ACETYLCHOLINE INCREASES THE LEVEL OF DIGLYCERIDE IN MOUSE PANCREAS

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Summary. Incubation of mouse pancreas with 10^{-5}M or 10^{-4}M acetylcholine (plus eserine) resulted in increased tissue diglyceride levels. Diglyceride rose from 0.5 μmoles per gram fresh tissue in unstimulated pancreas to 1.2 and 3.6 μmoles, respectively, in acetylcholine-stimulated tissue. The linear increase in diglyceride level with time at 10^{-4}M acetylcholine was blocked and reversed by 10^{-5}M atropine. When atropine was added, the level of diglyceride fell to the control, unstimulated value. The increase in diglyceride level was greater than could be accounted for by hydrolysis of phosphatidylinositol or phosphatidic acid.

ACh* increases the incorporation of [^{32}P] into phospholipids, especially PI and PA, in pancreas slices (1); a similar "phospholipid effect" has been observed in response to ACh and some other agents in a variety of tissues, including brain (2). ACh has recently been shown to cause a net decrease in the level of PI and a net increase in the level of PA in mouse pancreas tissue incubated in vitro (3). ACh also stimulates the breakdown of PI which has been pre-labeled with [2-^{3}H] myo-inositol or [^{32}P] in avian salt gland slices (4) and mouse pancreas (5), and of [^{32}P]phosphatidic acid in brain synaptosomes (6). If the enzymes PI-inositolphosphohydrolase and PA-phosphatase are involved in these reactions, then DG would be expected to be a product of ACh-stimulated PI and PA breakdown. This possibility, together with the recent development of a rapid, sensitive radiometric procedure for the quantitative assay of DG (7), prompted this investigation of the effect of ACh on the pool size of DG in mouse pancreas in vitro. ACh was found to significantly increase the level of DG, and this increase was reversed by

*Abbreviations are: ACh - acetylcholine; DG - diglyceride; PA - phosphatidic acid; PI - phosphatidylinositol; TLC - thin-layer chromatography.

atropine. However, it is not clear how this increase in tissue DG relates to changes in PI and PA pool size.

MATERIALS AND METHODS

Male mice which weighed approximately 25 grams, from an inbred Swiss-Webster strain were used. The mice were killed and the pancreas was quickly removed, chilled, weighed, and incubated; the tissue was not sliced (8). For each incubation vessel, one pancreas (approximately 125 mg. fresh weight) was incubated at 38°C, with shaking, in 1 ml. of Krebs-Henseleit bicarbonate saline medium with added glucose (1 mg./ml.). ACh and atropine were added to give final concentrations as indicated. Eserine, final concentration, 10^{-4}M, was added to all vessels to which ACh was added. After incubation, each pancreas was removed from the incubation medium and rapidly frozen in a glass tube in a dry ice alcohol bath. The frozen tissue was extracted as described by Folch et al. (9); the extracts were washed with 0.1M KCl. To preserve unsaturated species of fatty acids, 0.1% (v/v) 2-mercaptoethanol (Eastman Kodak Co., Rochester, N. Y.) was present in the extracting solvent and in all subsequent TLC solvent systems. Free DG in the extract was isolated using TLC (10) and quantified by acetylation with [^{3}H]acetic anhydride as described for [1-^{14}C]acetic anhydride acetylation of DG (7). Twenty five ml. of twice distilled acetic anhydride (136°) reagent grade (Baker Chemical Co., Phillipsburg, N. J.) were mixed with 100 mCi of [^{3}H]acetic anhydride, specific activity, 3 Ci/mmole (Amersham-Searle, Arlington Heights, Ill.). Aliquots (5 ml) were stored at -10°C over anhydrous sodium sulfate and under nitrogen in hypo-seal vials (Pierce Chemical Co., Rockford, Ill.). Acetylation was performed using 50 µl pyridine, 20 µl [^{3}H]acetic anhydride and 0.7 µl 72% $HClO_4$. The reaction was stopped by the addition of 0.4 ml. distilled water and the mixture was extracted twice with 0.5 ml. heptane. The [^{3}H] acetylated DG was isolated by TLC (11), counted and quantified by comparison with [^{3}H] cpm incorporated into 1,2-dipalmitin standard obtained from Applied Science Labs, Inc., State College, Pa.

