STIMULATION OF LIPOPROTEIN LIPASE ACTIVITY OF RAT ADIPOSE TISSUE AND POST-HEPARIN PLASMA BY N^6-(PHENYLISOPROPYL)ADENOSINE

Jorma J. OHISALO[+,*], Häkan STRANDBERG*, Ella KOSTIAINEN*, Timo KUUSI[†] and Christian EHNHOLM*

[+]*Department of Medical Chemistry, University of Helsinki, Siltavuorenpenger 10A, 00170 Helsinki 17,* [*]*Central Public Health Laboratory* and [†]*Third Department of Medicine, University Central Hospital, Helsinki, Finland*

Received 20 July 1981

1. Introduction

Adenosine has profound effects on adipose tissue metabolism. It is an inhibitor of β-adrenergic stimulation of cyclic AMP accumulation [1,2] and lipolysis [2–10] in the isolated rat fat cell. It also opposes the effects of adrenaline on phosphate uptake [11]. In vivo, the nucleoside has been reported to lower plasma free fatty acid and glycerol concentrations [8]. The effects of adenosine are thought to be mediated by two membrane receptors, one requiring an intact ribose (R-site) and the other one an intact purine moiety [12–14] (P-site). Adenosine is rapidly metabolized but its effects on the R-site [12–14] are shared by purine-substituted analogs such as N^6-(phenylisopropyl)adenosine that are not deaminated by the enzyme adenosine deaminase (EC 3.5.4.4) [15].

Lipoprotein lipase (EC 3.1.1.3.4) is the enzyme responsible for the hydrolysis and uptake of triglycerides from circulating chylomicrons and very low density lipoproteins [16–19]. The enzyme is thought to be located on the endothelial surface of the capillary bed of extrahepatic tissues and it can be released into the circulation by intravenous injection of heparin [16–20]. Another heparin-releasable ectoenzyme is probably located on the outer surface of the endothelial cells of the liver and it is therefore called hepatic lipase [20]. This enzyme has been suggested to hydrolyze high density lipoprotein phospholipids and triglycerides [21].

This work was undertaken to find out if N^6-(phenylisopropyl)adenosine has effects on lipoprotein lipase activity and, consequently, on the uptake of circulating triglycerides into tissues.

2. Materials and methods

Male Lewis albino rats (150–250 g body wt) were used. They were fed ad libitum with a standard chow until injected intraperitoneally with 50–300 μl isotonic NaCl with/without N^6-(phenylisopropyl)adenosine, at which time food was withheld. At the times indicated, the animals were anaesthetized with ether and blood was drawn by cardiac puncture into heparinized syringes. For post-heparin plasma lipase assays, blood was drawn 2 min after intravenous injection of heparin (2000 units/kg body wt). The blood was centrifuged and the resulting plasma was stored at −20°C until assayed.

Free glycerol was estimated enzymatically by using the commercial kit no. 125 032 of Boehringer-Mannheim GmbH. The assays for porst-heparin plasma lipases and the antibody to rat hepatic lipase have been described in [22]. The method used to measure heparin-releasable lipoprotein lipase activity of adipose tissue will be presented in detail in the text.

N^6-(phenylisopropyl)Adenosine was a gift from Dr Harald Stork of Boehringer-Mannheim GmbH. Glyceroltri-[^{14}C]oleate (49 mCi/mmol) was from the Radiochemical Centre (Amersham). Heparin was from Medica (Helsinki). All other reagents were from Sigma (St Louis MO).

3. Results

First, the finding that N^6-(phenylisopropyl)adenosine lowers the plasma concentration of free glycerol [8] was confirmed (table 1). The decrease was dose-dependent and a significant effect was observed at

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.50

Table 1
Effect of N^6-(phenylisopropyl)adenosine on plasma free glycerol concentrations in the rat

Dose (μg/kg)	n	Plasma glycerol (μM ± SEM)
0.0	6	462 ± 23
1.2	3	343 ± 10
3.6	5	388 ± 17
12.0	3	267 ± 7
480.0	3	287 ± 3

Fed rats were injected intraperitoneally with different doses of N^6-(phenylisopropyl)adenosine and food was withheld. The animals were killed 90 min later and plasma glycerol was determined as described in the text. n = number of animals

1.2 μg/kg body wt. As noted in [8], the effect was transient with a maximum 1–2 h after injection. For subsequent studies, 12 μg/kg body wt was chosen.

