EFFECTS OF ACETYLCHOLINE ON PHOSPHATE TURNOVER IN PHOSPHOLIPIDES OF BRAIN CORTEX *IN VITRO**

by

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We previously reported that acetylcholine or carbamylcholine markedly stimulates the incorporation of ^{32}P into the phospholipides of pancreas slices[1,2]. Preliminary observations also indicated that acetylcholine stimulates the incorporation of ^{32}P into the phospholipides of brain cortex slices, but to a lesser extent than in pancreas[1,3]. This paper is concerned with a more detailed study of the effects of acetylcholine on the phospholipides of guinea pig brain cortex slices, using ^{32}P and glycerol-1-^{14}C. The stimulation of ^{32}P incorporation is shown to occur in several phospholipide fractions, and appears to be due to an increased turnover of phosphate in preformed phospholipides rather than to a synthesis *de novo* of the phospholipide. The stimulation of phosphate turnover in phospholipides takes place in the nuclear, mitochondrial, microsomal and supernatant fractions of brain cortex. Low concentrations of atropine abolish the stimulatory effect of acetylcholine, reducing phosphate turnover in phospholipides about 15% below the level found without added acetylcholine or eserine.

EXPERIMENTAL

Guinea pigs were killed by decapitation, and the brain was removed immediately and placed in chilled saline. Slices were cut from the surface of the cortex only, to avoid white matter. Care was taken to add the same amount of tissue to each vessel in any one experiment. The slices were incubated in either modified[1] "Medium III" of KREBS[4] or in the bicarbonate saline of KREBS AND HENSELEIT[5], the gas phases being oxygen or 7% CO_2 in oxygen, respectively. Glucose (final concentration 200 mg per cent) was added to all vessels. Acetylcholine choride and atropine sulfate were added as indicated; eserine sulfate (final concentration $1.5 \cdot 10^{-4}$ *M*) was always added with acetylcholine. 10–20 μC of $NaH_2{}^{32}PO_4$ were added to each vessel, except in experiments with glycerol-1-^{14}C. In these experiments 3.25 μmol of glycerol-1-^{14}C (1 μC/μmol) were added to each vessel. The slices were incubated at 37° for 1–3 hours. Oxygen uptake was measured when modified KREBS Medium III was used.

Extraction and preparation of phospholipides. After incubation, the ether soluble phospholipides were isolated by previously described procedures[6,7,1] and their specific activities determined. The chloroform soluble phospholipides were prepared by the method of DAWSON[8]. The neutral and acidic phospholipide fractions were prepared from the ether soluble phospholipide extracts by a slightly modified[2] method of TAUROG, ENTENMAN, FRIES AND CHAIKOFF[9]. Glycerophosphate was isolated from the glycerophosphatides by alkaline hydrolysis of the ethanol-ether extracts followed by

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References p. 237.

ionophoresis on paper[2]. This glycerophosphate is derived from glycerophosphoryl choline, glycerophosphoryl ethanolamine, glycerophosphoryl serine, and possibly as yet unidentified glycerophosphatides.

All counts/min/μg P are corrected to 100,000 counts/min/μg P for the inorganic P in the medium. In experiments using glycerol-1-^{14}C the specific activity is expressed as ^{14}C counts/min/μg P of the isolated glycerophosphate.

The tissue phospholipide concentration was determined by making the combined ethanol and ethanol-ether extracts up to volume and estimating the total phosphorus in an aliquot.

Preparation of sub-cellular fractions by differential centrifugation. After incubation, the slices were homogenized for 30 seconds in 9 vol. of 0.25 *M* sucrose in a Teflon homogenizer (Arthur H. Thomas Co., Philadelphia, Pa.). The homogenate was separated into four fractions as follows: R_1 – The residue obtained after spinning at 600 *g* for 10 min; this was reddish and gelatinous in appearance. Microscopic examination revealed mainly nuclei with a few mitochondria and red blood corpuscles. R_2 – The residue obtained after spinning at 12,000 *g* for 10 min; this was whitish-tan and appeared to consist mainly of mitochondria. R_3 – The residue obtained after spinning at 23,000 *g* for 30 min; this was also whitish-tan and contained a few mitochondria and presumably the major portion of the microsomes. S – The supernatant fluid left after the last spinning. All operations were carried out at 0°. The above procedure for isolation of subcellular fractions by differential centrifugation combines features of methods published by SCHNEIDER[10] and BRODY AND BAIN[11].

