The Stimulation by Synaptic Transmitters of the Incorporation of Oleate into the Phospholipid of Synaptic Membranes

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1. Noradrenaline stimulated the incorporation of oleate into choline glycerophospholipids of guinea-pig brain synaptic membranes incubated in sodium phosphate buffer. In the presence of 1 mM-NaF, noradrenaline stimulated the incorporation of oleate into the choline glycerophospholipids, phosphatidylinositol, ethanolamine glycerophospholipids, phosphatidylserine and phosphatidic acid of synaptic membranes incubated in 10 mM-Tris-HCl buffer. 2. In Tris-HCl containing 1 mM-NaF, stimulation of incorporation of oleate into choline glycerophospholipids by noradrenaline was enhanced by ATP, CaCl_2 , MgCl_2 and CoA plus dithiothreitol. The optimum concentration of CaCl_2 for stimulation by 10 μM -noradrenaline was 10 μM . In the presence of CaCl_2 , the optimum concentration of ATP-2 MgCl_2 was in the range 0.1–1 mM. 3. Acetylcholine, carbamoylcholine, 5-hydroxytryptamine, dopamine, histamine and γ -aminobutyric acid also stimulated the incorporation of oleate into choline glycerophospholipids of synaptic membranes. Sigmoidal dose-response curves were obtained, similar to those obtained previously for stimulation by the same agonists of the hydrolysis of phosphatidylcholine by phospholipase A2 (Gullis & Rowe, 1975a). 4. The initial rate of transfer of oleate from oleoyl-CoA to choline glycerophospholipid was similar to the initial rate of transfer from oleate-albumin, stimulated by noradrenaline. Transfer of oleate from oleoyl-CoA was not appreciably stimulated by noradrenaline, but was stimulated by ATP and MgCl_2 .

During an investigation of the stimulation, by transmitters and putative transmitters of the central nervous system, of the hydrolysis of phosphatidylcholine by phospholipase A2 of the synaptic membranes of the cortex of guinea-pig brain (Gullis & Rowe, 1975a), it was observed that those agonists which normally stimulated hydrolysis depressed hydrolysis when used in high concentrations. It was also noted that, in the absence of theophylline, fatty acids released on stimulation were rapidly taken up again, although the stimulant was still present. Further investigation revealed that transmitters and putative transmitters, which stimulated hydrolysis of added phosphatidylcholine, under certain conditions also stimulated the acylation, by fatty acids, of endogenous phospholipid in the synaptic membrane. These investigations are now reported.

Experimental

Synaptic membranes were prepared, and the hydrolysis of phosphatidylcholine by phospholipase A2 was determined, as described previously (Gullis & Rowe, 1975a).

Preparation of [9,10- ^3H]oleate-albumin complex and oleoyl-CoA

Oleate-albumin complex was prepared from 1.2 μmol of [9,10- ^3H]oleate (20 Ci/mol) and 12.5 mg of

bovine serum albumin as described by Webster (1967). Oleoyl-CoA was prepared as described by Goldman & Vagelos (1961).

Determination of uptake of fatty acids and the acylation of membrane phospholipids

All incubations were conducted in buffered mixtures at pH 7.4. In experiments where membrane was incubated with radioactive phosphatidylcholine containing a trace of [9,10- ^3H]oleate (see Table 1), the fall in the radioactivity of the 'blank' was a measure of the uptake of oleic acid. This uptake was not calibrated for each experiment. Results presented are standardized such that, if it is assumed that the specific radioactivity of total unesterified fatty acid was 101 Ci/mol as indicated in Table 1, then the scale for fatty acid uptake given in the Figures (Figs. 1, 2, 3 and 5) is in pmol/mg of protein.

In other experiments, synaptic membranes were incubated with 16 nmol of [9,10- ^3H]oleate-albumin or 16 nmol of oleoyl-CoA. Cofactors (including NaF), substrate and neurohormones were added as described previously (Gullis & Rowe, 1975a) for the determination of phospholipid hydrolysis. The reaction was stopped by the addition of 2.0 ml of chloroform-methanol (2:1, v/v). Choline glycerophospholipid was isolated by t.l.c. and counted for radioactivity.

