ESTERS OF ADENOSINE 5'-PHOSPHATE WITH LIPOID HYDROXY COMPOUNDS (ADENOSINE NUCLEOLIPIDS) AND THEIR EFFECTS ON THE ACTIVITY OF ENZYMES OF CYCLIC AMP SYSTEM

Sixtus HYNIE[a] and Jiří SMRT[b]

[a] *Institute of Pharmacology, Faculty of General Medicine, Charles University, 128 40 Prague 2 and*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

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Esters of adenosine 5'-phosphate with lipoid hydroxy compounds exhibit strong inhibitory effect on adenylate cyclase activity. The activities of cyclic AMP phosphodiesterase and protein kinase are moderately inhibited.

Crude preparations of adenylate cyclase from various tissues, exposed to higher concentrations of several hydroxy compounds, form corresponding esters of adenosine 5'-phosphate[1-5]. The physiological significance of these compounds remains undefined and some speculations about their formation under physiological and pathophysiological conditions were expressed[2-6]. In order to evaluate the properties of AMP esters we were studying the influence of chemically synthetized ethyl and 2,3-dihydroxypropyl esters on the activity of enzymes of cyclic AMP system[6]. The only observed effects in concentrations up to 1 mM were those on the protein kinase.

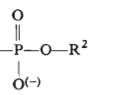
Further speculative possibility is the formation of AMP esters with mono- and diglycerides formed as intermediate products of lipolysis. On the basis of this hypothesis we synthetized adenosine 5'-esters of glycerol monostearate and glycerol monooleate. Since these compounds revealed strong inhibitory effects on the activity of adenylate cyclase, we have synthetized further lipoid residues containing esters of AMP (adenosine nucleolipids) and tested their effects on the activity of enzymes of cyclic AMP system. We now report some representative results of our studies along this line.

EXPERIMENTAL

Table I shows the structure and abbreviations of the nucleolipids used in this study. They were prepared by condensation of corresponding hydroxy compound with per-acetylated AMP

TABLE I

Chemical Structure and Abbreviations of Some Nucleolipids



Abbreviations	R^1	R^2
OG-AMP	3′-oleyl-2,3-dihydroxypropyl	adenosine-5′-yl
SG-AMP	3′-stearoyl-2,3-dihydroxypropyl	adenosine-5′-yl
C_{16}-AMP	hexadec-1-yl	adenosine-5′-yl
C_{18}-AMP	octadec-1-yl	adenosine-5′-yl
PEA-AMP	2-palmitamidoethyl	adenosine-5′-yl
PEA-AMP-Ac	2-palmitamidoethyl	N^6-acetyl-2′,3′-di-O-acetyladenosine-5′-yl
PEA-UMP	2-palmitamidoethyl	uridine-5′-yl

TABLE II

The Effects of Nucleolipids on the Activity of Adenylate Cyclase from Various Tissues, Activated by Different Stimulants

Source of enzyme	Activator	Nucleolipid	Adenylate cyclase activity (%) + S.E.				
			none	0·1	0·32	1·0	3·16 mM
Adipose tissue	none	OG-AMP	100[a]	84 ± 4	56 ± 5	34 ± 2	1 ± 0
	isoproterenol, 0·1 mM	OG-AMP	100[b]	92 ± 5	77 ± 4	36 ± 3	0 ± 0
Liver	sodium fluoride, 10 mM	SG-AMP	100[c]	95 ± 4	86 ± 4	37 ± 2	14 ± 1
	Gpp(NH)p, 0·1 mM	C_{18}-AMP	100[d]	85 ± 6	64 ± 5	43 ± 3	—
	0·1 mM	PEA-AMP	100[d]	70 ± 3	55 ± 3	23 ± 1	
	0·05 mM	PEA-AMP-Ac	100[e]	84 ± 5	40 ± 2	33 ± 0	—
	0·05 mM	C_{16}-AMP	100[e]	90 ± 5	79 ± 5	39 ± 2	—
	0·05 mM	PEA-UMP	100[e]	103 ± 2	105 ± 4	92 ± 5	—
Fibroblasts	PGE_1, 0·05 mM	OG-AMP	100[f]	108 ± 3	60 ± 3	26 ± 1	7 ± 1
	cholera toxin[h]	OG-AMP	100[g]	60 ± 3	47 ± 2	23 ± 2	2 ± 0

[a–g] pmol of cyclic AMP formed/mg protein/10 min: [a] 42 ± 2; [b] 111 ± 3; [c] 203 ± 8; [d] 467 ± 5; [e] 320 ± 2; [f] 416 ± 8; [g] 670 ± 10; [h] 25×10^6 cells of L-fibroblasts were exposed for 24 h to the action of 10 μg of choleragen.

in the presence of N,N'-dicyclohexylcarbodiimide[7], deblocked by methanolic ammonia and purified by silica gel chromatography. All other materials used in this study were mentioned in the previous papers[2,6,8].

