THE INHIBITORY EFFECTS OF SOME ADENOSINE NUCLEOLIPIDS ON THE LIPOLYSIS IN RAT EPIDIDYMAL FAT PADS *in vitro**

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3′-Oleoyl-2,3-dihydroxypropyl-AMP, 3′-stearoyl-2,3-dihydroxypropyl-AMP, octadecyl-AMP and palmitamidoethyl-AMP inhibited in comparison with adenosine or fatty acids much stronger the lipolysis in rat epididymal fat pads *in vitro* stimulated by isoproterenol, theophylline and dibutyryl cyclic AMP. The inhibition of the effects of the two latter drugs suggest that the described effect is caused not only by the inhibition of the cyclic AMP production but also by the inhibition of its effect on the following steps in process of lipolysis.

There have been described several compounds that might serve as physiological regulators of cyclic AMP-dependent lipolysis. In addition to the "feedback regulator" (FR) of Ho and coworkers[1,2] whose structure is yet unknown, the most frequently discussed drugs in this context are adenosine[3-7] and fatty acids[8-10]. The present study was designed to investigate the nature of the inhibitory effects of new drugs called adenosine nucleolipids[11,12] which are esters of adenosine 5′-phosphate with lipoic hydroxy compounds. Since these drugs revealed very strong inhibitory effects on the activity of adenylate cyclase from several tissues[11,12] and since due to their increased lipophility they may penetrate into the intact cells, their effects were tested on the lipolysis in rat epididymal fat pads *in vitro* stimulated either by isoproterenol or theophylline or dibutyryl cyclic AMP. These effects were compared with the inhibitory effects of adenosine, fatty acids and glycerol-monooleate.

EXPERIMENTAL

Following adenosine nucleolipids, prepared as described earlier[12], were used in this study: 3′-oleoyl-2,3-dihydroxypropyl ester of 5′-AMP (OG-AMP), 3′-stearoyl-2,3-dihydroxypropyl ester of 5′-AMP (SG-AMP), octadec-1-yl ester of 5′-AMP (C_{18}-AMP) and 2-palmitamidoethyl ester of 5′-AMP (PEA-AMP). Adenosine and monoolein were products of Sigma, Saint Louis, Missouri, U.S.A. Theophylline, palmitic and stearic acids were from Lachema, Brno, Czechoslovakia. Isoproterenol was purchased from Winthrop Laboratories, N.Y., U.S.A. and dibutyryl cyclic AMP from C. F. Boehringer und Soehne, Mannheim, Germany. Human serum

albumin was obtained from Sevac, Šarišské Michalany, Czechoslovakia. All listed and unlisted chemicals were commercial preparations and were used without further purification.

Lipolysis in adipose tissue: Male rats used in this study (Wistar strain), weighing 150—200 g and fed *ad libitum*, were obtained from Velaz (Prague, Czechoslovakia).

Lipolysis in adipose tissue was determined by the rate of glycerol release from minces of epididymal fat pads[13]. Minced adipose tissue in the amount of 50 mg was incubated for 1 h at 37°C, in 1 ml of Krebs-Riger phosphate buffer (pH 7·4) containing 2·4% (0·34 mM) purified human albumin and tested substances. The reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid. After centrifugation, the lipolytic activity was estimated by the measurement of glycerol[14] in the supernatant.

RESULTS

In the series of experiments (Table I) we examined the influence of OG-AMP in comparison with that of glycerol-monooleate (OG) on lipolysis in epididymal adipose tissue stimulated by (1) isoproterenol or (2) theophylline or (3) dibutyryl-cyclic AMP. The results showed that nucleolipid OG-AMP in 1 mM concentration inhibited lipolysis stimulated by isoproterenol by about 75% and lipolysis stimulated by theophylline and dibutyryl-cyclic AMP by about 60%. Glycerol-monooleate in 1 mM concentration was without any inhibitory effect in cyclic AMP dependent lipolysis. In the second series of experiments (Table II) the antilipolytic effects of another two nucleolipids, SG-AMP and PEA-AMP, were tested in comparison with the effects of adenosine. PEA-AMP revealed very strong inhibitory effect on the lipolysis stimulated by all three activating agents tested while the inhibitory effect of SG-AMP was evident only on lipolysis stimulated by theophylline and dibutyryl-cyclic AMP. Adenosine in 1 mM concentration was without any antilipolytic effect.

TABLE I

The Lipolytic Effect of Isoproterenol or Theophylline or Dibutyryl-cyclic AMP in Rat Epididymal Adipose Tissue *in vitro* in the Presence of 3′-Oleoyl-2,3-dihydroxypropyl-AMP (OG-AMP) or Glycerol-monooleate (OG)

The results are expressed in μmol of glycerol g/hour released after subtraction of basal values (1·0 ± 1 μmol/g/hour); given mean values ±S.E., $n = 6$.

