THE EFFECTS OF NORADRENALINE, ACETYLCHOLINE, CYCLIC AMP, CYCLIC GMP, AND OTHER AGENTS ON THE CONCENTRATION OF UNESTERIFIED FATTY ACIDS IN SYNAPTOSOMES AND SYNAPTIC MEMBRANES

Robert J. GULLIS and Charles E. ROWE

Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT UK

Received 16 June 1976

1. Introduction

It has been shown previously that the phospholipase A2-acylation system of isolated synaptic membranes of guinea pig cerebral cortex was stimulated, in the presence of appropriate cofactors, by a range of transmitter substances and putative transmitter substances [1,2]. Moreover these effects were mimicked by cyclic nucleotides, including cyclic AMP and cyclic GMP [3]. The previous investigations were carried out using added substrates, namely phosphatidylcholine and oleate-albumin. This communication reports the stimulation of the release or uptake of endogenous fatty acid in isolated synaptic membranes, and in synaptosomes, by noradrenaline, acetylcholine, cyclic AMP, cyclic GMP and other agents.

2. Experimental

Synaptosomes were prepared from guinea-pig cerebral cortex by the method of Eichberg et al. [4]. Synaptic membranes were prepared from osmotically-ruptured synaptosomes by the procedure of Whittaker et al. [5].

2.1. *Determination of unesterified fatty acids*

Lipids were extracted and the extracts washed by the procedure of Folch et al. [6]. Unesterified fatty acids were isolated by t.l.c. on Kieselgel G, using the solvent system light petroleum (b.p. 40–60°C)–diethyl ether–acetic acid (90:53:7, by vol). Fatty acids were methylated with diazomethanol [7] for 1 h. Excess diazomethane was removed by distillation in a rotary evaporator and the methyl esters were stored in light petroleum 40–60°C under N_2 at 0°C. Methyl esters were analysed by g.l.c. on a Pye argon gas chromatography apparatus. Argon at 0.54 kg/cm^2 pressure was passed through columns packed with acid-washed and silanised 'Supasorb', 80–100 mesh, coated with 5% w/w S.E. 30. Determinations were made, from measurements of the areas of recorded peaks, using methyl heptadecanoate as an internal standard.

3. Results and discussion

The effects of noradrenaline, acetylcholine, cyclic AMP, cyclic GMP and other agents including ATP + $MgCl_2$, $CaCl_2$, NaF, AMP, NaCl and KCl, on the concentrations of arachidonic, linoleic and oleic acids in synaptic membranes, and in synaptosomes, are shown in table 1. The changes in concentrations of arachidonic acid in three additional different preparations of synaptic membranes incubated with a selection of the abovementioned agents are compared in table 2.

The acid most affected by the agents used was arachidonic acid which varied from 0.06–3.61 nmol/mg of protein in synaptic membranes and 0.03–3.77 nmol/mg of protein in synaptosomes. Changes in the concentrations of linoleic and oleic acids were much less, and no changes in the concentrations of stearic and palmitic acids were detected. Changes in synaptosomes and synaptic membranes were similar, suggesting

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that the changes in synaptosomes were attributable mainly to the outer membrane.

The concentration of arachidonate was decreased by incubation with a 1:2 molar mixture of ATP and $MgCl_2$ (table 1), and the decrease was greater when time of incubation was extended to 5 min. Such decrease might be expected since ATP promotes the formation of acyl-CoA, and both ATP and $MgCl_2$ stimulate the transfer of acyl groups from acyl-CoA to phospholipid of synaptic membranes [2].