The activity of adenylate cyclase was determined according to Ramachandran[9] with modifications described earlier[6]. The phosphodiesterase activity was determined by the modification of Brooker's method[6,10] and the activity of protein kinase was estimated by the slight modicafition of the method of Miyamoto and coworkers[6,11]. Enzyme preparations from rat liver, heart, adipose tissue and from mouse L-fibroblasts were prepared according to published procedures[6,8]. All results are representative experiments carried out in triplicate or duplicate.

RESULTS AND DISCUSSION

The effects of adenosine 5'-phosphate esters with lipoid hydroxy compounds (adenosine nucleolipids) were tested on the activity of adenylate cyclase from rat adipose tissue, rat liver and mouse L-fibroblasts. Table II shows the representative experiments in which the effects of nucleolipids were tested on the basal adenylate cyclase activity or the activity stimulated by the hormone or Gpp(NH)p or sodium fluoride, and in the preparation from L-fibroblasts on the enzyme activity stimulated by the previous exposure of the cells to the action of choleragen for 24 hours. The data in Table II demonstrate that all adenosine nucleolipids tested revealed in concentrations above 1 mM very strong inhibitory effects on adenylate cyclase activity, regardless to the enzyme activity and to the type of the stimulatory agent used. Uridine nucleolipid PEA-UMP was without any inhibitory effect on the hepatic adenylate cyclase stimulated by the addition of Gpp(NH)p. The effects of guanosine nucleolipid PEA-GMP were inconclusive for the poor solubility of the drug.

Table III shows the results of the experiments in which the effects of adenosine nucleolipids were tested on the activity of cyclic AMP phosphodiesterase from rat adipose tissue and liver. Cyclic AMP at 1 μM concentration was used as substrate. The effects of adenosine nucleolipids were compared with the effects of theophylline, AMP and adenosine. With the exception of OG-AMP and PEA-AMP-Ac the inhibitory effects of adenosine nucleolipids were not stronger than the inhibitory effects of adenosine. In experiments with hepatic phosphodiesterase the detergent Lubrol PX did not have any inhibitory effect.

The effects of adenosine nucleolipids were further tested on the activity of protein kinase from rat adipose tissue and heart. In all cases the enzyme was stimulated by the addition of 1 μM cyclic AMP (Table IV). The effects of adenosine nucleolipids were mostly moderately inhibitory with the exception of PEA-AMP-Ac which had somewhat stronger inhibitory effects and C_{18}-AMP which showed some stimulation of protein kinase. The detergents Lubrol PX and Triton X-100 did not have any inhibitory or stimulatory effects on the enzyme activity.

The inhibitory effects of adenosine nucleolipids on the activity of adenylate cyclase seem to be quite specific and in concentrations above 0·1 mM are very strong. Our

unpublished data show that the effect is dependent on the whole molecule of adenosine nucleolipid. Free fatty acids or monoglycerides corresponding to the tested nucleolipids were much slighter inhibitors of adenylate cyclase. Also adenosine revealed lower inhibitory effects than adenosine nucleolipids and moreover, its effects are antagonized by the addition of adenosine deaminase to the assay system. All nucleolipids are to some extent detergents and this might explain their inhibitory effects on adenylate cyclase which is membrane bound enzyme. Nevertheless, the inhibitory effects of corresponding concentrations of Lubrol PX and Triton X-100 were weaker than those of nucleolipids and moreover, equally detergently effective uridine nucleolipid PEA-UMP was without any inhibitory effect. Acylation of PEA--AMP increased the inhibitory activity of the corresponding compound. This intervention did not modify the detergent properties of the drug but probably changed

TABLE III

The Effects of Theophylline, Adenosine, AMP and Adenosine Nucleolipids on the Activity of Phosphodiesterase from Rat Liver and Adipose Tissue