RESULTS

Effect of acetylcholine on the incorporation of ^{32}P into different phospholipide fractions of brain slices

Acetylcholine stimulated the incorporation of ^{32}P into all the phospholipide fractions of brain cortex slices studied, but to an unequal degree (Table I). The ether soluble phospholipides had a lower net specific activity than the chloroform soluble phospholipides, but the ether soluble phospholipides showed a greater percentage stimulation (Expt. 1, Table I). This suggests that those phospholipides which are insoluble in ether, but soluble in chloroform (possibly sphingomyelins and other as yet unidentified phospholipides), are less responsive to acetylcholine. In the ether soluble phospholipide fraction the acidic fraction (cephalins) had a higher specific activity than the neutral fraction (lecithins) (Expt. 2, Table I). This is in agreement with the findings of other workers on the brain[12,13]. The incorporation of ^{32}P into both the cephalin and lecithin fractions was stimulated by acetylcholine. In pancreas acetylcholine stimulates the cephalins to a much greater extent than the lecithins[14,2]. This striking difference was not found with brain, although it is probable that with the phospholipides from brain a less satisfactory separation of the two fractions was achieved.

TABLE I

THE SPECIFIC ACTIVITIES OF THE PHOSPHORUS OF DIFFERENT PHOSPHOLIPIDE FRACTIONS FROM BRAIN SLICES INCUBATED WITHOUT AND WITH ACETYLCHOLINE

Expt. No.	*Fraction*	*Specific activity (counts/min/μg P)*	
	Types of phospholipide	*Control*	*+ ACh*
1	Ether soluble (Lecithins and Cephalins)	131	272
	Chloroform soluble (Lecithins, Cephalins and Sphingomyelins)	295	400
2	Ether soluble, neutral (Lecithins)	82	138
	Ether soluble, acidic (Cephalins)	189	324
3	Ether soluble (Lecithins and Cephalins)	118	304
	Ether soluble glycerophosphatides	108	176

ACh – acetylcholine (10^{-2} *M* in Expts. 1 and 3; 10^{-3} *M* in Expt. 2). Eserine ($1.5 \cdot 10^{-4}$ *M*) was added with acetylcholine. Slices incubated in modified KREBS medium III for 2 hours.

References p. 237.

The specific activity of the glycerophosphatides from slices incubated without acetylcholine was approximately the same as the specific activity of the ether soluble phospholipides from the same slices (Expt, 3, Table I). However, the specific activity of the ether soluble phospholipides was increased by acetylcholine more than twice as much as the specific activity of the glycerophosphatides of this fraction. This suggests that the incorporation of ^{32}P into some ether soluble phospholipide which does not yield glycerophosphate on alkaline hydrolysis is markedly stimulated by acetylcholine. The brain diphosphoinositide described by FOLCH[15,16] is a constituent which might fit these conditions. It is present with the other cephalins in the ether soluble fraction and, although it contains glycerol, acid hydrolysis yields all the phosphate of the molecule as inositol metadiphosphate.

There was no appreciable change in the total phospholipide concentration on incubation of brain slices either anaerobically or aerobically for as long as three hours in the presence or absence of acetylcholine.

Effects of eserine and increasing concentrations of acetylcholine on the incorporation of ^{32}P into brain phospholipides

The stimulatory effect of acetylcholine on the incorporation of ^{32}P into the phospholipides increased with increasing concentrations of acetylcholine (Fig. 1). The minimum effective acetylcholine concentration, observed in Expt. 1 and obtained by extrapolation in Expts. 2 and 3, was between 10^{-6} and 10^{-5} M. With incubations of 2 or 3 hours duration(Expts. 2 and 3) the specific activities of the ether soluble phospholipides and the glycerophosphatides increased linearly with the logarithm of the acetylcholine concentration, within the range 10^{-5} to 10^{-2} M. With one hour incubation (Expt. 1) the maximum increase in the specific activity of the ether soluble phospholipides was obtained in the presence of 10^{-4} M acetylcholine. Eserine alone increased the incorporation of ^{32}P into the glycerophosphatides about 10% (Expt. 3); although this effect is small it was consistently observed. 10^{-5} M acetylcholine was only slightly more effective than eserine.