Noradrenaline, acetylcholine, cyclic AMP and cyclic GMP increased the concentration of unesterified fatty acid (table 1). The greatest increase was obtained with cyclic AMP which increased the concentration of arachidonic acid approx. eight-fold (table 1). These observations parallel the stimulations by the same

Table 1
The effects of noradrenaline, acetylcholine, cyclic AMP, cyclic GMP and other agents on the concentration of unesterified fatty acids in isolated synaptic membranes and synaptosomes

		Unesterified fatty acid (nmol/mg of protein)					
		Synaptic membrane			Synaptosomes		
	Conditions	Fatty acid			Fatty acid		
No.	Agent	20:4	18:2	18:1	20:4	18:2	18:1
1	Standard conditions	0.47	0.14	0.13	0.53	0.16	0.24
2	ATP, $MgCl_2$ and theophylline omitted	0.38	0.14	0.13	0.44	0.16	0.24
3	Theophylline omitted	0.19	0.10	0.10	0.28	0.14	0.20
4	Norad· ·naline	2.54	0.14	0.13	2.26	0.16	0.24
5	Acetylc. .ine[a]	2.04	0.14	0.13	1.98	0.16	0.24
6	Cyclic AMP	3.61	0.14	0.13	3.77	0.16	0.24
7	Cyclic GMP	2.70	0.14	0.13	2.89	0.16	0.24
8	NaF	0.16	0.10	0.13	0.19	0.14	0.17
9	$CaCl_2$	0.13	0.10	0.13	0.22	0.14	0.17
10	Noradrenaline + $CaCl_2$	0.19	0.10	0.13	0.28	0.16	0.24
11	Noradrenaline + AMP	0.66	0.14	0.13	0.75	0.16	0.24
12	Noradrenaline + CoA + NaF	0.06	0.10	0.10	0.09	0.10	0.13
13	Noradrenaline + CoA + $CaCl_2$	0.25	0.10	0.13	0.38	0.14	0.17
14	Cyclic AMP + CoA + NaF	0.03	0.07	0.10	0.03	0.07	0.13
15	Cyclic AMP + CoA + $CaCl_2$	0.14	0.07	0.10	0.06	0.07	0.13
16	Cyclic AMP + 100 mM NaCl	0.88	0.14	0.13	1.95	0.16	0.24
17	Cyclic AMP + 100 mM KCl	0.88	0.14	0.13	1.28	0.16	0.24
18	Preincubation with ATP, $MgCl_2$ and $CaCl_2$ (1.0 μM) for 5 min	0.09	0.07	0.10	0.15	0.10	0.17
19	18 + $CaCl_2$ (0.50 mM)	0.06	0.07	0.10	0.13	0.10	0.17
20	18 + AMP	0.60	0.14	0.13	0.69	0.14	0.20
21	18 + 50 mM NaCl	0.63	0.14	0.13	0.78	0.14	0.20
22	18 + 50 mM KCl	0.50	0.14	0.13	0.66	0.14	0.20

[a]GTP was used in place of ATP in the incubation mixture.

Incubations were carried out in 10 mM Tris-HCl buffer, pH 7.4. Conditions 1, 4–15; Tissues were incubated in mixtures containing ATP, 0.20 mM; $MgCl_2$, 0.40 mM; $CaCl_2$, 1.0 μM; theophylline, 0.50 mM; as described previously [1–3]. Concentrations of other agents when present were noradrenaline, 10 μM; acetylcholine, 10 μM; cyclic AMP 1.0 nM; cyclic CMP, 1.0 nM; NaF, 1.0 mM; AMP, 1.0 mM, CoA (1.0 μM). Where indicated (conditions 9, 10, 13 and 15) the concentration of $CaCl_2$ in the cofactors was increased to 0.50 mM. Conditions 11, 16 and 17; AMP, NaCl and KCl were added to the tissue immediately before the addition of the other cofactors. Conditions 18–22; theophylline was ommitted, $CaCl_2$ (0.50 mM) was added in addition to that already present.