To investigate the effect of N^6-(phenylisopropyl)-adenosine on post-heparin plasma lipolytic activities, rats were anaesthetized and injected intravenously with 2000 U heparin/kg body wt 2 h after intraperitoneal injection of the nucleoside analog (12 μg/kg body wt) or saline and the animals were exsanguinated 2 min later. It is evident from fig.1 that post-heparin plasma lipoprotein lipase activity was increased by the nucleoside analog. The increase was 57% over control and it was statistically significant at $p < 0.001$ Student's t-test). In contrast, hepatic lipase activity was not affected by N^6-(phenylisopropyl)adenosine. This finding was confirmed in two similar experiments, one of which was performed using rats that had fasted overnight.

Table 2
Effect of N^6-(phenylisopropyl)adenosine on adipose tissue lipoprotein lipase activity

	n	Lipase activity (± SEM)
Saline	5	170 ± 36
N^6-(phenylisopropyl)-adenosine	5	480 ± 94[a]

[a] Different from control at $p < 0.05$

Rats were injected intraperitoneally with N^6-(phenylisopropyl)-adenosine (12 μg/kg) or isotonic saline and were killed 90 min later. Heparin-releasable lipoprotein lipase activity was determined as described in the text. The activities are expressed as nmol unesterified fatty acids released . g tissue^{-1} . h^{-1}. n = number of animals

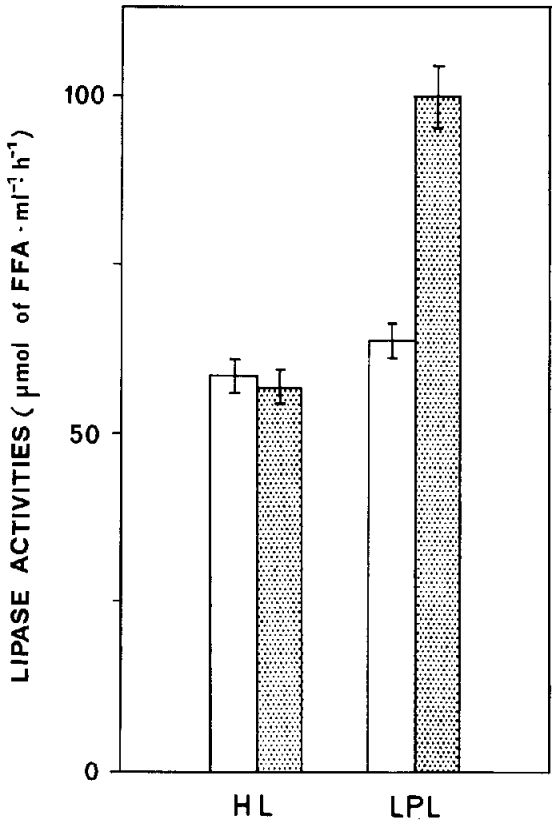

Fig.1. Stimulation of post-heparin plasma lipoprotein lipase activity by N^6-(phenylisopropyl)adenosine. Groups of 8 fed male rats (av. 168 g body wt) were injected intraperitoneally with N^6-(phenylisopropyl)adenosine (12 μg/kg) or isotonic NaCl under ether anaesthesia, and food was withheld. After 2 h the animals were anaesthetized again and injected intravenously with heparin (2000 U/kg); 2 min later the animals were exsanguinated and plasma lipolytic activities were determined as described in the text. Vertical bars indicate SEM; white columns, control; shaded columns, N^6-(phenylisopropyl)-adenosine; HL, hepatic lipase; LPL, lipoprotein lipase.

In subsequent experiments, we investigated the effect of the nucleoside analog on adipose tissue heparin-releasable lipase activity. N^6-(phenylisopropyl)-Adenosine (12 μg/kg) or saline were injected intraperitoneally to fed male rats. The animals were killed 90 min later, and small pieces (≤500 mg) of perigonadal adipose tissue were shredded and incubated for 40 min in 1000 μl 25 mM Tris–125 mM NaCl–5 mM KCl–1 mM $CaCl_2$–2.5 mM $MgCl_2$–1 mM KH_2PO_4–4 mM glucose–2% bovine serum albumin at pH 8.4, in the presence of heparin (50 U/ml) at constant shaking at 120 Hz at +37°C; 100 ml medium was then assayed

for lipoprotein lipase activity as in [23]. The results summarized in table 2 show that the activity was elevated >2.8-fold after intraperitoneal injection of N^6-(phenylisopropyl)adenosine.

4. Discussion

Adenosine mimicks the action of insulin on catecholamine-stimulated lipolysis and cyclic AMP accumulation [1–10] and on glucose metabolism [24]. It also opposes the effect of adrenaline on phosphate uptake [11]. These results show that adenosine analogs also elevate adipose tissue lipoprotein lipase activity and therefore may enhance the uptake of circulating triglycerides into the fat cells. These effects are elicited by a purine-substituted analog and therefore they are probably mediated by the postulated R-site [12]. The R-site adenosine effect may thus prove to be useful in the treatment of some forms of diabetes.

