INSULIN-LIKE EFFECT OF SOME POLYAMINES ON LIPOPROTEIN LIPASE FROM RAT ADIPOSE TISSUE

Yves GIUDICELLI, Marie-Christine REBOURCET, Roger NORDMANN and Joseph NORDMANN

Groupe de Recherches de l'INSERM sur le Métabolisme Intermédiaire, 47, Boulevard de l'Hôpital, 75013 Paris, France, and Service de Biochimie de la Faculté de Médecine de Paris-Ouest, France

Received 25 November 1975

1. Introduction

In recent reports, it has been shown that the polyamines, spermidine and spermine, but not putrescine mimicked the action of insulin on lipid and glucose metabolism as well as on lipolysis in isolated rat adipocytes [1,2]. As insulin plays an important role in the regulation of the clearing factor lipase activity in isolated fat cells [3,4], we have attempted to determine, in the present studies, whether spermidine and spermine may enhance, like insulin [3,4], the lipoprotein lipase activity in rat adipose tissue.

The present results indicate that both spermidine and spermine stimulate the lipoprotein lipase activity in rat epididymal adipose tissue, whereas putrescine has no effect under the same experimental conditions.

2. Materials and methods

Epididymal fat pads were obtained from male albino Wistar rats (150–250 g body weight), fasted for 18 hours. After decapitation and excision of adipose tissue, pairs of fat pads from individual animals were distributed equally between control and experimental groups as previously described [5]. Before incubation, the pads (350–450 mg) were weighed and immediately transferred into stoppered Erlenmeyer flasks containing 10 ml of a glucose free Krebs-Ringer bicarbonate buffer [6], pH 7.4, added with 4% (w/v) bovine albumin (Fraction V, fatty acid poor, Miles Pentex).

Incubations were carried out with gentle shaking at 37°C for 2 h under air as the gas phase. Addition of spermidine (sigma), spermine (Calbiochem) or putrescine (Merck) to the medium was done at zero time. At the end of the incubations, fat pads were quickly removed, homogenized in 0.25 M sucrose (pH 8.1) containing heparin (2 IU/ml) and the heparin eluted lipoprotein lipase activity determined using Intralipid (Vitrum) as substrate, following the experimental conditions previously described [7]. The amount of free fatty acids (FFA) released during 60 min in each assay (carried out in duplicate) was determined according to Dole and Meinertz [8] and taken as a measure of lipoprotein lipase activity, which was expressed as micromoles of FFA released per hour and per g adipose tissue wet weight. All results are given as mean values ± S.E.M. and Student's 't' test was used for comparison of mean values.

3. Results

Spermidine and spermine were tested using concentrations ranging from 10^{-6} to 10^{-4} M, which were previously shown to elicit maximal inhibitory effects on epinephrine stimulated lipolysis and stimulatory effects on glucose oxidation and conversion into neutral lipids [2].

As shown in table 1, 2 h exposure to spermidine or spermine, but not to putrescine, significantly stimulated the lipoprotein lipase activity of rat adipose tissue. As a matter of fact, spermidine or spermine (10^{-6} M) induced a 2- to 2.2-fold increase of this activity, but no further enhancement occurred when the concen-

North-Holland Publishing Company – Amsterdam

Table 1
Effects of different concentrations of spermidine, spermine and putrescine on the lipoprotein activity of rat epididymal adipose tissue incubated in vitro

Expt. No.	Lipoprotein lipase activity (μmoles of FFA $h^{-1} \cdot g^{-1}$ wet wt.)			
1	Control	Spermidine		
		10^{-6} M	10^{-5} M	10^{-4} M
	(12)	(4)	(4)	(4)
	4.8 ± 1.1	10.6 ± 1.4	12.8 ± 1.6	11.8 ± 1.3
		$P < 0.001$	$P < 0.001$	$P < 0.001$
2	Control	Spermine		
		10^{-6} M	10^{-5} M	10^{-4} M
	(17)	(2)	(7)	(8)
	3.7 ± 0.9	7.5 ± 1.6	7.3 ± 1.9	7.2 ± 2.3
		$0.001 < P < 0.01$	$P < 0.001$	$0.001 < P < 0.01$
3	Control	Putrescine		
		–	–	10^{-4} M
	(6)			(6)
	4.0 ± 1.2			4.0 ± 1.2
				$P > 0.5$

Each value represents the mean ± S.E.M. with the number of determinations in parentheses.

tration of spermidine or spermine was raised up to 10^{-4} M, showing thus that maximal lipoprotein lipase stimulation was achieved under these experimental conditions.

