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

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1. Introduction

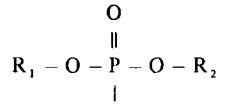
It has been shown by several investigators that crude preparations of adenylate cyclase, exposed to higher concentrations of several hydroxy compounds, form corresponding esters of adenosine 5'-phosphate [1–6]. Esters of this type might be theoretically formed in vivo after ethanol digestion or from glycerol released during the lipolysis [6,7]. The physiological significance of these compounds, however, remains undefined.

Since mono- and di-glycerides are formed as intermediate products during lipolysis we have synthetized, as further theoretically possible alcohol esters of AMP formed in the body, the adenosine 5'-esters of glycerol-monooleate, glycerol-monostearate and further lipoid residues containing drugs. We report here some representative results following the effects of these compounds (adenosine nucleolipids) on the activity of enzymes of cyclic AMP system.

2. Materials and methods

Table 1 shows the chemical structure and abbreviations of the adenosine nucleolipids used in this study. They were prepared by condensation of corresponding hydroxy compound with peracetylated AMP in the presence of *N,N'*-dicyclohexylcarbodiimide, purified after deblocking by preparative paper chromatography and characterized by elemental analysis. Corresponding guanosine nucleolipids were water insoluble. 2-Palmitamidoethyl ester of uridine 5'-phosphate was prepared similarly to PEA-AMP.

Table 1
Chemical structure and abbreviations of some adenosine nucleolipids



Abbreviations	R_1	R_2
OG-AMP	3-oleoyl-2',3'-dihydroxy-propyl	adenosine-5'-yl
SG-AMP	3-stearoyl-2',3'-dihydroxy-propyl	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

All other materials used in this study were as in [2,7,8].

The activity of adenylate cyclase was determined by using [α-^{32}P]ATP [7] and the product was separated on alumina [9]. The activity of phosphodiesterase and protein kinase were determined by the slight modifications of the methods in [7,10] and [7,11] respectively. Enzyme preparations from rat adipose tissue, liver, heart and mouse L-fibroblasts were prepared by the methods in [7,8]. All results are representative experiments carried out in triplicate or duplicate.

3. Results

The effects of adenosine nucleolipids were tested on the activity of adenylate cyclase, phosphodiesterase and protein kinase from several rat tissues (table 2). The data in table 2 demonstrate that all adenosine nucleolipids tested at 0.32–1.0 mM revealed inhibitory effects on adenylate cyclase activity, regardless of the degree of enzyme activity and of the type of the stimulatory agent used (hormone, sodium fluoride, guanylylimidodiphosphate, cholera toxin). Uridine nucleolipid PEA-UMP was without any inhibitory effect on the hepatic adenylate cyclase and the effects of PEA-GMP were inconclusive because of the poor solubility of the drug (results not shown).

Adenosine nucleolipids in concentrations which

Table 2
The effects of adenosine nucleolipids on the activity of adenylate cyclase, phosphodiesterase and protein kinase from various tissues

Enzyme source	Enzyme activity in % ± SE			
	Additions	None	0.1 mM	0.32 mM	1.0 mM
Adenylate cyclase					
	OG-AMP	100[a]	84 ± 4	56 ± 5	34 ± 2
Adipose tissue	OG-AMP	100[b]	92 ± 5	77 ± 4	36 ± 3
Liver	SG-AMP	100[c]	95 ± 4	86 ± 4	37 ± 2
	C_{16}-AMP	100[d]	90 ± 5	79 ± 5	39 ± 3
	C_{18}-AMP	100[e]	85 ± 6	64 ± 5	43 ± 3
	PEA-AMP	100[e]	70 ± 3	55 ± 3	23 ± 1
Fibroblasts	OG-AMP	100[f]	108 ± 3	60 ± 3	26 ± 1
	OG-AMP	100[g]	60 ± 3	47 ± 2	23 ± 2
Phosphodiesterase					
	OG-AMP	100[h]	74 ± 3	43 ± 3	12 ± 1
Adipose tissue	SG-AMP	100[h]	87 ± 4	85 ± 3	83 ± 3
Liver	C_{16}-AMP	100[i]	92 ± 3	93 ± 5	80 ± 6
	C_{18}-AMP	100[j]	87 ± 6	89 ± 4	84 ± 6
	PEA-AMP	100[i]	92 ± 4	95 ± 6	77 ± 7
	PEA-AMP	100[k]	102 ± 3	108 ± 7	90 ± 6
Protein kinase					
	OG-AMP	100[l]	–	115 ± 7	100 ± 3
Adipose tissue	SG-AMP	100[l]	–	120 ± 6	94 ± 6
	C_{18}-AMP	100[m]	100 ± 5	122 ± 5	181 ± 8
	PEA-AMP	100[n]	85 ± 4	76 ± 3	64 ± 5
Heart	C_{18}-AMP	100[o]	90 ± 4	109 ± 5	130 ± 4
	PEA-AMP	100[o]	98 ± 4	97 ± 7	94 ± 7