Acetylcholine has no effect on the oxygen uptake or the specific activities of the acid-soluble phosphate ester fractions of brain slices[1], indicating that the effects on phospholipides are not secondary to an increase in the permeability of the cell to phosphate ions nor to a general increase in the rate of phosphorylation.

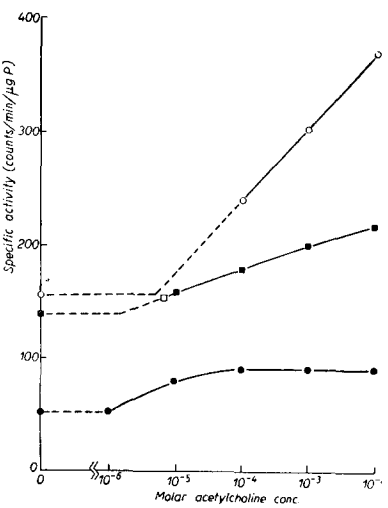

Fig. 1. Effects of eserine and increasing concentrations of acetylcholine on the incorporation of ^{32}P into brain phospholipides. ●——● Expt. 1. 110 mg wet wt tissue in 3 ml of modified KREBS medium III incubated for one hour. Specific activities of the ether soluble phospholipides measured. ○——○ Expt. 2. 100 mg wet wt tissue in 3 ml modified KREBS Medium III incubated for two hours. Specific activities of ether soluble phospholipides measured. ■——■ Expt. 3. 60 mg wet wt tissue in 2 ml bicarbonate saline incubated for three hours. □ – $1.5 \cdot 10^{-4}$ M eserine without acetylcholine. Specific activities of the glycerophosphatides measured. $1.5 \cdot 10^{-4}$ M eserine was added with acetylcholine in all cases.

References p. 237.

Time course of the incorporation of ^{32}P into glycerophosphatides

Acetylcholine increased the incorporation of ^{32}P into the glycerophosphatides only 12% during one hour's incubation as compared to 68% and 82% during two and three hours' incubation, respectively (Fig. 2). It will be noted that this increasing stimulation was primarily due to a decrease in the rate of incorporation of ^{32}P into the phospholipides in the unstimulated tissue. It is possible that the initial rate of incorporation of ^{32}P into the phospholipides in the tissue incubated without added acetylcholine is somewhat stimulated as a result of a release of acetylcholine during the slicing procedure and that the rate of incorporation of ^{32}P in the second and third hours (when the liberated acetylcholine would presumably have been destroyed by choline esterase) more closely represents the true unstimulated rate. It is also possible that the apparent lag in the action of acetylcholine is due to a low permeability of brain cortex slices to this substance. In this connexion it is of interest that in pancreas there is no lag in the action of acetylcholine; furthermore, a maximal stimulation of phosphate turnover in phospholipides is obtained with one-thousandth the concentration of acetylcholine as compared with brain cortex.

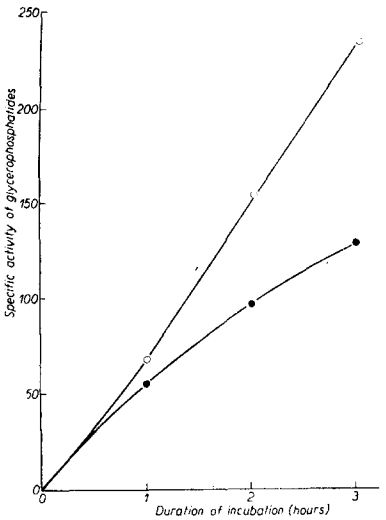

Fig. 2. Time course of the incorporation of ^{32}P into brain glycerophosphatides. O – Slices incubated with acetylcholine (10^{-2} *M*). ● – Control slices. Specific activities are expressed as counts/min/μg P. 80 mg wet wt of tissue was incubated in 2 ml bicarbonate saline. Eserine ($1.5 \cdot 10^{-4}$ *M*) was added with acetylcholine.