Table 1.

Effect of ACh on Total DG Level in Mouse Pancreas In Vitro

ACh	DG Conc.			P
(M. conc.)	(μmoles/g. fresh tissue)			
	Mean	SD	N	
0	0.492	±.035	5	-
10^{-7}	0.548	±.035	3	n.s.
10^{-6}	0.496	±.038	3	n.s.
10^{-5}	1.23	±.14	3	<.005
10^{-4}	3.58	±.15	3	<.005

Mouse pancreas tissue was incubated as described in the text. The incubation time was 80 minutes. P values were derived using a Student's t test, two-tailed.
n.s. - no significant difference from the control ($P>0.05$).

RESULTS

The level of DG in mouse pancreas did not change significantly from the control level during incubation with 10^{-7}M or 10^{-6}M ACh. However, a highly significant increase in the level of tissue DG was observed during incubation with 10^{-5}M or 10^{-4}M ACh (Table 1). The increase with 10^{-4}M ACh was much larger than the increase with 10^{-5}M ACh. In the presence of 10^{-4}M ACh, the level of tissue DG increased linearly with time over an 80 minute incubation period (Fig. 1).

Atropine is a specific pharmacological antagonist of the physiological effects of ACh. When atropine (10^{-5}M) was added after 40 minutes of incubation with 10^{-4}M ACh, the DG level fell to the control, unstimulated level during the following 40 minutes; atropine added in a similar manner to control, unstimulated pancreas had no effect on the level of tissue DG (Fig. 1). These results support the conclusion that ACh exerts a specific effect in raising the level of DG in mouse pancreas.

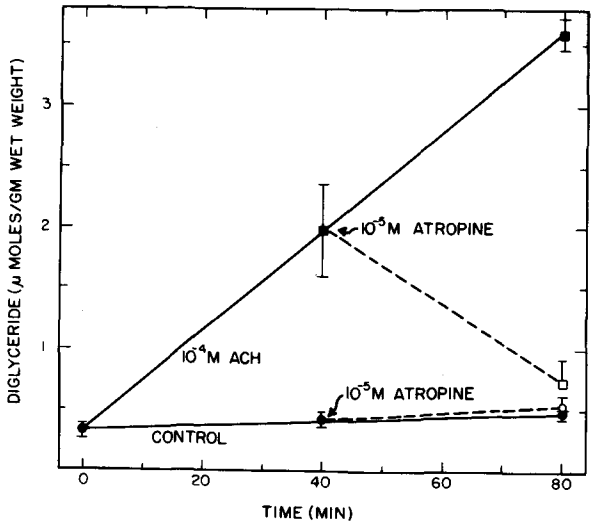

Fig. 1. Time course of the effect of ACh on mouse pancreas DG level, and reversal by atropine. Open square and circle are values for tissue to which atropine was added after 40 minutes of incubation. Data are reported as means ±SD of at least 3 values.

DISCUSSION

It is difficult to correlate the ACh induced changes in DG levels reported here with the ACh induced changes in PI and PA levels previously reported for mouse pancreas (3). A decrease in the level of PI and an approximately equal increase in the level of PA were observed in response to ACh concentrations of 10^{-5}M or higher; however, these changes appeared to be near maximal with 10^{-5}M ACh. In contrast, the level of tissue DG shows a big increase with 10^{-4}M ACh, as compared with 10^{-5}M ACh (see Table 1). It has been suggested that the hydrolysis of PI (12, 13) or PA (6), with the formation of DG, may be the point of action of ACh on phosphatide metabolism. The absolute levels of PI and PA in unstimulated mouse pancreas are 1.31 and 0.45 μmoles per g. fresh tissue, respectively. Even a complete breakdown of these two phosphatides could not account for the increase in DG level of 3.08 μmoles per g. fresh tissue which was observed here with 10^{-4}M ACh. The source of this DG and its fate when the tissue returns to the unstimulated

state remain to be investigated. The best interpretation of these results at the moment seems to be that the ACh induced changes in the level of pancreas DG may not be directly linked to changes in PA and PI metabolism, but that these changes in DG level may represent a separate effect of ACh which might be expected to change the properties of the pancreatic membranous elements in the cell in the highly stimulated state.

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