Time-courses were determined for periods of up to 10 min after the addition of substrate and stimulant. The values given in the Tables are calculated from the incorporation of oleate into choline glycerophospholipid after 1 min.

Calculations of concentrations of Ca^{2+} and Mg^{2+} in solution

Calculations were made from the dissociation constants and solubility products given by Sillén & Martell (1964). The data used were as follows. Solubility products: CaHPO_4 , $10^{-6.4}$; $\text{Ca}_3(\text{PO}_4)_2$, $10^{-32.5}$; MgHPO_4 , $10^{-4.5}$; $\text{Mg}_3(\text{PO}_4)_2$, $10^{-23.8}$; CaF_2 , $10^{-10.31}$; MgF_2 , $10^{-8.2}$. Dissociation constants: $\text{H}_3\text{PO}_4 \rightleftharpoons \text{H}^+ + \text{H}_2\text{PO}_4^-$, $10^{-12.4}$; $\text{H}_2\text{PO}_4^- \rightleftharpoons \text{H}^+ + \text{HPO}_4^{2-}$, $10^{-7.0}$ for calcium salts, $10^{-6.7}$ for magnesium salts.

Results

Change of stimulation of phospholipid hydrolysis to stimulation of uptake of oleate

In early experiments, synaptic membranes were incubated with phosphatidylcholine containing traces of radioactively labelled oleic acid. This acid was present owing to its complete removal after preparation of the radioactive phosphatidylcholine, and possibly from breakdown of phospholipid during

Table 1. Unesterified fatty acid present in preparations of phosphatidylcholine used as substrate for the determination of hydrolysis by phospholipase A2

Phosphatidylcholine (1-acyl-2-[9,10- ^3H]oleoyl-*sn*-glycero-3-phosphorylcholine; 10 μmol) was analysed by t.l.c. in the solvent chloroform-methanol-acetic acid-water (25:14:4:2, by vol.). Unesterified fatty acid was extracted from the chromatogram with 50 ml of methanol-water (24:1, v/v) and methylated with diazomethane. Methyl esters were separated by g.l.c. on a column of 5% (w/w) SE-30 at 200°C by using a Pye 104 chromatograph fitted with a stream-splitter. Individual esters were collected by passage of the column effluent through glass wool soaked in light petroleum (b.p. 60–80°C), and their radioactivities were determined. Values are means of two analyses by g.l.c. The recovery of [9,10- ^3H]oleic acid by the procedure was 90%. Calculated specific radioactivity of total unfractionated fatty acid was 101 Ci/mol.

Fatty acid	Yield of phospholipid		Radioactivity (d.p.m.)
	(pmol/ 10 nmol)	(% of total)	
Palmitic	5.40	48.7	0
Stearic	1.25	10.4	0
Oleic	3.00	26.1	2570
Arachidonic	1.80	15.7	0
Total	11.45	—	—

storage. The analysis of unesterified fatty acid in a typical preparation is given in Table 1. The phosphatidylcholine contained 0.06% (mol/mol) of unesterified fatty acid.

In the presence of ATP, MgCl_2 , CaCl_2 and theophylline, noradrenaline stimulated the hydrolysis of phosphatidylcholine by synaptic membranes incubated in 10 mM-Tris-HCl buffer (Fig. 1). This effect has been reported previously (Gullis & Rowe, 1975a). When, however, 10 mM-Tris-HCl buffer was replaced by 10 mM-sodium phosphate, noradrenaline stimulated the uptake of oleate during the first 30 s. This was followed by stimulation of a net release of oleate from phosphatidylcholine, the second stimulation being complete after 2½ min. In 100 mM-sodium phosphate, uptake of oleate was the only effect observed. At 50 mM, sodium phosphate gave effects intermediate between these obtained with 10 mM- and 100 mM-sodium phosphate respectively. In Tris-HCl,

Fig. 1. Effect of sodium phosphate on the stimulation, by noradrenaline, of the hydrolysis of phosphatidylcholine by phospholipase A2, and the uptake of oleate

All incubation mixtures contained the cofactors ATP (0.20 mM), MgCl_2 (0.40 mM), CaCl_2 (1.0 μM), theophylline (0.50 mM) and buffer at pH 7.4. Synaptic membranes were incubated in: ○, 10 mM Tris-HCl; ◇, 10 mM-sodium phosphate; △, 50 mM-sodium phosphate; □, 100 mM-sodium phosphate; ●, 10 mM-Tris-HCl and 10 μM -noradrenaline; ◆, 10 mM-sodium phosphate and 10 μM -noradrenaline; ▲, 50 mM-sodium phosphate and 10 μM -noradrenaline; ■, 100 mM-sodium phosphate and 10 μM -noradrenaline.

