±10,87*
		447,20±18,18**
Bile (20)		Not determined
Gastric juice (10)		» »
Saliva (10)		» »

Legend. Number of determinations given in parentheses; *pancreozymin, **pancreozymin + secretin.

Consequently, the results confirm the hypothesis that ChE activity of pancreatic origin is determined by the method used in the duodenal contents. Further confirmation is given by the significant increase in ChE activity after administration of stimulators of pancreatic secretion. It will be clear from Table 1 that the ChE output during the 20 min after stimulation by pancreozymin and secretin was increased fivefold. Incidentally, the ChE concentration in the duodenal contents varies over a wider range than the absolute output. Concentration depends on possible dilution of the pancreatic juice by other components of the duodenal contents. Determination of the absolute ChE output after stimulation is the most informative parameter.

Thus ChE activity can be determined in human duodenal contents by the use of the cholesteryl ester of o-coumaric acid as substrate. Values obtained for ChE activity can be used to evaluate the external secretory function of the pancreas in clinical and experimental investigations.

LITERATURE CITED

1. C. Erlanson and B. Bargstrom, Scand. J. Gastroenterol., 5, 395 (1970).
2. D. Lombardo, O. Guy, and C. Figarella, Biochim. Biophys. Acta, 527, 142 (1978).
3. G. E. Palade, P. Siekevitz, and L. C. Coro, The Exocrine Pancreas, Boston (1961), p. 23.
4. C. R. Treadwell and G. V. Vahouny, Handbook of Physiology, Vol. 3, Baltimore (1968), p. 1407.
5. G. V. Vahouny and C. R. Treadwell, Meth. Biochem. Anal., 16, 219 (1968).