Source of enzyme	Additions	Phospodiesterase activity (%) ± S. E.				
		none	0·1	0·32	1·0	3·16 mM
Adipose tissue	theophylline	100[a]	95 ± 3	85 ± 4	63 ± 3	31 ± 2
	adenosine	100[a]	87 ± 6	100 ± 7	110 ± 6	92 ± 4
	AMP	100[a]	80 ± 3	91 ± 5	97 ± 4	57 ± 3
	OG-AMP	100[a]	74 ± 3	43 ± 3	12 ± 1	2 ± 0
	SG-AMP	100[a]	87 ± 4	85 ± 3	83 ± 6	74 ± 3
Liver	adenosine	100[b]	95 ± 4	80 ± 3	50 ± 4	49 ± 5
	C_{18}-AMP	100[b]	87 ± 6	89 ± 4	84 ± 6	68 ± 6
	PEA-AMP	100[b]	93 ± 6	66 ± 5	60 ± 4	46 ± 3
	PEA-AMP-Ac	100[b]	68 ± 2	49 ± 3	38 ± 3	15 ± 1
Liver	theophylline	100[c]	97 ± 4	80 ± 4	55 ± 2	—
	AMP	100[c]	96 ± 5	95 ± 6	96 ± 7	—
	C_{16}-AMP	100[c]	92 ± 3	93 ± 5	80 ± 6	—
	PEA-AMP	100[c]	92 ± 4	95 ± 6	77 ± 7	—
	PEA-AMP-Ac	100[c]	92 ± 3	78 ± 3	27 ± 4	—
Liver	theophylline	100[d]	95 ± 5	82 ± 2	60 ± 3	27 ± 1
	Lubrol PX	100[d]	106 ± 2	90 ± 5	94 ± 5	82 ± 6
	PEA-AMP	100[d]	102 ± 3	108 ± 7	90 ± 6	82 ± 5
	PEA-AMP-Ac	100[d]	68 ± 5	50 ± 3	38 ± 2	15 ± 2

[a–d] pmol of cyclic AMP hydrolysed/mg protein/20 min (cyclic AMP used at 1 μM concentration); [a] 825 ± 15; [b] 1 250 ± 12; [c] 1 080 ± 20; [d] 1 150 ± 18.

the affinity of the drug to the affected site. Further studies are necessary to disclose the site of action of adenosine nucleolipids which inhibit adenylate cyclase regardless of the degree of enzyme activity and the type of the stimulatory agent. It is also evident that the degree of the inhibition is dependent on the lipoid component and on the intermediate bridge (glycerol, ethanolamine *etc.*).

The activity of phosphodiesterase seems to be influenced by adenosine nucleolipids relatively weekly. The only drug with a substantial inhibitory effect was PEA-AMP-Ac. Thus, it seems that suitable modification of the nucleolipid moiety could change the effects of nucleolipids on the activity of phosphodiesterase. From the experiments with Lubrol PX it is evident that the observed effects are not caused by the detergent properties of the drug used.

The activity of adenosine nucleolipids on protein kinase is inconsistent. Most of these drugs revealed any effect only at very high concentrations, and the effects cannot be caused by the detergent action of the drugs tested. We do not yet have any reasonable explanation for the stronger inhibitory effect of PEA-AMP-Ac and the stimulatory effect of certain concentrations of C_{18}-AMP.

TABLE IV

The Effects of Adenosine and Adenosine Nucleolipids on the Activity of Protein Kinase from Rat Adipose Tissue and Heart

Source of enzyme	Additions	Protein kinase activity (%) ± S.E.				
		none	0·1	0·32	1·0	3·16 mM
Adipose tissue	adenosine	100[a]	—	69 ± 2	40 ± 2	5 ± 1
	SG-AMP	100[a]	—	120 ± 6	94 ± 6	13 ± 1
	OG-AMP	100[a]	—	115 ± 7	100 ± 3	51 ± 4
Adipose tissue	adenosine	100[b]	108 ± 4	101 ± 6	50 ± 2	15 ± 1
	Lubrol PX	100[b]	114 ± 2	118 ± 4	126 ± 4	120 ± 3
	Triton X-100	100[b]	109 ± 3	117 ± 2	126 ± 6	113 ± 5
	PEA-AMP	100[b]	85 ± 4	76 ± 3	64 ± 5	32 ± 4
	PEA-AMP-Ac	100[b]	76 ± 4	32 ± 2	8 ± 2	8 ± 3
Adipose tissue	PEA-AMP	100[c]	55 ± 8	67 ± 8	69 ± 9	—
	PEA-AMP-Ac	100[c]	67 ± 7	76 ± 9	74 ± 8	—
	C_{18}-AMP	100[c]	100 ± 5	123 ± 5	181 ± 8	—
Heart	PEA-AMP	100[d]	98 ± 4	97 ± 5	94 ± 7	82 ± 5
	PEA-AMP-Ac	100[d]	102 ± 7	97 ± 6	83 ± 2	23 ± 3
	C_{18}-AMP	100[d]	90 ± 4	109 ± 5	130 ± 4	14 ± 2

[a–d] nmol of ^{32}P incorporated into histone/mg protein/15 min (in the presence of 1 μM cyclic AMP); [a] 3·1 ± 0·1; [b] 3·1 ± 0·1; [c] 2·6 ± 0·2; [d] 3·2 ± 0·2.

Our results show that adenosine nucleolipids might be useful compounds which would be able to antagonize the increased formation and action of cyclic AMP. In accordance with this suggestion, we have shown in our preliminary experiments that these compounds are strong inhibitors of hormonally stimulated lipolysis[12].

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