Fig. 2. Effects of different concentrations of noradrenaline on the phospholipase A2 fatty-acid uptake system. Cofactors were as described for Fig. 1. Buffers (a, 10mM-Tris-HCl; b, 100mM-sodium phosphate) were at pH7.4. Concentrations of noradrenaline were: $\circ$, zero; Δ , 0.10 μ M; $\blacktriangle$, 1.0 μ M; $\blacksquare$, 10 μ M; $\bullet$, 100 μ M. $\square$, Membrane without cofactors or noradrenaline.

Fig. 3. Effect of oleic acid and oleate-albumin on the hydrolysis of phosphatidylcholine by phospholipase A2. Synaptosomes were incubated in 150mM-sodium phosphate, pH7.4. $\circ$, Oleate-albumin; $\bullet$, oleic acid.

increasing concentrations of noradrenaline increasingly stimulated the release of oleate from phosphatidylcholine (Fig. 2). In 100mM-sodium phosphate, however, the uptake of oleate was increasingly stimulated. The effects of oleate and oleate-albumin on the hydrolysis of phosphatidylcholine, in the absence of noradrenaline, are shown in Fig. 3.

In 50mM-sodium phosphate the net stimulation of the phospholipase A2-fatty acid uptake system by noradrenaline was sensitive to the concentrations of CaCl_2 and MgCl_2 (Fig. 4). CaCl_2 (1 μ M) enhanced the stimulation of the uptake of oleate by noradrenaline. Higher concentrations of CaCl_2 (10 μ M–5 mM) enhanced the stimulation of the hydrolysis of added phospholipid. EGTA [ethanedioxybis(ethylamine)-tetra-acetate], which complexes Ca^{2+} , decreased the

uptake of oleate. MgCl_2 (6mM) changed the stimulation of uptake of oleate to stimulation of hydrolysis of phospholipid (Fig. 4). The effects obtained by using 100mM-sodium phosphate were also obtained with membranes incubated in 10mM-Tris-HCl containing 1mM-NaF, CoA and dithiothreitol, as shown in Fig. 5.

Stimulation of the transfer of oleate from albumin to membrane phospholipid

The transfer of oleate from albumin-oleate to choline glycerophospholipid of synaptic membrane is shown in Fig. 6. In the presence of cofactors, choline glycerophospholipid was acylated at a rate

Fig. 4. Effects of CaCl_2 (a) and MgCl_2 (b) on the stimulation of the phospholipase A2-fatty acid uptake system by noradrenaline in phosphate buffer

Membranes were incubated in 50 mM-sodium phosphate, pH 7.4, with cofactors as described in Fig. 1, except where indicated otherwise. The concentration of noradrenaline was 10 μM . (a) $\circ$, Noradrenaline absent; $\square$, noradrenaline; $\bullet$, noradrenaline and 1.0 mM-EGTA; $\diamond$, noradrenaline and 1.0 μM - CaCl_2 ; Δ , noradrenaline and 0.10 mM- CaCl_2 ; $\triangle$, noradrenaline and 0.50 mM- CaCl_2 . (b) $\circ$, Noradrenaline absent; Δ , noradrenaline present, theophylline omitted; $\square$, noradrenaline with 6.0 mM- MgCl_2 ; $\bullet$, noradrenaline with 6.0 mM- MgCl_2 , and theophylline omitted.

of 0.05 nmol/min per mg of protein. Sodium phosphate slightly decreased and NaF slightly increased this rate. Noradrenaline completely prevented the net transfer of oleate from albumin. However, in the presence of sodium phosphate, noradrenaline stimulated the initial rate of transfer approximately 30-fold. In the presence of NaF, noradrenaline stimulated the initial rate of transfer approximately 40-fold.

