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INCREASED DE NOVO PHOSPHOLIPID BIOSYNTHESIS IN THE STIMULATED RAT EXOCRINE PANCREAS

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SUMMARY

Sincalide significantly stimulated the incorporation of uniformly labelled ^{14}C-glucose into pancreatic phospholipids, but not into neutral lipids. Incorporation into phosphatidyl inositol was stimulated to the greatest degree followed by phosphatidyl ethanolamine, and phosphatidyl choline and lysophosphatidyl choline. Incorporation of label into the phospholipid glycerol backbone was enhanced suggesting increased de novo phospholipid biosynthesis.

It is suggested that in the stimulated exocrine pancreas phospholipid metabolism may be altered in a number of ways.

Increased incorporation of labelled precursors into phospholipids upon stimulation of exocrine pancreas was first described by Hokin and Hokin more than twenty years ago (1 - 4). Stimulation of incorporation was greatest into phosphatidyl inositol, but was also significant into various other phospholipids and results were interpreted as being consistent with an agonist induced increase in de novo biosynthesis of phosphatidyl inositol (5). However, subsequent work from a number of laboratories in pancreas and other tissues has resulted in the currently accepted hypothesis that the initial event in the phospholipid effect is an agonist induced breakdown of phosphatidyl inositol to diacylglycerol followed by resynthesis to phosphatidyl inositol (6 - 11).

Although largely neglected, increased de novo biosynthesis of phospholipids upon stimulation of target tissue has been the subject of a few reports since the early publications of the Hokins (12 - 14).

In the present study, the influence of sincalide, the carboxy terminal active octapeptide of pancreozymin, on the incorporation of uniformly labelled ^{14}C-glucose into rat pancreas lipids in vitro has been examined.

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Evidence is presented to demonstrate that stimulation of the exocrine pancreas by sincalide leads to a dramatic disproportionate increase in de novo phospholipid biosynthesis, with synthesis of phosphatidyl inositol being influenced to the greatest extent.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing 220-250g were fasted for 20 hours, killed by cervical dislocation and exsanguination and pancreas was removed. Incubations were carried out as previously described (15). Briefly, pancreatic slices were incubated in 25ml Ehrlenmeyer flasks containing 5.0ml of Krebs-Ringer-Bicarbonate buffer pH 7.4 with glucose added to 5.6mM, 5μCi of ^{14}C-U-glucose (0.17mCi/mmol final specific activity, Searle Nucleonics, Sydney Australia), and sincalide at 7.5 Ivy Dog Units/ml or no sincalide for 2 hours at 37° in a 95% O_2-5% CO_2 atmosphere. Sincalide, the carboxy-terminal octapeptide of pancreozymin, was a generous gift from E.R. Squibb & Sons, Noble Park, Australia. At the end of incubation, tissue was rinsed with excess ice-cold buffer, lipids were extraced with chloroform : methanol (2:1) and washed (16). Individual lipids were separated by thin layer chromatography as previously described (16), and phosphatidyl inositol was isolated by two-dimensional thin layer chromatography (17). Lipids were visualized with iodine, scraped into scintillation vials, scintillation fluid (18) was added and radioactivity measured in a Packard Tri-Carb Liquid Scintillation Spectrometer. Quench corrections were made using the external standardization method. Saponification was carried out as previously described (16). Statistical comparisons were performed using the paired t-test.

RESULTS

As shown in Table 1, sincalide at a concentration of 7.5 Ivy Dog Units per ml of incubation medium significantly stimulated the incorporation of uniformly labelled ^{14}C-glucose into a number of phospholipids of rat pancreas in vitro, with incorporation into phosphatidyl inositol being influenced to the greatest extent (Table 1). Labelling of neutral lipids, on the other hand, was not affected by sincalide (Table 1). Saponification data show that in all glyceride phospholipids affected, the greatest proportion of incorporated label appeared in the glycerol backbone and incorporation into this moiety was stimulated by sincalide (Table 2). A smaller fraction of label appeared in the glyceride - fatty acid moiety, into which sincalide appeared to stimulate incorporation of label in the case of phosphatidyl inositol and phosphatidyl choline, but not phosphatidyl ethanolamine (Table 2).

