EFFECTS OF ACETYLCHOLINE ON THE TURNOVER OF PHOSPHORYL UNITS IN INDIVIDUAL PHOSPHOLIPIDS OF PANCREAS SLICES AND BRAIN CORTEX SLICES*

by

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Earlier studies showed that acetylcholine stimulates the turnover of phosphate in the total phospholipids of slices of pancreas, parotid gland, submaxillary gland and brain cortex[1,2,3,4]. In pancreas slices maximal stimulation (up to ten-fold) of phosphate turnover in phospholipids was achieved with a relatively low concentration of acetylcholine ($10^{-5}M$), whilst in brain cortex slices this stimulation increased with increasing concentrations of acetylcholine, being approximately two to three-fold at $10^{-2}M$ acetylcholine. Chemical fractionation procedures showed that in pancreas the turnover of phosphate in the cephalins was stimulated to a much greater extent than in lecithin[2]. This marked difference in the behaviour of the cephalins as compared to lecithin was not found in brain cortex slices. In brain cortex the stimulation of phosphate turnover was greater in the total ether-soluble phospholipids than in the glycerophosphate isolated after strong alkaline hydrolysis of the lipid extract[4]. This indicated that in brain cortex, phosphate turnover in some ether-soluble phospholipid which did not yield glycerophosphate on strong alkaline hydrolysis was particularly responsive to acetylcholine. It was suggested that this phospholipid might be diphosphoinositide.

Naturally, the isolation of the individual phospholipids in pure form would be preferable to the somewhat crude chemical fractionation procedures employed in our earlier studies. Recently, DAWSON[5] described chromatographic procedures for isolating the water-soluble hydrolysis products of the individual phospholipids. The hydrolysis products which were identified on paper were glycerylphosphorylcholine, glycerylphosphorylethanolamine, glycerylphosphorylserine, a phosphorus-containing hydrolysis product of diphosphoinositide, and glycerophosphate. These hydrolysis products were derived from phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, diphosphoinositide and phosphatidic acid, respectively. Quantities of tissue as small as 150 mg were found satisfactory for this procedure. With the use of this method, DAWSON[5] was able to determine the specific activities of the individual phospholipids of brain dispersions after incubation with ^{32}P under appropriate conditions. We have found the procedure of DAWSON[5] to be well suited for studies on the effect of acetylcholine on the turnover of phosphate in the individual phospholipids in small quantities of tissue slices incubated *in vitro*. The data reported below are from such studies.

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MATERIALS AND METHODS

Procedures already described[1,2,4] were used for the preparation and incubation of slices of pigeon pancreas and guinea pig brain cortex. In experiments with brain cortex, equal portions of surface slices from each of two guinea pig brains were added to the control vessel and to the vessel containing acetylcholine (plus eserine). In experiments with pancreas, slices from the pancreas of one pigeon were added to control and to experimental vessels. 50 to 100 mg of tissue were added per ml of incubation medium. The slices were incubated in conical flasks with shaking. The volume of the flask was approximately ten times the volume of the incubation medium.

The following isotopes were used in the various experiments: $NaH_2{}^{32}PO_4$ (obtained from Atomic Energy of Canada, Ltd., Chalk River, Ontario), glycerol-1-^{14}C (obtained from the Radiochemical Centre, Amersham, England), and ethanolamine-2-^{14}C ($HOCH_2{}^{14}CH_2NH_2$) which was synthesised by Dr. D. E. DOUGLAS of the Research Institute, the Montreal General Hospital.

After incubation the tissues were homogenised in 5% trichloroacetic acid and treated according to the method of DAWSON[5], with the following modifications. Instead of ascending chromatography, descending chromatography was used in the second dimension with the *tert*-butanol/trichloroacetic acid solvent. The phosphorus compounds were identified on paper by the method of WADE AND MORGAN[6]. This method does not decompose the compounds, so they can be eluted from the paper. The spots were cut out and eluted with molar ammonia by a previously described technique[7]. Counts and total phosphorus were determined on aliquots of the eluate. Phosphorus determinations on the samples of glycerylphosphorylserine, the hydrolysis product of diphosphoinositide, and glycerophosphate were carried out by the method of BERENBLUM AND CHAIN[8]. Glycerylphosphorylcholine and glycerylphosphorylethanolamine were obtained in larger amounts; the phosphorus in these samples was determined by the method of FISKE AND SUBBAROW[9]. Specific activities after incubation with either ^{32}P or ^{14}C labelled compounds are expressed as counts/min/μg phospholipid P (corrected in the case of ^{32}P to a specific activity of 100,000 counts/min/μg inorganic P in the medium).