Acetylcholine and the incorporation of glycerol-1-^{14}C into brain glycerophosphatides

Acetylcholine stimulates the incorporation of ^{32}P into the glycerophosphatides of pancreas slices over ninefold but does not increase the incorporation of glycerol-1-^{14}C by more than 20%[2]. In brain cortex slices acetylcholine also failed to increase the incorporation of glycerol-1-^{14}C into the glycerophosphatides under conditions in which the incorporation of ^{32}P was increased (Table II). In fact, 10^{-2} *M* acetylcholine consistently inhibited the incor-

TABLE II

COMPARISON OF THE EFFECT OF ACETYLCHOLINE ON THE INCORPORATION OF GLYCEROL-1-^{14}C AND ^{32}P INTO BRAIN GLYCEROPHOSPHATIDES

Expt. No.	*Isotope added*	*Specific activity of glycerophosphatides (counts/min/μg P)*	
		Control	*+ ACh*
1	Glycerol-1-^{14}C	26.0	15.5
	$NaH_2{}^{32}PO_4$	257	375
2	Glycerol-1-^{14}C	34.8	21.8
	$NaH_2{}^{32}PO_4$	249	334

ACh – acetylcholine (10^{-2} *M*). Eserine ($1.5 \cdot 10^{-4}$ *M*) was added with acetylcholine. 100 mg wet wt tissue incubated in 3 ml modified KREBS medium III for 2 hours.

References p. 237.

poration of glycerol-1-^{14}C by about 40%. This inhibition was not found with concentrations of acetylcholine lower than 10^{-2} *M*. From these data it appears that in brain cortex, as well as in pancreas, acetylcholine stimulates the independent turnover of phosphate (and presumably the base attached to it) in preformed phospholipides. The inhibition of the incorporation of glycerol-1-^{14}C by high concentrations of acetylcholine indicates that under these conditions acetylcholine may inhibit the synthesis of glycerophosphatides whilst at the same time it stimulates the turnover of ^{32}P in the molecule.

The incorporation of ^{32}P into the glycerophosphatides of various cell fractions of brain cortex slices

It was thought that fractionation of brain cortex cells by differential centrifugation of slices homogenized after incubation might give some indication as to the site of action of acetylcholine. The stimulation in the presence of acetylcholine was approximately the same in all cell fractions, *i.e.*, nuclear, mitochondrial, microsomal, and soluble (Table III). This suggests that acetylcholine under these conditions affects the phospholipides in more than one cell component. Admittedly, the fractions isolated are not homogeneous or entirely free of contamination from other fractions. But if the incorporation of ^{32}P into phospholipides were stimulated in only one cellular component, it is difficult to see how this component or fragments of this component could so distribute itself in the various fractions to give an approximately equal stimulation in all fractions.

TABLE III

EFFECT OF ACETYLCHOLINE ON THE INCORPORATION OF ^{32}P INTO THE GLYCEROPHOSPHATIDES OF VARIOUS CELL FRACTIONS OF BRAIN CORTEX SLICES

Fraction	*Specific activity of glycerophosphatides (counts/min/μg P)*	
	Control	*+ ACh*
Whole	255	537
R_1 – Nuclei	289	679
R_2 – Mitochondria	184	426
R_3 – Microsomes	200	500
S – Supernatant fluid	292	750

ACh – acetylcholine (10^{-2} *M*). Eserine ($1.5 \cdot 10^{-4}$ *M*) was added with acetylcholine. Guinea pig brain cortex slices (330 mg wet wt) were incubated in 3 ml of modified KREBS Medium III for 2 hours before fractionation.

Effect of atropine on the incorporation of ^{32}P into the phospholipides of brain cortex slices

We have previously shown that atropine abolishes the stimulatory effect of acetylcholine on phosphate turnover in the phospholipides of pancreas slices[1]. As is shown in Table IV, atropine in one-hundredth the concentration of acetylcholine abolished the stimulatory effect of the latter substance on phosphate turnover in the phospholipides, reducing the incorporation of ^{32}P into the phospholipides about 13% below that of the control slices incubated without acetylcholine or eserine.