Thus 100 mM-sodium phosphate and 1 mM-NaF had similar effects in that they changed the stimulation of the hydrolysis of phosphatidylcholine by noradrenaline to stimulation of incorporation of fatty acid to form choline glycerophospholipid. In view of the complexities of the interactions of phosphate with Ca^{2+} and Mg^{2+} , discussed below 1 mM-NaF was used

as a routine in the investigation of the stimulation of acylation of endogenous lipid. The effects of different concentrations of NaF itself on the acylation of membrane choline glycerophosphate, and on the hydrolysis of phosphatidylcholine by phospholipase A2, are shown in Fig. 7. At 1 mM it slightly enhanced acylation and phospholipid hydrolysis. In other experiments (not shown) 1 mM-NaF slightly decreased phospholipid hydrolysis. The stimulation of phospholipid hydrolysis by 50 mM-NaF was not dependent on ATP and MgCl_2 and thus differed from the stimulations by transmitters (Gullis & Rowe, 1975a).

Effects of cofactors

The effects of ATP, MgCl_2 , CoA + dithiothreitol and noradrenaline on the incorporation of oleate

Fig. 5. Effect of 1 mM-NaF on the stimulation of the phospholipase A₂-fatty acid uptake system of synaptic membranes

Synaptic membranes were incubated in 10 mM-Tris-HCl buffer (pH 7.4) containing ATP (0.20 mM), MgCl₂ (0.40 mM), CaCl₂ (1.0 μM), CoA (1.0 μM), dithiothreitol (10 μM) and theophylline (0.50 mM). Concentrations shown in the Figure refer to noradrenaline. Δ, Noradrenaline absent; □, 0.1 mM-noradrenaline present; ○, noradrenaline present in the concentrations shown; ▲, 1 mM-NaF present, noradrenaline absent; ●, 1 mM-NaF present, noradrenaline present in the concentrations shown.

into choline glycerophospholipid of synaptic membranes are shown in Table 2. A mixture of ATP and MgCl₂ increased the incorporation fivefold. The inclusion of CoA and dithiothreitol in the mixture of cofactors increased the rate of incorporation of oleate tenfold (Expt. 1). In the presence of all the cofactors, including NaF, noradrenaline stimulated incorporation 12-fold (Expt. 2). In the presence of cofactors, the optimum concentration of CaCl₂ for the stimulation by 10 μM-noradrenaline was 10 μM (Fig. 8). Maximum stimulation was obtained with a 1:2 molar mixture of ATP and MgCl₂ in the concentration range 0.10 mM–1 mM ATP (Fig. 9). With membranes incubated with 0.50 mM-ATP, 3.0 mM-MgCl₂, 1.0 μM-CaCl₂, 1.0 μM-CoA and 10 μM-dithiothreitol, the apparent *K_m* for the incorporation of oleate into choline glycerophospholipid was 0.1 mM.

Stimulation of acylation of other classes of phospholipids

In the presence of cofactors, including NaF, oleate was transferred from albumin-oleate into the major

Fig. 6. Effects of sodium phosphate and NaF on the stimulation by noradrenaline of the incorporation of oleate into choline glycerophospholipid of synaptic membrane

Synaptic membranes were incubated with oleate-albumin in the presence of the cofactors ATP (0.50 mM), MgCl₂ (3.0 mM), CaCl₂ (1.0 μM), CoA (1.0 μM), dithiothreitol (10 μM) and theophylline (0.50 mM). ○, Membranes in 10 mM-Tris-HCl; □, membranes in 100 mM-sodium phosphate; ■, membranes in 100 mM-sodium phosphate in the presence of noradrenaline (10 μM); ▲, membranes in 10 mM-Tris-HCl with 1 mM-NaF; △, membranes in 10 mM-Tris-HCl with 1 mM-NaF and noradrenaline (10 μM); ●, membranes in Tris-HCl plus noradrenaline (10 μM), NaF absent.

phospholipid classes in synaptic membranes, the highest incorporation in terms of mg of membrane protein being into choline glycerophospholipids (Fig. 10). The molar concentrations of phosphatidic acid and phosphatidylinositol in synaptic membranes are approx. 2% and 10% respectively that of choline glycerophospholipid (Breckenridge *et al.*, 1972). Thus the greatest incorporations of oleate per mol of phospholipid class were into phosphatidic acid and phosphatidylinositol. Noradrenaline (10 μM) stimulated the incorporation of oleate into all phospholipid classes.