RESULTS

Effects of acetylcholine on the incorporation of ^{32}P into the individual phospholipids of pigeon pancreas slices

The paper chromatogram obtained from the lipids of pancreas slices showed phosphorus-containing spots corresponding in R_F values to each of the spots illustrated by DAWSON[5] on a chromatogram obtained from the lipids of a guinea pig brain dispersion. The products of mild alkaline hydrolysis of the lipids of these two tissues would appear therefore to be qualitatively identical. The material from brain tissue which has the lowest R_F in each of the solvents was shown by DAWSON[5] to be hydrolysis product of brain diphosphoinositide. The similarity in R_F values of the corresponding spot on the chromatogram obtained from pancreas material suggests that the inositol-containing phospholipids of pancreas give rise to the same hydrolysis product as that obtained from brain diphosphoinositide. For convenience, we refer to the parent material of this hydrolysis product as pancreas phosphoinositide, although it should be pointed out that there is at this stage no rigorous proof of identification of the material isolated. The parent material of the small quantity of glycerophosphate obtained after mild alkaline hydrolysis of the lipid extracts is referred to as "phosphatidic acid". The existence of phosphatidic acid in the tissue may be an *in vitro* artefact since DAWSON[5] was unable to find free glycerophosphate in alkaline hydrolysates of the lipids extracted from fresh liver or brain.

Of the five phospholipids studied, "phosphatidic acid" and phosphoinositide had the highest ^{32}P specific activity in slices of pigeon pancreas incubated without acetylcholine. The specific activity of phosphatidyl ethanolamine was intermediate; the specific activities of phosphatidyl choline and phosphatidyl serine were by comparison very low. Acetylcholine ($10^{-3}M$) stimulated the incorporation of ^{32}P into each of the five phospho-

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lipids—but to an unequal degree. The average per cent increases in specific activity as compared with the controls in three experiments were as follows: phosphatidyl choline 73%, phosphatidyl ethanolamine 75%, phosphatidyl serine 293%, phosphoinositide 1093%, and "phosphatidic acid" 50%. A representative experiment is shown in Table I.

TABLE I

EFFECTS OF ACETYLCHOLINE ON THE INCORPORATION OF ^{32}P INTO THE PHOSPHOLIPIDS OF PIGEON PANCREAS SLICES

Phospholipid type	*Specific activity (counts/min/μg P)*	
	Control	*With acetylcholine (10^{-3} M)*
Phosphatidyl choline	41	55
Phosphatidyl ethanolamine	236	433
Phosphatidyl serine	9	25
Phosphoinositide	440	7480
"Phosphatidic acid"	754	1250
Calculated overall specific activity*	135	782

* Calculated from the relative quantities of phosphorus in each of the five derivatives isolated (see text).

Approximately 650 mg tissue incubated for 3 hours at 39° C in 14 ml Krebs-Henseleit bicarbonate saline containing 200 mg per cent glucose and $NaH_2{}^{32}PO_4$ (20 μc/ml). Eserine sulphate (final concentration $6 \cdot 10^{-4}$ M) was added with the acetylcholine. All counts are corrected to a specific activity of 100,000 counts/min/μg P for the inorganic P in the medium.