Table 2
The effects of noradrenaline, acetylcholine, cyclic AMP, cyclic GMP and other reagents on the concentration of arachidonic acid in different preparations of synaptic membranes

Conditions		Arachidonate (nmol/mg of protein) Preparation		
No.	Agent	1	2	3
1	Standard conditions	1.54	2.64	3.45
2	ATP, $MgCl_2$ and theophylline omitted	1.31	2.17	3.45
3	Theophylline omitted	0.66	1.32	2.14
4	Noradrenaline	2.26	2.89	3.45
5	Acetylcholine	2.04	2.83	3.45
6	Cyclic AMP	2.51	3.45	3.45
7	Cyclic GMP	2.26	3.14	3.45
8	NaF	0.53	0.75	1.63
9	$CaCl_2$	0.63	0.63	1.57

reagents of the hydrolysis of added phosphatidylcholine by phospholipase A2 of synaptic membranes [1].

$CaCl_2$ (0.05 mM) decreased the concentration of arachidonate and also changed the stimulation of the release of arachidonate by noradrenaline to stimulation of uptake of arachidonate. Likewise combined $CaCl_2$ and CoA changed the stimulated release of arachidonate by cyclic AMP to stimulation of uptake. These observations correlate with the observations that 0.50 mM $CaCl_2$ diminished net hydrolysis of phosphatidylcholine by phospholipase A_2 [1] and enhanced the acylation of membrane phospholipid [2]. These correlate also with the observations that, in the presence of cyclic AMP, 0.50 mM $CaCl_2$ suppressed phospholipid hydrolysis and stimulated phospholipid acylation [3].

NaF (1.0 mM) had effects similar to those given by $CaCl_2$. Thus in the presence of NaF, the concentration of arachidonate obtained after incubation for 1 min was diminished. Moreover, when combined with CoA, it reversed the direction of the effects of noradrenaline and cyclic AMP. The latter phenomenon parallels the effect of NaF on the stimulated phospholipase A2-acylation system of synaptic membranes [1–3].

AMP, when added to membranes which had been preincubated with ATP + $MgCl_2$, slightly increased the concentration of arachidonate. This contrasts with the observations obtained with added lipid substrates, in which AMP, when added after preincubation, completely suppressed the hydrolysis of phosphatidylcholine and stimulated the uptake of fatty acid (Gullis and Rowe, unpublished observations). On the other hand, AMP, 100 mM NaCl and 100 mM KCl diminished the cyclic AMP-stimulated increase of arachidonate (table 1) in a manner similar to their reduction of the cyclic AMP-stimulated hydrolysis of added phosphatidylcholine (Gullis and Rowe, unpublished observations).

Thus, with the exception of one effect of AMP, conditions leading to an increase of endogenous unesterified fatty acid were those which stimulated hydrolysis of phosphatidylcholine by phospholipase A2 of synaptic membranes. Also conditions which lead to a decrease of unesterified fatty acid were those leading to acylation of membrane phospholipid. The correlations are in accord with the view that the observed changes in the concentrations of unesterified fatty acids were due to stimulated changes in the phospholipase A2-acylation cycle.

Membranes, which, after incubation for 1 min in the absence of agents, contained high concentrations of arachidonic acid, were most affected by those agents stimulating fatty acid uptake such as ATP + $MgCl_2$, $CaCl_2$ and NaF as shown in table 2. This is illustrated for ATP + $MgCl_2$ in fig.1. Conversely the same membranes were least affected by agents stimulating the release of fatty acids such as noradrenaline, cyclic AMP or cyclic GMP (table 2). This is shown for

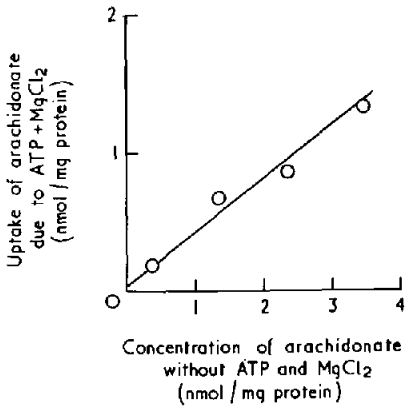

Fig.1. The effect of the concentration of arachidonate on the stimulation of the uptake of arachidonate by ATP + $MgCl_2$. Values are calculated from data given in tables 1 and 2, by subtracting the concentrations of arachidonic acid obtained under condition 2 from those obtained under condition 3.

noradrenaline in fig.2. Thus the effectiveness of the agents depended upon the state of the membranes, and the observations are consistent with the agents stimulating the uptake or release of fatty acid towards an equilibrium value.