Fig. 3 shows the effect of increasing concentrations of atropine on the incorporation of ^{32}P into the phospholipides of brain cortex slices incubated in the absence of acetylcholine. 10^{-6} *M* atropine reduced the phosphate turnover in the phospholipides about 15%. Although this reduction was small it was found to be statistically significant (P =

References p. 237.

0.03). This suggests that under the conditions of our experiments the concentration of endogenous acetylcholine in non-eserinized tissue produces a slight stimulation of phosphate turnover in phospholipides.

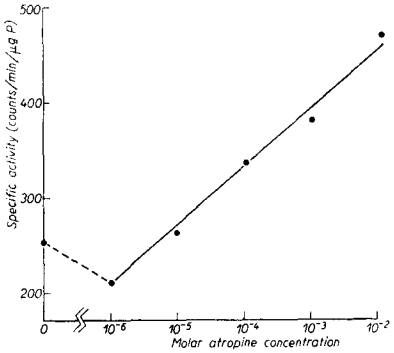

Fig. 3. Effect of atropine on the incorporation of ^{32}P into brain phospholipides. Each point represents an average value from four separate experiments. Approx. 60 mg wet wt tissue were incubated in 2 ml of modified KREBS Medium III for 3 hours. The specific activities of ether soluble phospholipides were measured.

TABLE IV

EFFECT OF ATROPINE ON THE INCORPORATION OF ^{32}P INTO THE PHOSPHOLIPIDES IN THE PRESENCE OF ACETYLCHOLINE

Acetylcholine concentration (Molar)	*Atropine concentration (Molar)*	*Specific activity of ether soluble phospholipides (counts/min/μg P)*
0	0	234
10^{-4}	0	358
10^{-4}	10^{-6}	204
10^{-4}	10^{-5}	227
10^{-4}	10^{-4}	297
10^{-4}	10^{-3}	411

Approximately 75 mg of guinea pig brain cortex slices were incubated in 2 mls of modified KREBS Medium III for 3 hours. Eserine ($1.5 \cdot 10^{-4}$ *M*) was added with acetylcholine.

At higher concentrations (10^{-4} to 10^{-2} *M*) atropine stimulated the incorporation of ^{32}P into the phospholipides (Table IV and Fig. 3); this stimulation increased linearly with the logarithm of the atropine concentration. This stimulatory effect of atropine is of interest, since in high doses this drug is a central nervous system stimulant.

DISCUSSION

Although it is possible that the concentration of acetylcholine attained in the tissue under the conditions of our experiments is considerably less than the external concentration, it is likely that the effects on phospholipides observed with the higher concentrations of acetylcholine represent an exaggerated response of the tissue. In physiological concentrations acetylcholine probably affects a relatively small number of specific "receptor" phospholipide or lipoprotein molecules. A dilution of these "receptor" phospholipides by the "non-receptor" phospholipides, which would also incorporate ^{32}P but which would not be responsive to physiological concentrations of acetylcholine, could account for the small stimulations observed with the lower concentrations of acetylcholine. The fact that low concentrations of atropine reduced to some extent the incorporation of ^{32}P into the phospholipides of brain cortex slices incubated in the absence of added acetylcholine of eserine suggests that the endogenous acetylcholine is sufficient to activate phosphate turnover in phospholipides.

There is good evidence that acetylcholine is a transmitter substance at some, but not all, synapses of the central nervous system, including the cerebral cortex (see FELDBERG[17,18] and ECCLES[19] for reviews; see also MACINTOSH AND OBORIN[20]; BROCK, COOMBS AND ECCLES[21]; and ECCLES, FATT AND KOKETSU[22]). It has been suggested before that phospholipides may be concerned with neural function[23,24], possibly by regulating

References p. 237.