Stimulations by other transmitters and putative transmitters

The incorporation of oleate into choline glycerophospholipid was stimulated by acetylcholine, carbamoylcholine, 5-hydroxytryptamine, dopamine, histamine and γ-aminobutyric acid. The dose-response curves for these stimulations, together with that for noradrenaline, are shown in Fig. 11. Sigmoidal curves were obtained. There was some indication that, at concentrations of stimulant higher than that required for maximum stimulation, there was some

Fig. 7. Effect of NaF on the hydrolysis of phosphatidylcholine by phospholipase A₂, and the incorporation of oleate into choline glycerophospholipid

Membranes were incubated in 10mM-Tris-HCl buffer. O, Hydrolysis of phosphatidylcholine in the presence of cofactors as described for Fig. 1; □, incorporation of oleate into choline glycerophospholipid in the presence of cofactors as described for Fig. 6.

decrease in stimulation. GTP was more effective than ATP in enhancing the stimulation by acetylcholine, but in the presence of GTP ATP was still effective (Table 3). ATP was more effective than GTP in enhancing the stimulation by noradrenaline (Table 3). The concentrations of stimulant giving 50% of the maximum stimulation are given in Table 4, and are compared with those giving maximum stimulation of the hydrolysis of added phosphatidylcholine by phospholipase A₂, in both Tris-HCl and sodium phosphate buffers.

Acylation with oleoyl-CoA

Oleate was readily transferred to choline glycerophospholipid when it was presented to the membrane as oleoyl-CoA (Table 2). ATP and MgCl₂ were necessary for a maximum rate of transfer (Expt. 3), but NaF stimulated transfer only slightly (Expt. 2). CaCl₂ had only slight stimulating and EGTA only slight depressant effects, whereas EDTA greatly decreased incorporation (Expt. 4). The stimulating effects of different concentrations of ATP and MgCl₂ are illustrated in Fig. 12.

Table 2. Effects of cofactors on the transfer of oleate from oleate-albumin and oleoyl-CoA to choline glycerophospholipids of synaptic membranes

All membranes were incubated with 0.50mM-theophylline and, except where stated otherwise, 1.0μM-CaCl₂. Concentrations of reagents were as follows: ATP, 0.50mM; MgCl₂, 3.0mM; CoA, 1.0μM; dithiothreitol, 10μM; NaF, 1.0mM; noradrenaline, 5.0μM.

Expt.	Components of incubation mixtures	Incorporation of oleate (nmol/min per mg of protein)
1	Oleate-albumin	0.10
	Oleate-albumin + ATP + MgCl ₂	0.48
	Oleate-albumin + ATP + MgCl ₂ + CoA + dithiothreitol	1.0
2	(a) Oleate-albumin + ATP + MgCl ₂ + NaF + CoA + dithiothreitol	0.50
	(b) As (a) + noradrenaline	6.0
	(c) Oleate-albumin + ATP + MgCl ₂ + NaF	0.20
	(d) As (c) + noradrenaline	0.38
	(e) Oleate-albumin + ATP + MgCl ₂	0.18
	Oleoyl-CoA + ATP + MgCl ₂	6.0
	Oleoyl-CoA + ATP + MgCl ₂ + NaF	6.5
3	Oleoyl-CoA	2.0
	Oleoyl-CoA + ATP + MgCl ₂	6.3
4	Oleoyl-CoA + ATP + MgCl ₂	7.0
	Oleoyl-CoA + ATP + MgCl ₂ + 0.10mM-CaCl ₂	7.5
	Oleoyl-CoA + ATP + MgCl ₂ + 1.0mM-EGTA	6.5
	Oleoyl-CoA + ATP + MgCl ₂ + 10mM-EDTA	2.0
5	Oleoyl-CoA + ATP + MgCl ₂ + NaF	6.1
	Oleoyl-CoA + ATP + MgCl ₂ + NaF + noradrenaline	6.1

