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Nonparallel Courses of Intrapancreatic Levels of Exportable Enzymes after a Fatty Meal (39270)

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(Introduced by J. MORISSET)

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It has been shown by Mott *et al.* (1), in man, and Go *et al.* (2) and Meyer *et al.* (3), in dog, that an intraduodenal perfusion of oleic acid induced a pancreatic secretory stimulation probably mediated by the release of cholecystokinin-pancreozymin. The aim of the present work has been to study the response of rat pancreas to an intragastric intubation of oleic acid. Our results have shown that after this kind of fatty meal, intrapancreatic levels of amylase, chymotrypsin, trypsin, and lipase had nonparallel courses. The concomitant variations of the rate of pancreatic protein biosynthesis that we have found are in good agreement with the hypothesis that nonparallelism in exportable enzyme levels is a consequence of a nonparallel regulation of the individual rates of enzyme biosynthesis by the stimulus.

Materials and methods. Male rats of the Wistar CF strain were fed *ad libitum*, as reported in a previous work (4), a homemade diet with 17% proteins (casein), 48% carbohydrates (starch), and 35% lipids (olive oil), expressed in percentage of total calories. All animals were maintained 2 months on this diet and were fasted 24 hr before experimentation. The fatty meal consisted in an intragastric intubation of 2 ml of pure oleic acid (4). Ten minutes before death each animal received 20 μCi of [^{14}C]valine (140 μCi/mmole CEA Saclay). The animals were killed by decapitation, their pancreas removed, trimmed free of fat, then homogenized in 10 ml of 0.1 *M* sodium phosphate buffer, pH 8.1, using a glass-Teflon Potter apparatus.

Enzymatic assays were performed on the high-speed supernatant of an aliquot of the homogenate, and activities were determined as previously described (4) using soluble starch, olive oil emulsion, acetyl-tyrosine-ethyl-ester and tosyl-arginine-methyl-ester as specific substrates, respectively, for amylase, lipase, chymotrypsin, and trypsin. Enzyme levels were expressed as enzyme units per microgram of DNA.

The remainder of the pancreatic homogenate was fractionated according to Schneider (5). The specific radioactivity (SRA) of intrapancratic free valine was determined from the cold TCA soluble fraction, using an automated amino-acid analyser (Jeol) with norvaline as internal standard. DNA was extracted in the hot TCA soluble fraction and determined using the diphenylamine method. Radioactivity incorporated into pancreatic proteins was measured in the hot TCA insoluble fraction of the homogenate. Assuming that the intrapancreatic free amino-acid pool SRA is actually the best estimation of the amino-acyl-tRNA pool SRA (6, 7), the rate of biosynthesis has been expressed as picmoles of valine incorporated into proteins per minute and per microgram of DNA.

To control the reproducibility of our results, we compared in the G.I. Unit laboratories of the Sherbrooke University (Québec, Canada) the enzymatic levels of amylase and chymotrypsin in rats 30 min after oleic acid intubation and in control animals. The experimental protocol was identical to the previous one, with the exception that rats were of the Sprague-Dawley strain, and chymotrypsin was analyzed according to Hümmel (8).

Results were expressed as the arithmetical mean ± SE for each concerned group. Statistical significance of the differences between groups were tested using the nonparametric rank sum test of Wilcoxon (9).

Results. Table I shows the time-course of intrapancreatic levels of amylase (Am), lipase (Lip), chymotrypsinogen (ChTg), and

trypsinogen (Tg) following the intubation of oleic acid. Within the first 50 min, the level of ChTg increased significantly, whereas the levels of the three other enzymes were not significantly modified. On the other hand, from the 70th min, levels of Am and Tg were significantly lower than those of controls. ChTg decreased down to control values, while lipase did not change. It appears that the kinetics of Am, Tg, and ChTg levels can be divided into two periods: before and after 50 min of intubation. Within the first 50 min, Am and Tg remained unchanged, while the ChTg level was continuously increasing. After 50 min the three enzymes showed a parallel behavior: all levels decreased. ChTg was the only enzyme whose level was significantly increased by the oleic acid treatment. In an effort to evaluate the magnitude of the nonparallelism, which appeared between the different enzyme courses, for each time we measured the ratios of Am, Lip, and Tg levels to that of ChTg. There was a significant decrease in the three ratios between control and the 30-min period; after 30 min only the Am/ChTg ratio remained significantly lowered (Table I). In the control experiment realized in Sherbrooke (see Material and Methods), we have found that the Am/ChTg levels was 29.1% lowered after a 30-min stimulation. This result is in good agreement with the 31.6% decrease, which can be calculated from Table I.