Group	Isoproterenol 0·1 mM	Theophylline 10 mM	Dibutyryl cyclic AMP 2 mM
Controls	2·8 ± 0·2	6·2 ± 0·5	5·6 ± 0·5
OG-AMP, 1 mM	0·7 ± 0·1	2·7 ± 0·2	2·3 ± 0·2
OG, 1 mM	2·7 ± 0·2	6·0 ± 0·5	6·6 ± 0·7

In the third series of experiments (Table III) the antilipolytic effects of PEA-AMP and C_{18}-AMP were compared with the effects of palmitic and stearic acids. Both fatty acids caused only insignificant decrease of glycerol formation in all three types

TABLE II

The Comparison of the Antilipolytic Effect of Adenosine, 3'-O-Steoroyl-2,3-dihydroxypropyl--AMP (SG-AMP) and Palmitamidoethyl-AMP (PEA-AMP) in Rat Epididymal Adipose Tissue *in vitro*

The results are expressed in absolute values of glycerol released after subtraction of basal values (0·9 ± 0·1 μmol/g/hour); given mean values ±S.E. from 6 experiments.

Group	Isoproteronol 0·1 mM	Theophylline 10 mM	Dibutyryl cyclic AMP 2 mM
Controls	4·1 ± 0·3	8·5 ± 0·7	7·8 ± 0·8
Adenosine, 1mM	3·5 ± 0·4	8·0 ± 0·7	6·9 ± 0·7
SG-AMP, 1 mM	4·7 ± 0·3	6·2 ± 0·5[a]	5·4 ± 0·4[a]
PEA-AMP, 1 mM	0·7 ± 0·1[b]	3·1 ± 0·2[b]	2·7 ± 0·2[b]

[a] Nonsignificant decrease in comparison with the effect of lipolytic agents in the controls. [b] Statistically significant differences in comparison with the effects of lipolytic agents in the controls for $P < 0·01$.

TABLE III

The Comparison of the Antilipolytic Effect of Palmitamidoethyl-AMP (PEA-AMP) and Octadecyl-AMP (C_{18}-AMP) with the Effects of Palmitic and Stearic Acids in Rat Epididymal Adipose Tissue *in vitro*

The results are expressed in absolute values of glycerol released after subtraction of basal values (1·0 ± 0·1 μmol/g/hour); given mean values ±S.E. from 6 experiments.

Group	Isoproterenol 0·1 mM	Theophylline 10 mM	Dibutyryl cyclic AMP 2 mM
Controls	4·5 ± 0·3	6·7 ± 0·4	6·5 ± 0·5
PEA-AMP, 1 mM	0·4 ± 0·1[a]	1·3 ± 0·1[a]	1·0 ± 0·1[a]
Palmitic acid, 1 mM	3·6 ± 0·4[b]	6·2 ± 0·5[b]	6·1 ± 0·6[b]
C_{18}-AMP, 1 mM	2·0 ± 0·1[a]	3·0 ± 0·2[a]	3·1 ± 0·2[a]
Stearic acid, 1 mM	3·6 ± 0·4[b]	6·4 ± 0·7[b]	5·4 ± 0·5[b]

[a] Statistically significant differences in comparison with the effects of lipolytic agents in control group for $P < 0·01$. [b] Nonsignificant decrease in comparison with the effect of lipolytic agents in the controls.

of lipolysis activation. Both PEA-AMP and C_{18}-AMP significantly decreased lipolysis stimulated by isoproterenol, theophylline and dibutyryl-cyclic AMP. In all cases the antilipolytic effect of PEA-AMP was about 2–3 times stronger than that of C_{18}-AMP.

DISCUSSION

Adenosine nucleolipids are a new group of compounds which showed high inhibitory properties on activity of adenylate cyclase from several tissues[11,12]. These compounds due to their increased lipophility may penetrate the cell membrane and regulate the level of cyclic AMP within the cell. To test this hypothesis a series of experiments was undertaken on the lipolysis in rat epididymal fat pads which is known to be mediated by cyclic AMP and where the inhibition of adenylate cyclase should inhibit the lipolysis. The first aim of our experiments was to disclose whether adenosine nucleolipids have any inhibitory effect on lipolysis and, in the positive case, to compare their effect with very well known inhibitory effects of adenosine[3–7] and fatty acids[8–10]. The second aim was to try to elucidate, at least partially, the site of their action.