TABLE 1.

INFLUENCE OF SINCALIDE ON THE INCORPORATION OF ^{14}C-U-GLUCOSE INTO LIPIDS OF RAT PANCREAS IN VITRO.

	^{14}C-U-Glucose Incorporation (dpm/100 mg dry tissue weight)			
	Sincalide	Control	P	Percent of Control
Free Cholesterol and Diacylglycerol	2,840 ± 290	3,430 ± 610	NS	——
Free Fatty Acid	710 ± 70	750 ± 150	NS	——
Triacylglycerol	17,200 ± 2,170	15,280 ± 1,480	NS	——
Phosphatidyl Inositol	2,640 ± 470	1,040 ± 140	<0.01	261.7 ± 34.6
Phosphatidyl Ethanolamine	2,080 ± 360	1,070 ± 170	<0.01	200.8 ± 19.6
Phosphatidyl Choline	12,710 ± 1,280	9,900 ± 990	<0.01	129.8 ± 7.2
Lysophosphatidyl Choline	480 ± 50	340 ± 30	<0.01	143.6 ± 11.6
Phosphatidyl Serine	740 ± 260	420 ± 110	NS	——
Sphingomyelin	340 ± 90	300 ± 100	NS	——

Results presented as mean ± SEM of at least eight experiments.

Sincalide concentration equals 7.5 Ivy Dog Units per ml of incubation medium.

Statistical comparisons by paired t-test.

TABLE 2.

INFLUENCE OF SINCALIDE ON THE INCORPORATION OF ^{14}C-U-GLUCOSE INTO PHOSPHOGLYCERIDE FATTY ACID AND GLYCEROL MOIETIES IN RAT PANCREAS IN VITRO

	^{14}C-U-GLUCOSE INCORPORATION (dpm/100 mg dry tissue weight)			
	Glyceride Fatty Acid		Glyceride Glycerol	
	Sincalide	Control	Sincalide	Control
Phosphatidyl Inositol	646 ± 287	285 ± 74	2,353 ± 454	624 ± 160
Phosphatidyl Ethanolamine	407 ± 95	765 ± 418	1,591 ± 258	933 ± 62
Phosphatidyl Choline	1,016 ± 286	471 ± 148	11,508 ± 280	9,261 ± 1,217

Results presented as Mean ± SEM of three experiments.

Sincalide concentration equals 7.5 Ivy Dog Units/ml of incubation medium.

DISCUSSION

Extensive study of pancreas and a variety of other tissues in a number of laboratories has led to the currently accepted model of the phospholipid effect as a cycle of phosphatidyl inositol breakdown and resynthesis (6 - 11). The model proposes that upon tissue stimulation phosphatidyl inositol is broken down to 1,2-diacylglycerol, inorganic phosphate and inositol. During the secondary resynthetic phase, phosphatidyl inositol is reconstituted by re-phosphorylation of the diacylglycerol to phosphatidic acid followed by conversion to phosphatidyl inositol.

The present study has demonstrated increased incorporation of labelled glucose primarily into the glycerol, but also the fatty acid, moiety of phosphatidyl inositol in the stimulated rat pancreas, suggesting de novo synthesis of phosphatidyl inositol. In addition, the data indicate a sincalide stimulation of de novo synthesis of a number of other phospholipids. Clearly, results from the present study are not compatible with the currently accepted model of the phospholipid effect, but rather suggest a selective stimulation of membrane synthesis in response to the action of agonist on pancreatic tissue.

In view of the present and previously published data (9, 19, 20), it may be suggested that more than one phospholipid effect is operative in the stimulated exocrine pancreas: (i) a phosphatidyl inositol effect involving an increased turnover of inositol on a stable phosphatidyl inositol glycerol backbone, and (ii) a phospholipid effect involving differential stimulation of de novo phospholipid synthesis.

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