Arachidonic acid is a precursor of prostaglandin E2 and F2 [8,9,10] which are both found in brain. Prostaglandin E2 increases the concentration of cyclic AMP in incubated slices of rat cerebral cortex, [11]. It also antagonises the electrical effects of noradrenaline on rat cerebrellar Purkinje cells [12] and the effects of dopamine of hyperpolarising rabbit cervical ganglion [13]. There is evidence that prostaglandin inhibits the release of noradrenaline from the sympathetic nervous system [14]. The results presented in this communication therefore suggest a possibility that noradrenaline- and acetylcholine-stimulated release of arachidonate could lead to the formation of prostaglandins which themselves modulate cerebral activity.

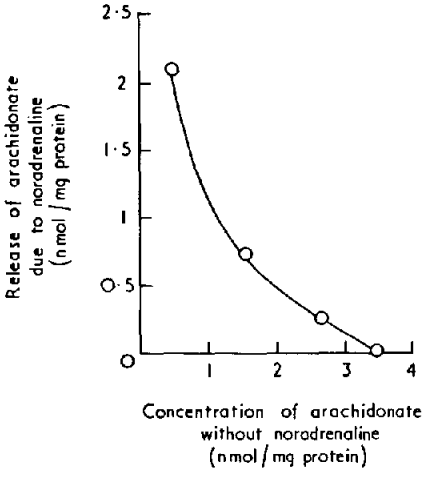

Fig.2. The effect of the concentration of arachidonate on the noradrenaline-stimulated release of arachidonate. Values are calculated from the data given in tables 1 and 2, by subtracting the concentrations of arachidonic acid obtained under condition 1 from those obtained under condition 4.

Acknowledgements

We are grateful to the Medical Research Council for a grant for expenses. This work was done during the tenure by R. J. G. of a Guinness Research Studentship.

References

[1] Gullis, R. J. and Rowe, C. E. (1975) Biochem. J. 148, 197–208.
[2] Gullis, R. J. and Rowe, C. E. (1975) Biochem. J. 148, 557–565.
[3] Gullis, R. J. and Rowe, C. E. (1975) Biochem. J. 148, 567–581.
[4] Eichberg, J., Whittaker, V. P. and Dawson, R. M. C. (1964) Biochem. J. 92, 91–100.
[5] Whittaker, V. P., Michaelson, I. A. and Kirkland, R. J. A. (1964) Biochem. J. 90, 293–303.
[6] Folch, J., Lees, M. and Sloan-Stanley, G. H. (1957) J. Biol. Chem. 226, 497–509.
[7] Lunt, G. G. and Rowe, C. E. (1968) Biochim. Biophys. Acta 152, 681–693.
[8] Van Dorp, D. A., Beerthuis, R. K., Nugteren, D. H. and Vonkeman, H. (1964) Biochim. Biophys. Acta 90, 204–207.
[9] Bergström, S., Danielsson, H. and Samuelsson, B. (1964) Biochim. Biophys. Acta 90, 207–210.
[10] Anggord, E. and Samuelsson, B. (1965) J. Biol. Chem. 240, 3518–3521.
[11] Berti, F., Trabucchi, M., Bernaraeggi, V., Fumagalli, R. (1972) Proc. Int. Conf. on Prostagl. pp. 475–480. Vienna, Pergamon Press-Viewg.
[12] Hoffer, B., Siggins, G. R. and Bloom, F. E. (1969) Science 166, 1418–1420.
[13] Greengard, P. and McAfee, D. A. (1972) Biochem. Soc. Symp. 36, 87–102.
[14] Smith, A. D. (1972) Biochem. Soc. Symp. 36, 103–131.