ACETYLCHOLINE CAUSES A NET DECREASE IN PHOSPHATIDYLINOSITOL AND A NET INCREASE IN PHOSPHATIDIC ACID IN MOUSE PANCREAS

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Summary: Stimulation of mouse pancreas *in vitro* with acetylcholine in concentrations of 10^{-5}M or higher led to a net decrease in phosphatidylinositol and a net increase in phosphatidic acid. The phosphatidylinositol level fell from 1.31 ±0.10 to 0.93 ±0.08 μmoles per gram of fresh tissue; the phosphatidic acid level rose from 0.45 ±0.05 to 0.82 ±0.09 μmoles per gram of fresh tissue. When atropine was added to acetylcholine-stimulated tissue, to cause its return to the unstimulated state, these changes were reversed; the level of phosphatidic acid fell and the level of phosphatidylinositol rose. The levels of the other phosphatides which were measured did not change significantly under these conditions.

ACh[1] produces an increased turnover of phospholipids in pancreas tissue, as measured by the incorporation of radioisotopes; the turnover of PI and PA are the most markedly stimulated (1,2). The present experiments were undertaken in order to see whether there were any net changes in the levels of phospholipids in response to ACh in the pancreas.

METHODS

Mice were killed and the pancreas was quickly removed, chilled, weighed and incubated; the tissue was not sliced (3). Incubations were carried out for 40, 60, or 80 minutes in Krebs-Henseleit bicarbonate saline medium with added glucose (1mg. per ml) at 38°C, with shaking; the gas phase was 95% O_2 + 5% CO_2. Approximately 100 mg. of tissue was incubated in 1 ml. of medium per vessel. [^{32}P]-Orthophosphate was present in the incubation medium in order to label the phospholipids. This was used to check the efficiency of recovery of each phosphatide in each sample, as

[1]Abbreviations are: ACh - acetylcholine; PA - phosphatidic acid; PI - phosphatidylinositol.

described below. Other additions were made as indicated. In all vessels to which ACh was added, eserine sulfate, 10^{-4}M, was also added in order to inhibit ACh-esterase activity. After incubation, the tissues were rapidly frozen in glass tubes in a dry ice alcohol bath. They were homogenized from the frozen state in 5% trichloroacetic acid and the lipids were extracted as described elsewhere (4). PI and PA are relatively minor components of the total phosphatides of the tissue; together they account for about 7% of the total phospholipid P. In order to obtain sufficient material for analysis without overloading the chromatograms with major constituents such as phosphatidylcholine, the lipid extracts were first fractionated by chromatography on small silicic acid columns; fractions were eluted in four mixtures of chloroform and methanol, in the ratios: 12:1; 6:1; 5:2; 3:2. The separate fractions were then submitted to two-dimensional thin layer chromatography. Care was taken to ensure that all PI spots were well separated from phosphatidylserine and lysophosphatidylethanolamine, which can be frequent contaminants. Phosphorus content of the separated lipids was determined by a slight modification of the method of Bartlett (5). Values for PI levels were corrected with reference to the percentage recovery of ^{32}P radioactivity after fractionation and two-dimensional chromatography, as compared with the total radioactivity in PI which had been separated by one-dimensional chromatography of the unfractionated lipid extract on silicic acid-impregnated paper (6). The average recovery of PI after column fractionation and two-dimensional chromatography was 60%; recovery of PA after these procedures was usually quantitative.

RESULTS

There were no significant changes in the levels of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, or lysophosphatidylcholine after incubation of mouse pancreas tissue with either 10^{-7}M or 10^{-5}M ACh. There were no significant changes in the levels of PI or PA with 10^{-7}M ACh. With 10^{-5}M ACh, the level of PI fell from

1.31 ±0.10 (21) to 0.93 ±0.08 (15) μmoles per g. fresh tissue, and the PA level rose from 0.43 ±0.05 (21) to 0.82 ±0.09 (15) μmoles per g. fresh tissue. (Figures are, in order, mean ±SE and, in parentheses, number of observations.) The changes appeared to be near maximal at 10^{-5}M ACh; the sum of the PI and PA in each sample did not change significantly under these conditions (Fig. 1).