For this reason we have tested the effects of all above mentioned substances on the lipolysis stimulated by three different ways, namely by the hormonal agonist which occupies membrane receptor and stimulates adenylate cyclase, by the inhibitor of phosphodiesterase which increases intracellular concentration of cyclic AMP and finally by direct application of dibutyryl cyclic AMP whose effects are at least

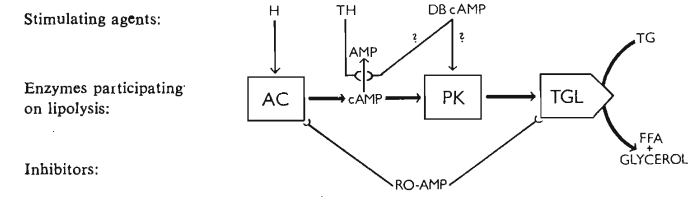

FIG. 1

Schematic Representation of Cyclic AMP-Dependent Lipolysis in Rat Epididymal Adipose Tissue *in vitro*, Stimulated by Hormonal Agonists (H) or Theophylline (TH) or Dibutyryl Cyclic AMP (DBc AMP), and its Inhibition by Adenosine Nucleolipids (RO-AMP)

Explanations: AC adenylate cyclase, PK protein kinase, TGL triglyceride lipase, TG triglycerides, → activation, —(inhibition ➡ conversion of the substrates.

partially caused by the direct effect of this compound on the activity of protein kinase[15]. Data presented in our work clearly demonstrate that adenosine nucleolipids penetrate into the fat tissue and modify the events inside their cells in a manner that manifests itself as a decreased glycerol production. Their effect is observable in concentration where well known inhibitors of lipolysis, adenosine and fatty acids (the critical ratio[10] fatty acid: albumine = 3 was not exceeded) were actually inactive. The comparison of the effects of some nucleolipids showed that the activity is strongly dependent on the lipoid moiety and can be changed by chemical modification of this part of the molecule.

As the site of action of adenosine nucleolipids is concerned, it follows from the inhibition of lipolysis *in vitro* stimulated by the activation of adenylate cyclase by the hormonal agonist or by the increase of cyclic AMP due to the inhibition of phosphodiesterase or finally by the direct effect of dibutyryl cyclic AMP that these compounds act on at least two different sites. Fig. 1 shows schematically the possible sites of action of adenosine nucleolipids. The inhibition of lipolysis stimulated by the application of phosphodiesterase inhibitor or dibutyryl cyclic AMP suggest that the described effect is caused not only by the inhibition of cyclic AMP production, as could be expected from the inhibitory effects of adenosine nucleolipids on the activity of adenylate cyclase[11,12], but also by the inhibition of its effect on the following steps in the process of lipolysis. The stronger inhibition of lipolysis caused by the stimulation of adenylate cyclase by the hormonal agonist suggest that the inhibition of this enzyme contributes to the overall inhibitory effect. Inhibition of protein kinase does not seem to be decisive for the antilipolytic effects because adenosine nucleolipids revealed only very weak inhibitory effects on protein kinase activity from various tissues[11,12]. One of the possible targets of the inhibitory effects of adenosine nucleolipids is, in addition to adenylate cyclase, the triglyceride lipase.

REFERENCES

1. Ho R. J., Sutherland E. W.: J. Biol. Chem. *246*, 6822 (1971).
2. Ho R. J., Russell T. R., Asakawa T., Hucks M. W.: J. Cycl. Nucl. Res. 1, 81 (1975).
3. Davies J. I.: Nature (London) *218*, 349 (1968).
4. Fain J. N., Pointer R. H., Ward W. F.: J. Biol. Chem. *247*, 6866 (1972).
5. Fain J. N.: Pharmacol. Rev. *25*, 67 (1973).
6. Schwabe U., Ebert R., Erbler H. C.: Naunyn-Schmiedeberg's Arch. Pharmacol. *276*, 133 (1973).
7. Schwabe U., Ebert R.: Naunyn-Schmideberg's Arch. Pharmacol. *282*, 33 (1974).
8. Rodbell M.: Ann. N. Y. Acad. Sci. *131*, 302 (1965).
9. Burns T. W., Langley P. E., Robinson G. A.: Metabolism *24*, 265 (1975).
10. Fain J. N., Shepherd R. E.: J. Biol. Chem. *250*, 6586 (1975).
11. Hynie S., Smrt J.: FEBS (Fed. Eur. Biochem. Sect.) Lett. *94*, 339 (1978).
12. Hynie S., Smrt J.: This Journal *44*, 1645 (1979).

13. Schusterová D., Krčíková D., Mühlbachová E., Hynie S., Wenke M.: Int. J. Neuropharmacol. *3*, 129 (1964).
14. Lambert M., Neish A. C.: Can. J. Res., Sect B *28*, 83 (1950).
15. Robinson G. A., Butcher R. W., Sutherland E. W.: *Cyclic AMP*, p. 286. Academic Press, New York and London 1971.

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