the movements of ions[25,26,27,28,29]. Assuming that acetylcholine is a transmitter at some synapses in the cerebral cortex the evidence presented here would suggest that phospholipides may be concerned with its mechanism of action. As to how acetylcholine activates phosphate turnover in phospholipides and what function this activation might serve in relation to synaptic transmission, we can only speculate at the present stage of the work. It is quite possible that the initial reaction of acetylcholine is with a receptor lipoprotein, leading to a splitting or an alteration in the structure of the latter, which renders the phospholipide portion of the lipoprotein accessible to enzymes which catalyze the turnover of its phosphate[2]. An alteration in structure of lipoproteins in the cell membrane could in itself increase the cation permeability of the membrane and allow the initial influx of sodium, which is presumably responsible for the depolarization of the post-synaptic cell membrane (see ECCLES[19]). But if the effects of acetylcholine observed here are related to its physiological function the changes brought about by this substance would not end at the cell membrane. Our finding that acetylcholine stimulates phosphate turnover approximately to the same extent in the phospholipides of all the cell fractions studied (nuclear, mitochondrial, microsomal, and soluble) suggests that elements within the cell, rather than the cell membrane alone, contain acetylcholine receptors. This view is further supported by the recent observations of BURGEN[30] that in the neurones of the central nervous system (*e.g.* the anterior horn cells) true cholinesterase is concentrated in the cytoplasm of the cell body rather than at the cell membrane.

The effects of acetylcholine on phospholipides within neuronal cells may be concerned with the outflow of potassium from these cells during the repolarization phase of the postsynaptic potential. LING[31] has shown that part of the potassium of skeletal muscle is non-exchangeable. STANBURY AND MUDGE[32] and BARTLEY AND DAVIES[33] have found that a certain amount of potassium is firmly bound to the mitochondria of liver and kidney cortex. Under the conditions of STANBURY AND MUDGE energy is required for exchange of this potassium with extra-mitochondrial potassium. By using 2,4-dinitrophenol these workers showed that this exchange was correlated with the esterification of orthophosphate into some unknown mitochondrial phosphate ester, which was hydrolyzed by 1 *N* HCl at 100° C in 40 minutes. No studies of the physical state of potassium within brain cortex have been carried out, but it is reasonable to assume that a "non-exchangeable"[32] fraction is present here as in skeletal muscle, liver and kidney cortex. Cephalins bind potassium and sodium (CHRISTENSEN AND HASTINGS[25]). It is possible that cephalins and other phospholipides contained in mitochondria, as well as in other intracellular structures, hold potassium in an insoluble ionic complex in the same manner as an acidic ion exchange resin would bind this cation. A splitting of the phosphate from the phospholipide as a result of the action of acetylcholine would liberate this bound potassium, which would then diffuse from the cell. The rephosphorylation of the phospholipide, presumably through the participation of adenosine triphosphate or some such energy-rich phosphate donor, would restore the binding site for potassium and allow its reaccumulation.

SUMMARY

1. The incorporation of ^{32}P into the ether soluble phospholipides of guinea pig brain cortex slices is increased up to three-fold in the presence of acetylcholine (with eserine). The chloroform soluble phospholipides and the glycerophosphatides show a lesser stimulation. The phospholipide content is not appreciably changed during incubation in the presence or absence of acetylcholine.

References p. 237.

2. The minimum effective external concentration of acetylcholine lies between 10^{-6} and 10^{-5} *M*. With 2 to 3 hours incubation the specific activities of the brain phospholipides increase linearly with the logarithm of the external acetylcholine concentration over the range tested (10^{-5} *M* to 10^{-2} *M*).

3. The increased incorporation of ^{32}P into the glycerophosphatides is due to an increased turnover of phosphate in preformed phospholipides rather than to an increased synthesis *de novo* of phospholipides.

4. The stimulation of phosphate turnover in glycerophosphatides in the presence of 10^{-2} *M* acetylcholine is approximately the same in the nuclear, mitochondrial, microsomal and supernatant fractions.

5. Atropine, in very low concentrations (10^{-6} *M*), abolishes the stimulatory effect of acetylcholine (10^{-4} *M*) on phosphate turnover in phospholipides, reducing the turnover about 15% below the level found without acetylcholine or eserine. In higher concentrations (10^{-4} to 10^{-2} *M*) atropine stimulates the incorporation of ^{32}P into the phospholipides.