[a–g] Activity of adenylate cyclase in pmoles of cyclic AMP formed/mg protein/10 min (the activator used in the particular experiment is given in the bracket): [a] 42 ± 2 (basal value); [b] 111 ± 3 (0.1 mM isoproterenol); [c] 203 ± 8 (10 mM sodium fluoride); [d] 320 ± 2 (0.05 mM Gpp(NH)p); [e] 467 ± 5 (0.1 mM Gpp(NH)p); [f] 416 ± 8 (0.05 mM PGE_1); [g] 670 ± 10 (25×10^6 cells of L-fibroblasts were exposed for 24 h to the action of 10 μg choleragen)

[h–k] Activity of phosphodiesterase in pmol cyclic AMP hydrolysed/mg protein/20 min (cyclic AMP 1 μM): [h] 825 ± 15; [i] 1080 ± 20; [j] 1250 ± 12; [k] 1150 ± 18

[l–o] Activity of protein kinase in nmoles of ^{32}P incorporated into histone/mg protein/15 min (with 1 μM cyclic AMP): [l] 1.1 ± 0.1; [m] 2.6 ± 0.2; [n] 3.1 ± 0.1; [o] 3.2 ± 0.2

inhibited the activity of adenylate cyclase revealed, with the exception of OG-AMP, only weak inhibitory effects on the phosphodiesterase activity from rat adipose tissue and liver (table 2). Their inhibitory effect never exceeded the effect of adenosine (data not shown).

The activity of the protein kinase from rat adipose tissue and heart, stimulated by 1 μM cyclic AMP, was affected by adenosine nucleolipids quite inconsistently. Only high concentrations of most adenosine nucleolipids revealed a slight inhibition of the enzyme, and C_{18}-AMP showed some stimulation of protein kinase (table 2).

4. Discussion

Alcohol esters of AMP are formed by adenylate cyclase preparations [1–6] and therefore these compounds might have a feedback regulatory role on enzymes of cyclic AMP system [7]. For the same reason we have tested the effects of adenosine nucleolipids, on the activity of adenylate cyclase, phosphodiesterase and protein kinase. It is not yet known whether such compounds might be formed under physiological or pathophysiological conditions. Nevertheless, these compounds due to their increased lipophilicity penetrate cells and might be effective drugs which could modify the formation of cyclic AMP.

The inhibitory effects of adenosine nucleolipids on the activity of adenylate cyclase seem to be quite specific, and at > 0.1 mM have inhibitory effects. 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 less inhibitory. Also adenosine was much less inhibitory than adenosine nucleolipids. The inhibitory effects of these compounds are dependent on the presence of adenosine moiety because the corresponding uridine nucleolipid is without any inhibitory effect on adenylate cyclase activity. 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. Also the elucidation of structure–activity relationships will require further studies.

The activity of phosphodiesterase seems to be influenced by adenosine nucleolipids only weakly. Unpublished experiments showed that the strong inhibitory effects of OG-AMP are caused by a parallel inhibitory effect of unsaturated oleic acid.

The effects of adenosine nucleolipids on protein kinase are inconsistent. We do not yet have a reasonable explanation for the stimulatory effect of certain concentrations of C_{18}-AMP.

Our results show that adenosine nucleolipids might be useful into antagonizing the increased formation of cyclic AMP. In accordance with this suggestion, we have shown in preliminary experiments that these compounds are strong inhibitors of hormonally stimulated lipolysis in rat epididymal adipose tissue in vitro [12]. Further experiments along this line are in progress.

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