In the presence of ATP and MgCl₂, the rate of transfer of oleate from oleoyl-CoA was approximately the same as that from the noradrenaline-stimulated transfer from oleate-albumin in the presence of cofactors. Moreover acyl transfer from oleoyl-CoA was not stimulated by noradrenaline (Table 2, Expt. 5). These observations imply that the stimulation of acylation by noradrenaline was due to the stimulation of the formation of acyl-CoA.

Discussion

Since, in phosphate buffer, stimulation of uptake of oleate could be changed to stimulation of hydrolysis of phospholipid by the addition of CaCl₂ and MgCl₂, it is possible that predilection of the membrane to stimulation of acylation, by phosphate, could have

Fig. 8. Effect of CaCl_2 on the stimulation of the incorporation of oleate into choline glycerophospholipid by noradrenaline. Cofactors other than CaCl_2 were as described for Fig. 6, with the addition of 1.0mM-NaF. $\circ$, Noradrenaline ($10\mu\text{M}$) present; $\square$, noradrenaline absent.

Fig. 9. Effects of ATP-2MgCl_2 on the stimulation of the incorporation of oleate into choline glycerophospholipid by noradrenaline.

Cofactors other than ATP and MgCl_2 were as described for Fig. 6, with the addition of 1.0mM-NaF. ATP and MgCl_2 were present in the molar ratio 1:2. Concentrations shown on abscissa refer to ATP . $\circ$, Noradrenaline ($10\mu\text{M}$) present; $\square$, noradrenaline absent.

Fig. 10. Comparison of the incorporation of oleate, and the stimulations of the incorporation of oleate by noradrenaline, into different classes of the phospholipids of synaptic membranes.

(a) Noradrenaline absent; (b) noradrenaline present. Cofactors were as described for Fig. 6, with the addition of 1.0mM-NaF. Phospholipid classes were isolated by t.l.c. in two dimensions on Kieselgel H as described by Pumphrey (1969). $\circ$, Choline glycerophospholipid; Δ , phosphatidylinositol; $\square$, ethanolamine glycerophospholipid; $\blacksquare$, phosphatidic acid; $\bullet$, phosphatidylserine.

been due in part to the complexing of Ca^{2+} and Mg^{2+} . Calculated concentrations of Ca^{2+} and Mg^{2+} in solution in 50mM-sodium phosphate are $0.3\mu\text{M}$ and $28\mu\text{M}$ respectively, which are below the optima for stimulation of either phospholipid hydrolysis (Gullis & Rowe, 1975a) or the incorporation of oleate into

choline glycerophospholipid, in Tris-HCl, indicating that complexing of the cation by phosphate might affect the system. On the other hand, with 1mM-NaF the calculated maximum concentrations of Ca^{2+} and Mg^{2+} in solution are $50\mu\text{M}$ and 6.5mM respectively. These are above the optima of added CaCl_2 and

Fig. 11. Dose-response curves for the stimulation of the incorporation of oleate into choline glycerophospholipids by transmitter substances and putative transmitter substances

○, Cofactors as described for Fig. 6, with the addition of 1.0 mM-NaF; □, ATP and $MgCl_2$ omitted. (b) and (c): ○, 0.50 mM-GTP included in the cofactors; □, GTP omitted.

Table 3. Effects of GTP and ATP on the stimulation of the incorporation of oleate into choline glycerophospholipid by acetylcholine and noradrenaline

Membranes were incubated in the presence of 0.50 mM-theophylline. Concentrations of reagents used were as follows: acetylcholine, 10 μ M; noradrenaline, 10 μ M; $MgCl_2$, 3.0 mM; GTP, 0.50 mM; ATP, 0.50 mM.