Table II shows that the rate of pancreatic protein biosynthesis was significantly decreased during the 35th- to 75th-min period after oleic acid intubation.

Discussion. Our results show that the pancreatic secretory stimulation evoked by a fatty meal induced nonparallel courses of intrapancreatic enzyme levels. Because the

TABLE I. INTRAPANCREATIC ENZYMATIC LEVELS FOLLOWING OLEIC ACID ADMINSTRATION.[a]

	Time after intubation (min)				
	0	30	50	70	90
Amylase	13.95 ± 1	14.30 ± 3.7 NS	12.63 ± 1.02 NS	8.05 ± 0.62 $P < 0.01$	8.40 ± 1.51 $P < 0.01$
Lipase	22.1 ± 2.1	19.4 ± 1.1 NS	27.2 ± 5 NS	16.2 ± 3.4 NS	18.6 ± 3.1 NS
Trypsinogen	2.2 ± 0.11	2.05 ± 0.19 NS	2.56 ± 0.41 NS	1.61 ± 0.09 $P < 0.01$	1.60 ± 0.2 $P < 0.025$
Chymotrypsinogen	2.35 ± 0.27	3.3 ± 0.42 $P < 0.05$	3.45 ± 0.35 $P < 0.025$	2.1 ± 0.25 NS	2.15 ± 0.1 NS
Amylase Chymotrypsinogen		$P < 0.05$	$P < 0.01$	$P < 0.025$	$P < 0.025$
Trypsinogen Chymotrypsinogen		$P < 0.05$	NS	NS	NS
Lipase Chymotrypsinogen		$P < 0.05$	NS	NS	NS
Number of animals	11	6	7	6	4

[a] Values are means ± SE Statistical significance of the difference between the concerned group and control is performed according to Wilcoxon (see Materials and Methods).

TABLE II. RATE OF PANCREATIC PROTEIN SYNTHESIS FOLLOWING OLEIC ACID ADMINISTRATION.

	Time after intubation (min)				
	0	35	55	75	90
Picomoles Val incorporated per (min × μg of DNA) ± SE	11.80 ± 2.2	9.85 ± 1.5	4.75 ± 0.35	8.20 ± 1.17	9.04 ± 1.56
Number of animals	12	6	5	7	5
P values[a]		NS	< 0.01	NS	NS

[a] Statistical significance of the difference between the concerned group and control (see Materials and Methods).

intrapancreatic level of an exportable enzyme is the result of a steady state between synthesis and secretion, nonparallel courses of the levels of several enzymes involve nonparallelisms in their rates of synthesis and/or secretion. We do not have data concerning secretion, but a nonparallelism in the biosynthesis rates of the various enzymes seems plausible. First of all, Reboud *et al.* (6) and Christophe *et al.* (10) have shown that in the case of pancreatic adaptation to different diets, variations in the rates of pancreatic enzyme biosynthesis were responsible for the observed variations in intrapancreatic enzyme levels. Secondly, it was possible to establish a relationship between the variations in the enzyme levels and the total rate of biosynthesis that we observed. From this, it seems likely that the opposite variations in individual levels that have been observed reflect opposite variations in the individual rates of biosynthesis. This could be the reason why the decrease in the rate of total pancreatic protein biosynthesis is so small at 35 min. Furthermore, the levels of the four enzymes we studied show a parallel decrease from the 50th minute on, and this is in good agreement with the important decrease in the total rate of pancreatic protein biosynthesis noticed at 55 min.

The problem of the nonparallelism in the processing of pancreatic enzyme secretion has been recently reviewed (11). To our knowledge, there is no information concerning the courses of individual rates of biosynthesis following a stimulation. Actually, the nonparallelism in the processing of pancreatic proteins is never discussed in terms of synthesis, but only in terms of intracellular transport (12, 13) or exocrine pancreatic tissue heterogeneity (14).

Summary. Stimulation of pancreatic secretion by a fatty meal resulted in a nonparallel time-course of intrapancreatic amylase, lipase, trypsinogen, and chymotrypsinogen levels. Concomitant variations in the rate of pancreatic protein biosynthesis are observed. Those results support the hypothesis that nonparallelism in exportable enzyme levels is a consequence of a nonparallel regulation by the stimulus of the individual rates of enzyme biosynthesis.

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