6. The possible significance of the increased phosphate turnover in phospholipides in response to acetylcholine is discussed.

RÉSUMÉ

1. L'incorporation de ^{32}P dans les phospholipides éthérosolubles de coupes de cortex cérébral de cobaye augmente jusqu'à trois fois en présence d'acétylcholine (et d'ésérine). Les phospholipides solubles dans le chloroforme et les glycérophospholipides présentent une stimulation moins nette. La teneur en phospholipides ne varie pas sensiblement au cours de l'incubation en présence ou en l'absence d'acétylcholine.

2. La concentration externe efficace minimum en acétylcholine est entre 10^{-6} et 10^{-5} *M*. Après 2 à 3 heures d'incubation, les activités spécifiques des phospholipides cérébraux augmentent linéairement en fonction du logarithme de la concentration externe en acétylcholine, dans le domaine de concentrations utilisé (10^{-5} *M* à 10^{-2} *M*).

3. L'incorporation accrue de ^{32}P dans les glycérophosphatides est liée à un "turnover" plus rapide des phosphates dans les phospholipides préformés, plutôt qu'à un accroissement de la synthèse *de novo* des phospholipides.

4. La stimulation du "turnover" des phosphates des glycérophosphatides en présence d'acétylcholine 10^{-2} *M* est approximativement la même dans les fractions nucléaire, mitochondriale, microsomale et surnageante.

5. L'atropine, à très faible concentration (10^{-6} *M*), supprime l'effet stimulant de l'acétylcholine (10^{-4} *M*) sur le "turnover" des phosphates des phospholides, amenant le "turnover" à une valeur 15% plus faible que celle mesurée en l'absence d'acétylcholine ou d'ésérine. A des concentrations plus élevées (10^{-4} à 10^{-2} *M*), l'atropine stimule l'incorporation de ^{32}P dans les phospholipides.

6. La signification possible de l'accroissement du "turnover" des phosphates des phospholipides en présence d'acétylcholine est discutée.

ZUSAMMENFASSUNG

1. Der Einbau von ^{32}P in die Äther-löslichen Phospholipoide der Rindenschnitte des Meerschweinchengehirns wächst auf das dreifache in Gegenwart von Acetylcholin (mit Eserin). Die Chloroform-löslichen Phospholipoide und die Glycerophosphatide zeigen eine geringere Beeinflussung. Der Phospholipoidgehalt ändert sich während der Inkubation in Gegenwart und Abwesenheit von Acetylcholin nicht merklich.

2. Die kleinste noch wirksame äussere Acetylcholinkonzentration liegt zwischen 10^{-6} und 10^{-5} *M*. Bei 2- bis 3-stündiger Inkubation steigen die spezifischen Aktivitäten der Gehirn-Phospholipoide linear mit dem Logarithmus der äusseren Acetylcholinkonzentration im untersuchten Konzentrationsbereich (10^{-5} bis 10^{-2} *M*) an.

3. Das Ansteigen des Einbaues von ^{32}P in Glycerophosphatide beruht mehr auf einem Anwachsen des Phosphataustausches in preformierte Phospholipoide als auf einer gesteigerten Synthese neuer Phospholipoide.

4. Die Anregung des Phosphataustausches der Glycerophosphatide in Anwesenheit von 10^{-2} *M* Acetylcholin ist ännahernd der gleiche in der nuklearen, mitochondralen, mikrosomalen und überstehenden Fraktion.

5. Atropin in sehr niedriger Konzentration (10^{-6} *M*) verhindert den anregenden Effekt des Acetylcholins (10^{-4} *M*) auf den Phosphataustausch in Phospholipoiden, indem es den Phosphataustausch um 15% unter den Wert erniedrigt, der ohne Acetylcholin oder Eserin gefunden wurde.

References p. 237.

In höheren Konzentrationen (10^{-4} bis 10^{-2} *M*) reizt Atropin den Einbau von ^{32}P in die Phospholipoide.

6. Die mögliche Bedeutung des gesteigerten Phosphataustausches in den Phospholipoiden unter dem Einfluss von Acetylcholin wird diskutiert.

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