The figures for the specific activity of the "phosphatidic acid" were more variable than for the other derivatives. It is of considerable interest that by far the greatest increase in specific activity after incubation with acetylcholine was found in the hydrolysis product of phosphoinositide. Calculations indicated that the increase in radioactivity of the phosphoinositide accounted on average for about 75% of the increase in the net radioactivity of the "total lipid phosphorus" (calculated by summation of the quantities of phosphorus in the five phospholipid derivatives isolated). It was previously observed that acetylcholine markedly stimulated (850%) the incorporation of ^{32}P into those phospholipids which yield glycerophosphate on strong alkaline hydrolysis[2]. The data in Table I indicate that this could not have been due to the stimulation of ^{32}P-incorporation into phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine. The only substance which shows an increase in specific activity comparable to that of the glycerophosphate obtained on strong alkaline hydrolysis is phosphoinositide. It seems likely therefore that under the conditions used previously (strong alkaline hydrolysis) a large part of the phosphoinositide was hydrolysed to give glycerophosphate. This does not apparently occur to the same extent with brain diphosphoinositide (see below), suggesting that the properties of pancreas phosphoinositide are somewhat different from those of brain diphosphoinositide. The marked stimulation by acetylcholine of ^{32}P incorporation into pancreas phosphoinositide can account for the much greater stimulation of ^{32}P incorporation into the total ether-soluble phospholipids of pancreas as compared with brain cortex slices (compare Tables I and III). This suggests that phosphoinositide may play an important role in pancreatic function; the suggestion is supported by the fact that pancreas has an unusually high inositol content (TAYLOR AND MCKIBBEN[10]). The marked stimulation of ^{32}P incorporation into pancreas phosphoinositide can also

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account for our earlier observation[2] that acetylcholine stimulates the incorporation of ^{32}P into the cephalins three to five times more than into the lecithins.

Although the specific activity of phosphatidyl choline was not high in pancreas slices, its total radioactivity was appreciable, since this substance accounted for 54% of the "total lipid phosphorus". The total activity of phosphatidyl ethanolamine was even greater; this substance had a high specific activity and accounted for 26% of the "total lipid phosphorus". It would be of interest to know whether the relatively high incorporation of ^{32}P into phosphatidyl ethanolamine is related to the high content of phosphorylethanolamine in the pancreas (AWAPARA, LANDUA AND FUERST[11]; TALLEN, MOORE AND STEIN[12].) Phosphatidyl serine, phosphoinositide and "phosphatidic acid" accounted for 10, 8 and 2%, respectively, of the "total lipid phosphorus".

Effect of acetylcholine on the incorporation of ethanolamine-2-^{14}C into phosphatidyl ethanolamine and phosphatidyl choline in pigeon pancreas slices

Earlier studies[2] showed that glycerol-1-^{14}C incorporation into glycerophosphatides in pancreas slices was not stimulated by acetylcholine, indicating that the increased ^{32}P incorporation was due to an accelerated rate of independent turnover of phosphate in the phospholipids. Since increased ^{32}P incorporation in response to acetylcholine in the pancreas takes place in glycerophosphatides in which the phosphate is esterified both to glycerol and to a base or amino acid (choline, ethanolamine or serine) it would be expected that in these phospholipid types the incorporation of the base or amino acid would be increased to the same extent as that of phosphate. In liver, radioactive ethanolamine has been shown to be incorporated into phosphatidyl ethanolamine and, by conversion to choline, into phosphatidyl choline (PILGERAM, GAL, SASSENRATH AND GREENBERG[13]). To test whether acetylcholine stimulates the turnover of the base, pancreas slices were incubated with ethanolamine-2-^{14}C in the presence and absence of acetylcholine ($10^{-3}M$). Table II shows that the ^{14}C was readily incorporated into phosphatidyl ethanolamine, and to a lesser extent into phosphatidyl choline; in slices incubated in the presence of acetylcholine the specific activities of both phosphatidyl ethanolamine and phosphatidyl choline were approximately doubled. These increases in specific activity were of the same order as found with ^{32}P—the average increases in specific activities in three experiments with ^{32}P were 75% for phosphatidyl ethanolamine and 73% for phosphatidyl choline. In view of this it seems likely that acetylcholine stimulates the turnover of phosphorylcholine and phosphorylethanolamine as units in their respective

TABLE II

EFFECT OF ACETYLCHOLINE ON THE INCORPORATION OF ETHANOLAMINE-2-^{14}C INTO THE PHOSPHOLIPIDS OF PIGEON PANCREAS SLICES

Phospholipid type	*Specific activity (counts/min/μg P)*	
	Control	*With acetylcholine (10^{-3} M)*
Phosphatidyl choline	0.9	2.2
Phosphatidyl ethanolamine	79.5	151

Approximately 300 mg tissue incubated for 3 hours at 39° C in 6 ml Krebs-Henseleit bicarbonate saline containing 200 mg per cent glucose and 2.5 μmol/ml ethanolamine-2-^{14}C·HCl (35,000 counts/min/μmol). Eserine sulphate (final concentration $6 \cdot 10^{-4}$ M) was added with the acetylcholine.