The heparin-releasable lipoprotein lipase activity of isolated fat cells and adipose tissue segments increases during incubation [16,25–29]. These results offer an explanation to this phenomenon. Isolated rat fat cells are known to release adenosine into their incubation medium [1] at pharmacologically effective concentrations. As N^6-(phenylisopropyl)adenosine enhanced the enzyme activity, it is probable that endogenous adenosine in tissue pieces has a similar effect. Therefore, the adenosine effect must be considered when studying the regulation of lipoprotein lipase activity of isolated fat cells or adipose tissue pieces.

Acknowledgements

The financial support from The Nutrition Research Foundation of Finnish Sugar Co. Ltd. and The Sigrid Jusélius Foundation is gratefully acknowledged.

References

[1] Schwabe, U., Ebert, R. and Erbler, H. C. (1973) Naunyn-Schmiedeberg's Arch. Pharmacol. 276, 133–148.
[2] Fain, J. N. (1973) Mol. Pharmacol. 9, 595–604.
[3] Turpin, B. P., Duckworth, W. C. and Solomon, S. S. (1977) J. Clin. Invest. 60, 442–448.
[4] Fredholm, B. B. (1978) Med. Biol. 56, 249–261.
[5] Ohisalo, J. J. and Stouffer, J. E. (1979) Biochem. J. 178, 249–251.
[6] Ohisalo, J. J. (1980) FEBS Lett. 116, 91–94.
[7] Ohisalo, J. J. (1981) J. Clin. Endocrinol. Metab. 52, 359–363.
[8] Westermann, E., Stock, K. and Bieck, P. (1969) Fettstoffwechsel 5, 68–73.
[9] Fredholm, B. B. (1978) Acta Physiol. Scand. 102, 191–198.
[10] Fredholm, B. B. and Sollevi, A. (1977) Acta Physiol. Scand. 99, 254–256.
[11] Ohisalo, J. J. and Stouffer, J. E. (1981) Horm. Metab. Res. 13, 222–224.
[12] Londos, C., Cooper, D. M. F., Schlegel, W. and Rodbell, M. (1978) Proc. Natl. Acad. Sci. USA 75, 5362–5366.
[13] Londos, C., Wolff, J. and Cooper, D. M. F. (1979) in: Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides (Baer, H. and Drummond, G. eds) pp. 271–281, Raven, New York.
[14] Londos, C., Cooper, D. M. F. and Wolff, J. (1980) Proc. Natl. Acad. Sci. USA 77, 2551–2554.
[15] Kalckar, H. M. (1947) J. Biol. Chem. 167, 445–459.
[16] Davies, P., Cryer, A. and Robinson, D. S. (1974) FEBS Lett. 45, 271–275.
[17] Robinson, D. S. (1970) Comprehens. Biochem. 18, 51–116.
[18] Kompiang, I. P., Bensadoun, A. and Yang, M. W. W. (1976) J. Lipid Res. 17, 498–505.
[19] Lithell, H. (1977) Academic dissertation, Uppsala.
[20] Nilsson-Ehle, P., Garfinkel, A. S. and Schotz, M. C. (1980) Annu. Rev. Biochem. 49, 667–693.
[21] Shirai, K., Barnhart, R. L. and Jackson, R. L. (1981) Biochem. Biophys. Res. Commum. 100, 591–599.
[22] Kuusi, T., Ehnholm, C. and Nikkilä, E. A. (1980) Atherosclerosis 35, 363–374.
[23] Ehnholm, C., Kinnunen, P. K. J., Huttunen, J. K., Nikkilä, E. A. and Ohta, M. (1975) Biochem. J. 149, 649–655.
[24] Schwabe, U., Schönhöfer, P. S. and Ebert, R. (1973) Eur. J. Biochem. 46, 537–545.
[25] Salaman, M. R. and Robinson, D. S. (1966) Biochem. J. 99, 640–647.
[26] Kornhauser, D. M. and Vaughan, M. (1975) Biochim. Biophys. Acta 380, 97–105.
[27] Wing, D. R., Salaman, M. R. and Robinson, D. S. (1966) Biochem. J. 99, 648–656.
[28] Cryer, A., Davies, P., Williams, E. R. and Robinson, D. S. (1975) Biochem. J. 146, 481–488.
[29] Parkin, S. M., Walker, K., Ashby, P. and Robinson, D. S. (1980) Biochem. J. 188, 193–199.