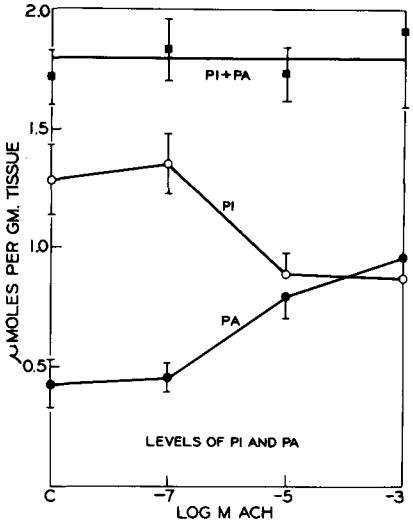

Fig. 1. Levels of PI and PA and the sums of these levels in mouse pancreas after incubation with different concentrations of ACh. Mouse pancreas tissue was incubated as described in the text; incubation time was 60 minutes. Eserine, 10^{-4}M, was present in all vessels which contained ACh. Values are the means ±SE of the following number of observations: Control = C, 21; ACh, 10^{-7}M, 11; ACh, 10^{-5}M, 15; ACh, 10^{-3}M, 3.

Atropine is a specific pharmacological antagonist of acetylcholine. When atropine was added after these changes, they were reversed. In the experiment shown in Table 1, mouse pancreas tissue was first stimulated with ACh, then after 40 minutes, atropine was added to the incubation medium. When the stimulation was "turned off" in this way by atropine, the level of PA fell and the level of PI rose towards the unstimulated, control level.

Table 1.

Effect of Atropine on PI and PA Levels in ACh-stimulated Pancreas

Incubation Conditions	Levels (μmoles/g.tissue)		
	PI	PA	*N*
Control, 0'-40'	1.45 ±.01	0.64 ±.01	2
Control, 0'-80'	1.55 ±.06	0.51 ±.04	4
Control, 0'-40'; Atropine, 40'-80'	1.40 ±.09	0.64 ±.01	2
ACh, 0'-40'	0.97 ±.15	0.90 ±.09	4
ACh, 0'-80'	0.90 ±.12	0.75 ±.05	4
ACh, 0'-80'; Atropine, 40'-80'	1.39 ±.16	0.60 ±.04	4

Mouse pancreas tissue was incubated as described in the text. Atropine was added, where indicated, after 40 min. of incubation, and the incubation was continued for a further 40 min. ACh concentrations were either 10^{-5}M or 10^{-4}M; atropine was added to the ACh vessels to give either 10^{-6}M or 10^{-5}M respectively; atropine was added to the control vessels to give 10^{-5}M. Eserine, 10^{-4}M was present in all vessels which contained ACh. Values are means ±SE.

Atropine did not itself give these effects when added to control, unstimulated tissue (Table 1).

DISCUSSION

A net decrease of PI and a net increase of PA appear to be part of the "phospholipid effect" in the pancreas. The data suggest that there is a net conversion of PI to PA in the stimulated state and a net conversion of PA to PI on return from the stimulated to the unstimulated state. A breakdown of PI could give rise to diglyceride, which could act as a precursor for the formation of PA. In turn, PA is a precursor for the synthesis of PI by the known metabolic pathway, which involves the intermediate formation of cytidinediphosphodiglyceride from cytidinetriphosphate and PA. By these pathways, interconversion of PI and PA, involving turnover of the phosphate and inositol moieties, could take place, with conservation of the diglyceride moiety. This might explain the reciprocal nature of the changes in levels of these two phosphatides. However, further work is necessary to

establish whether such a direct interconversion does indeed take place.

The work presented here was carried out using intact cells. Previous work with avian salt gland tissue, in which PI was prelabeled with inositol-2-[^{3}H] under suitable conditions, indicated that there was a breakdown of PI in response to ACh in salt gland slices (7). There have since been various suggestions that PI hydrolysis may occur in response to ACh in cell-free systems, although the evidence has been somewhat tenuous. Durell and Garland (8) discuss an increased liberation of radioactive organic phosphate compounds from both prelabeled PI and also polyphosphoinositides in response to ACh in crude brain mitochondrial fractions. However, Schacht and Agranoff (9) did not observe a breakdown of prelabeled PI in response to ACh in guinea pig synaptosomes. Canessa de Scarnati and Roderiquez de Lores Arnais (10) have reported a direct activation by ACh of PI-inositol-phosphohydrolase activity in both particulate and soluble fractions of brain synaptosomes; Lapetina and Michell (11) were unable to confirm this. White and Hawthorne (12) did not observe any increase in the activity of PI-inositolphosphohydrolase in the presence of cholinergic drugs in pancreas homogenates or sub-cellular fractions. It does not seem likely therefore that ACh directly activates PI-inositolphosphohydrolase in the pancreas. It is possible that this enzyme is activated indirectly, such as by a second messenger.

Approximately one third of the total PI of pancreas tissue disappears in the presence of 10^{-5}M ACh, and the PA content of the tissue doubles. Such a change might be expected to alter the funcional properties of the membranes in which these two acidic phosphatides are located. It remains to be determined which enzymes are responsible for these changes, and how their activity is changed in the presence of ACh.

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