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phospholipids. The specific activities after incubation with ^{32}P or ethanolamine-2-^{14}C are not, of course, directly comparable since the specific activities of ^{32}P and ^{14}C in the donor moieties are not known.

Effects of acetylcholine on the incorporation of ^{32}P into the individual phospholipids of guinea pig brain cortex slices

In brain cortex slices ^{32}P was incorporated very readily into diphosphoinositide and "phosphatidic acid" and to a lesser extent into phosphatidyl choline, but there was relatively little incorporation into phosphatidyl ethanolamine and phosphatidyl serine (Table III). Similar findings were made by DAWSON[5] in brain dispersions, except that he failed to find any significant incorporation of ^{32}P into phosphatidyl choline. As compared to the atropinised control, the incorporation ^{32}P into diphosphoinositide and into "phosphatidic acid" was increased about 100% in the presence of acetylcholine ($10^{-2}M$); the incorporation into phosphatidyl choline was increased by about 50%. The specific activities of phosphatidyl ethanolamine and phosphatidyl serine in these experiments were too low for the slight increases to be regarded as significant.

TABLE III

EFFECTS OF ACETYLCHOLINE ON THE INCORPORATION OF ^{32}P INTO THE PHOSPHOLIPIDS OF GUINEA PIG BRAIN CORTEX SLICES

Phospholipid type	*Specific activity (counts/min/μg P)*	
	*Control**	*With acetylcholine (10^{-2} M)*
Phosphatidyl choline	76	126
Phosphatidyl ethanolamine	9	12
Phosphatidyl serine	2	3
Diphosphoinositide	710	1650
"Phosphatidic acid"	1020	2180
Calculated overall specific activity**	70	135

* Atropine sulphate (final concentration 10^{-7} M) was added to the control vessel.

** Calculated from the relative quantities of phosphorus in each of the five derivatives isolated (see text).

Approximately 400 mg tissue incubated for 3 hours at 39° C in 4 ml Krebs-Henseleit bicarbonate saline containing 200 mg per cent glucose and $NaH_2{}^{32}PO_4$ (20 μc/ml). Eserine sulphate (final concentration $6 \cdot 10^{-4}$ M) was added with the acetylcholine. All counts are corrected to a specific activity of 100,000 counts/min/μg P for the inorganic P in the medium.

The observation that acetylcholine stimulated the incorporation of ^{32}P into the total ether-soluble phospholipids to a greater extent than into those ether-soluble phospholipids which yield glycerophosphate on strong alkaline hydrolysis[4] indicates that brain diphosphoinositide, which appears to be responsible for a large part of the overall stimulation, does not yield glycerophosphate to any great extent during strong alkaline hydrolysis. The "total lipid phosphorus" (calculated, as in the case of the pancreas, by summation of the quantities of phosphorus in the five phospholipid derivatives isolated) showed the following per cent composition: phosphatidyl choline, 47.4; phosphatidyl ethanolamine 27.8; phosphatidyl serine 19.3; diphosphoinositide 3.4; "phosphatidic acid" 0.7.

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Incorporation of glycerol-1-^{14}C into the individual phospholipids of guinea pig brain cortex slices.

When slices of brain cortex were incubated with glycerol-1-^{14}C and the specific activities of the various phospholipids determined it was found that these specific activities were all of the same order of magnitude (Table IV). KARNOVSKY AND GIDEZ[14] have provided good evidence that glycerol is an obligatory intermediate in the synthesis of phospholipids. The incorporation of glycerol-1-^{14}C should therefore be a good measure of phospholipid synthesis. If this is so it can be concluded from the data in Table IV that the rate of synthesis of each of the five phosphatides examined is approximately the same.