4. Discussion

Lockwood et al. [1,2] first demonstrated that spermidine and spermine act on rat fat cells in a manner similar to insulin, i.e. by facilitating glucose transport, by increasing glucose conversion into CO_2 and lipids and by inhibiting the lipolytic activity of epinephrine and theophylline but not of dibutyryl 3′-5′ cyclic AMP. These observations suggested that, in fat cells, these polyamines, like insulin, influence lipolysis by suppressing endogenous 3′-5′ cyclic AMP levels [1]. Despite the insulin-like actions of spermidine and spermine, Amatruda and Lockwood reported that these polyamines initiate these effects by interacting with the cell membrane at sites which are distinct from the insulin receptors [9].

The present communication provides an additional evidence for the insulin-like action of some polyamines; as a matter of fact, we found that spermidine and spermine enhance the activity of rat epididymal adipose tissue lipoprotein lipase, enzyme which is also stimulated by insulin [3,4]. This does not appear, however, to be a common feature for all polyamines, since, as shown by the present results, another polyamine, putrescine, fails to affect the lipoprotein lipase activity of rat adipose tissue. In connection with these negative findings, it must be noted that putrescine was also reported to possess no insulin-like effects on both glucose conversion to CO_2 and lipolysis in rat fat cells [1,2].

Any conclusions as to the mechanism by which some polyamines stimulate lipoprotein lipase in rat adipose tissue are at this time speculative. However, previous reports concerning the regulation of this enzyme allow to the following comments. Adipose tissue lipoprotein lipase has been shown to be inhibited by dibutyryl 3′-5′ cyclic AMP [10,11], lipolytic hormones such as catecholamines [12] or methylxanthines [4], suggesting that the intracellular level of 3′-5′ cyclic AMP may control, at least in part, the lipoprotein lipase activity [4,10]. Accordingly, one could be tempted to establish a relationship between the possible lowering effect of spermidine and spermine on adipose tissue 3′-5′ cyclic AMP level [1] and the presently reported stimulatory effect of these polyamines on rat adipose tissue lipoprotein lipase. Further investigations are currently in progress to verify this hypothesis.

Acknowledgements

These investigations were supported by grants from the Institut National de la Santé et de la Recherche Médicale and the Faculté de Médecine de Paris-Ouest.

References

[1] Lockwood, D. H., Lipsky, J. J., Meronk, F., Jr. and East, L. E. (1971) Biochem. Biophys. Res. Commun. 44, 600–607.
[2] Lockwood, D. H. and East, L. E. (1974) J. Biol. Chem. 249, 7717–7722.
[3] Salaman, M. R. and Robinson, D. S. (1966) Biochem. J. 99, 640–647.
[4] Robinson, D. S. and Wing, D. R. (1970) in: Adipose tissue, Regulation and Metabolic Functions (Jeanrenaud, B. and Hepp, D., eds.), pp. 41–46, Academic Press, New York, London.
[5] Giudicelli, Y., Nordmann, R. and Nordmann, J. (1972) Biochim. Biophys. Acta 280, 393–407.
[6] Umbreit, W. W., Burris, R. H. and Stauffen, J. F. (1949) in: Manometric techniques and tissue metabolism, 2nd Edn., pp. 119, Burgess Publishing Co., Minneapolis.
[7] Giudicelli, Y., Nordmann, R. and Nordmann, J. (1975) Clin. Sci. Molec. Med. 48, 153–156.
[8] Dole, V. P. and Meinertz, H. (1960) J. Biol. Chem. 235, 2595–2599.
[9] Amatruda, J. M. and Lockwood, D. H. (1974) Biochim. Biophys. Acta 372, 266–273.
[10] Wing, D. R. and Robinson, D. S. (1968) Biochem. J. 109, 841–849.
[11] Patten, R. L. (1970) J. Biol. Chem. 245, 5557–5584.
[12] Wing, D. R., Salaman, M. R. and Robinson, D. S. (1966) Biochem. J. 99, 648–656.