Reagents added	Incorporation of oleate (nmol/min per mg of protein)
Acetylcholine + GTP + $MgCl_2$	3.8
Acetylcholine + ATP + $MgCl_2$	2.4
Acetylcholine + ATP + GTP + $MgCl_2$	5.6
Noradrenaline + ATP + $MgCl_2$	5.2
Noradrenaline + GTP + $MgCl_2$	3.0

$MgCl_2$ for the stimulation of phospholipid hydrolysis or incorporation of oleate in Tris-HCl under the experimental conditions used. Thus complexing of Ca^{2+} or Mg^{2+} with fluoride is unlikely to have been the cause of predisposition of the membranes to stimulation of incorporation of oleate.

As with the stimulation of hydrolysis of phospholipid by phospholipase A₂, the dose-response curves for the stimulation of uptake of oleate were sigmoidal. Moreover, the theoretical dissociation constants of the hypothetical transmitter-receptor complexes, which are equal to the concentration of transmitters giving half-maximum stimulation (Rang, 1971), were similar for stimulations of both phospholipid hydrolysis and the incorporation of oleate. The dissociation constants for the different stimulants were also similar, although the dissociation constants

Table 4. Concentrations of stimulants giving half-maximum stimulation

Stimulant	-log(Molar concentration of stimulant giving half-maximum stimulation)		
	Hydrolysis of phosphatidylcholine by phospholipase A2		Incorporation of oleate into choline glycerophospholipid
	10mM-Tris-HCl*	150mM-sodium phosphate† pH7.4	10mM-Tris-HCl‡
Noradrenaline	6.2	6.3	6.1
Acetylcholine	5.8	6.3	6.3
Carbamoylcholine	6.2	—	5.9
5-Hydroxytryptamine	6.5	6.0	6.3
Histamine	6.2	6.3	6.7
Dopamine	6.3	6.2	5.8
γ-Aminobutyric acid	6.0	6.2	6.0

* Calculated from data reported previously (Gullis & Rowe, 1975a).
† Concentrations of cofactors were: ATP, 0.50mM; MgCl₂, 1.0mM; CaCl₂, 0.10mM; theophylline, 0.50mM.
‡ Calculated from data presented in Fig. 11.

Fig. 12. Effects of ATP and MgCl₂ on the transfer of oleate from oleoyl-CoA to choline glycerophospholipids of synaptic membranes

Membranes were incubated with oleoyl-CoA in the presence of 1.0mM-NaF and 0.50mM-theophylline. O, 3.0mM-MgCl₂ and ATP varied; □, 0.50mM-ATP and MgCl₂ varied.

for acetylcholine were less than those for carbamoylcholine. The values for acetylcholine were close to that calculated by Changeux *et al.* (1971) for the dissociation constant (0.8μM) for the interaction of the cholinergic agonist decamethonium with the cholinergic receptor isolated from *Electrophorus electricus*.

The main observation is that neurotransmitters and putative neurotransmitters, which were shown previously (Gullis & Rowe, 1975a) to stimulate the hydrolysis of phosphatidylcholine by phospholipase A2 of synaptic membranes, also stimulate, in the presence of sodium phosphate or NaF, the uptake of fatty acid and its incorporation into membrane phospholipid, forming, among other things, choline glycerophospholipid. The observed rates of deacylation and acylation are net rates which reflect a balance between opposing reactions. Stimulations by neurotransmitters reflect changes in this balance.

NaF and sodium phosphate, which switched net stimulation of deacylation by noradrenaline to net stimulation of acylation, are both inhibitors of cerebral protein phosphatase (Maeno & Greengard, 1972). Stimulations of the phospholipase A2-acylation system by cyclic nucleotides, which stimulate cerebral protein kinase (Miyamoto *et al.*, 1969), are reported elsewhere (Gullis & Rowe, 1975b).

The observation that neurotransmitters which stimulate the hydrolysis of phospholipid by phospholipase A2 of synaptic membranes can also, under certain circumstances, stimulate the acylation of membrane phospholipid suggests that the phospholipase A2-acylation cycle might be concerned in the molecular changes taking place in membranes during synaptic transmission. That two opposing reactions can be stimulated by the same transmitters emphasizes the possible importance of modulators, which could determine the direction of stimulation. Although these modulators are at present hypothetical, there is some evidence that Ca²⁺ and phosphate could be involved.

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