TABLE IV

THE INCORPORATION OF GLYCEROL-1-^{14}C INTO THE PHOSPHOLIPIDS OF GUINEA PIG BRAIN CORTEX SLICES

Phospholipid type	*Specific activity (counts/min/μg P)*
Phosphatidyl choline	41.0
Phosphatidyl ethanolamine	17.7
Phosphatidyl serine	38.8
Diphosphoinositide	28.8
"Phosphatidic acid"	27.0

Approximately 200 mg tissue incubated 3 hours at 37° C in 4 ml Krebs-Henseleit bicarbonate saline containing 200 mg per cent glucose, 2.5 μmol/ml glycerol-1-^{14}C (1 μc/μmol) and 10^{-7} *M* atropine sulphate.

The relatively equal rates of incorporation of glycerol-1-^{14}C into the phospholipids contrast sharply with the very unequal rates of incorporation of ^{32}P into these phospholipids. KORNBERG AND PRICER[15,16], KENNEDY[17,18] and KENNEDY AND WEISS[19] have shown alternative pathways for the incorporation of ^{32}P and choline into phospholipids in cell-free liver preparations—one in which glycerophosphate is an intermediate for phosphatidic acid synthesis[15,17], one in which free choline and presumably phosphatidic acid are the intermediates for lecithin formation[18] and one in which phosphorylcholine is an intermediate for lecithin formation[16,19]. If, in the present work, glycerophosphate was the intermediate for phospholipid synthesis it follows that the amount of ^{32}P incorporated into that phospholipid with the lowest ^{32}P/^{14}C ratio, *i.e.* phosphatidyl serine, would be the maximum amount of ^{32}P which could be incorporated as a result of phospholipid synthesis–unless it is postulated that the glycerophosphate moieties forming the different phospholipid types have different ^{32}P/^{14}C ratios. Thus with glycerophosphate as an intermediate the bulk of the incorporation of ^{32}P into the various phospholipids would be due to turnover of phosphoryl units on preformed diglyceride. The very high ^{32}P/^{14}C ratio of the glycerophosphate isolated argues against "phosphatidic acid" being a precursor for the incorporation of ^{32}P into phosphatidyl choline, phosphatidyl ethanolamine or phosphatidyl serine, since these all have lower ^{32}P/^{14}C ratios.

If the phospholipids were synthesised by esterification of diglycerides with various phosphoryl units such as phosphorylcholine, phosphorylethanolamine and phosphorylcholine or, in the case of "phosphatidic acid", with free phosphate it becomes more difficult to distinguish between synthesis (defined as including the incorporation of

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glycerol) and turnover (defined as the turnover of phosphoryl units in preformed glyceride). It would be possible to obtain the results shown in Tables III and IV if the phospholipids were synthesised by esterification of newly formed diglyceride with phosphoryl units having different individual specific activities (*e.g.* that of phosphorylserine would be the lowest). By this mechanism different $^{32}P/^{14}C$ ratios could be obtained in different glycerophosphatides without any independent turnover of phosphoryl units taking place on preformed diglyceride. However, in view of the fact that the increased incorporation of ^{32}P into the phospholipids of slices incubated in the presence of acetylcholine is exclusively due to an increased turnover of phosphoryl units in preformed lipid rather than to an increased synthesis of lipid[4], it seems rather likely that an appreciable incorporation of ^{32}P into the phospholipids of slices incubated in the absence of acetylcholine may also the due to this type of turnover. This idea is strengthened by the fact that, in general, phosphate turnover was stimulated most in those phospholipids which have the highest unstimulated rate of ^{32}P incorporation.

The exact chemical composition of the alkaline hydrolysis product of diphosphoinositide which is isolated on paper is unknown. DAWSON[5] was of the opinion that it might be inositol metadiphosphate or that it might contain a glycerol residue. The fact that the specific activity (^{14}C counts/min/μg P) of this substance after incubation with glycerol-1-^{14}C was of the same order of magnitude as that of the hydrolysis products of the glycerophosphatides suggests that this hydrolysis product of diphosphoinositide does contain glycerol.

DISCUSSION

It is of interest that in both pancreas slices and brain cortex slices the major stimulation by acetylcholine of phosphate turnover occurs in the inositol-containing phospholipids. FOLCH[20] has shown that all of the phosphoric acid of brain diphosphoinositide is present in the diesterified form. The structure of pancreas phosphoinositide has not yet been defined; however, there is as yet no evidence that the phosphate of phosphoinositides from other tissues is present in the monoesterified form. One can therefore predict that the stimulation by acetylcholine of the turnover of phosphate in the inositol-containing phospholipids of brain and probably of pancreas involves the turnover of phosphorylinositol as a unit in a manner analogous to the turnover of phosphorylethanolamine and phosphorylcholine.

It is apparent from the effects of acetylcholine on ^{32}P incorporation into phospholipids that enzymes exist which are capable of catalysing the turnover of phosphoryl units on preformed diglyceride. In view of this, the widely accepted concept of the measurement of synthesis of phospholipids by the incorporation of ^{32}P should perhaps be revised. If the synthesis of lipids is defined as including the condensation of fatty acids with glycerol, then in the absence of evidence that glycerol is being incorporated to the same extent as ^{32}P in any particular system, it would perhaps be better to described the incorporation of ^{32}P-labelled compounds as turnover rather than synthesis.

The physiological significance of the stimulation by acetylcholine of the turnover of phosphate and phosphoryl units in the phospholipids of pancreas and brain remains unknown, although these and earlier studies do point to the phospholipids as participating in the physiological events thrown into play by acetylcholine.

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SUMMARY

The incorporation of ^{32}P has been followed in five individual phospholipid types in slices of pigeon pancreas and guinea pig brain cortex, incubated in the presence and absence of acetylcholine. In pancreas slices the average ^{32}P-specific activities of the individual phospholipids show the following increases after incubation with acetylcholine (10^{-3} *M*) as compared with the control: phosphatidyl choline 73%, phosphatidyl ethanolamine 75%, phosphatidyl serine 293%, phosphoinositide 1093%, "phosphatidic acid" 50%. The increase in radioactivity of phosphoinositide accounts for about 75% of the increase in radioactivity of the "total phospholipid phosphorus". In brain cortex slices acetylcholine (10^{-2} *M*) stimulates ^{32}P incorporation into diphosphoinositide and "phosphatidic acid" by about 100% and into phosphatidyl choline by about 50%. The incorporation of ^{32}P into phosphatidyl ethanolamine and phosphatidyl serine under these conditions is too low for the slight increases to be regarded as significant.

The incorporation of ethanolamine-2-^{14}C into phosphatidyl ethanolamine and phosphatidyl choline was followed in pancreas slices incubated in the presence and absence of acetylcholine (10^{-3} *M*). Acetylcholine stimulates the incorporation of ^{14}C into these phospholipids to approximately the same extent as it stimulates the incorporation of ^{32}P. This suggests that acetylcholine stimulates the turnover of phosphorylcholine and phosphorylethanolamine as units in their respective phospholipids.

The rates of incorporation of glycerol-1-^{14}C into the various phospholipids of brain cortex slices are approximately the same, indicating that the total synthesis (or total turnover) of each of these phospholipids is of the same order of magnitude. The rates of ^{32}P incorporation into the various phospholipids in this tissue are highly disproportionate, suggesting that most of the incorporation of ^{32}P into the different phospholipids may be due to the same type of independent turnover of phosphoryl moieties as that which is found in the presence of acetylcholine.

RÉSUMÉ

L'incorporation de ^{32}P dans cinq types individuels de phospholipides a été suivie dans des coupes de pancréas de pigeon et de cortex cérébral de cobaye, incubées en présence et en absence d'acétylcholine. Dans des coupes de pancréas, les activités spécifiques moyennes de ^{32}P dans les phospholipides individuels présentent les augmentations suivantes, après incubation avec de l'acétylcholine (10^{-3} *M*), par rapport à un témoin: phosphatidyl choline 73%, phosphatidyl éthanolamine 75%, phosphatidyl sérine 293%, phosphoinositide 1093%, "acide phosphatidique" 50%. L'augmentation de la radioactivité du phosphoinositoside représente environ 75% de l'augmentation de la radioactivité du "phosphore phospholipidique total". Dans les coupes de cortex cérébral, l'acétylcholine (10^{-2} *M*) stimule l'incorporation de ^{32}P dans le phosphoinositoside et dans l'"acide phosphatidique" d'environ 100% et, dans la phosphatidyl choline d'environ 50%. L'incorporation de ^{32}P dans la phosphatidyl éthanolamine et la phosphatidyl sérine dans ces conditions est trop faible pour que les légers accroissements observés soient significatifs. L'incorporation de la 2-^{14}C-éthanolamine dans la phosphatidyl éthanolamine et dans la phosphatidyl choline a été suivie dans les coupes de pancréas incubées en présence et en absence d'acétyl choline (10^{-3} *M*). L'acétyl choline stimule l'incorporation du ^{14}C dans ces phospholipides à peu près dans les mêmes proportions que celle du ^{32}P. Ceci suggère que l'acétylcholine stimule le "turn-over" de la phosphorylcholine et de la phosphoryléthanolamine en tant que telles dans leurs phospholipides respectifs.

Les vitesses d'incorporation du 1-^{14}C-glycérol dans les divers phospholipides du cortex cérébral sont à peu près les mêmes, ce qui indique que la synthèse totale (ou le "turn-over" total) de chaque phospholipide est du même ordre de grandeur. Les vitesses d'incorporation de ^{32}P dans les divers phospholipides de ce tissu sont fortement disproportionnées, ce qui suggère que la majeure partie de l'incorporation de ^{32}P dans les divers phospholipides peut être due au même type de "turn-over" indépendant des moitiés phosphorylées, que celui qui a été trouvé en présence d'acétylcholine.

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ZUSAMMENFASSUNG

Man verfolgte die Einverleibung von ^{32}P in fünf individuelle Phospholipidtypen in Taubenpankreas- und Meerschweinchengehirnrindenschnitten, welche in Gegenwart und in Abwesenheit von Azetylcholin inkubiert wurden. Nach Inkubation mit Azetylcholin (10^{-3} *M*) wurde in Pankreasschnitten, verglichen mit dem Kontrollversuch, folgende Erhöhung der durchschnittlichen ^{32}P-spezifischen Aktivitäten der individuellen Phospholipide festgestellt: Phosphatidylcholin 73%, Phosphatidylethanolamin 75%, Phosphatidylserin 293%, Phosphoinositid 1093%, "Phosphatidsäure" 50%. Die in der Phosphoinositidfraktion gefundene Erhöhung der Radioaktivität stellt ungefähr 75% der Erhöhung dar, welche in der Radioaktivität des "in Phospholipiden enthaltenen totalen Phosphors" gefunden wurde. In Gehirnrindenschnitten wird durch Azetylcholin (10^{-2} *M*) die Einverleibung von ^{32}P in Diphosphoinositid und "Phosphatidsäure" ungefähr 100 prozentig und in Phosphatidylcholin ungefähr 50 prozentig gesteigert. Die Einverleibung von ^{32}P in Phosphatidylethanolamin und Phosphatidylserin ist unter diesen Bedingungen so gering, dass die kleinen Erhöhungen nicht als bedeutungsvoll angesehen werden können.

Die Einverleibung von Ethanolamin-2-^{14}C in Phosphatidylethanolamin und Phosphatidylcholin wurde in Pankreasschnitten verfolgt, welche in Gegenwart und in Abwesenheit von Azetylcholin (10^{-3} *M*) inkubiert worden waren. Azetylcholin steigert die Einverleibung von ^{14}C in diese Phospholipide ungefähr in gleichem Masse, wie die Einverleibung von ^{32}P. Dies führt zu der Annahme, dass Azetylcholin die Umsetzung von Phosphorylcholin und Phosphorylethanolamin als Einheiten innerhalb ihrer betreffenden Phospholipiden steigert.

Die Einverleibungsgeschwindigkeiten von Glyzerin-1-^{14}C in die verschiedenen Phospholipide der Gehirnrindenschnitten sind ungefähr die gleichen; dieses Ergebnis weist darauf hin, dass die gesamte Synthese (oder gesamte Umsetzung) eines jeden dieser Phospholipide von der gleichen Grössenordnung ist. Die Einverleibungsgeschwindigkeiten von ^{32}P in die verschiedenen Phospholipide dieser Gewebe sind sehr ungleichmässig; dies führt zu der Annahme, dass der grösste Teil der Einverleibung von ^{32}P in die verschiedenen Phospholipide durch denselben unabhängigen Umsetzungstypus der Phosphorylhälften verursacht wird, wie derjenige, welcher in Gegenwart von Azetylcholin gefunden wird.

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