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Effect of dibutyryl cyclic AMP and theophylline on lipoprotein lipase secretion by human monocyte-derived macrophages

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The effect of dibutyryl cyclic AMP and theophylline on lipoprotein lipase secretion was investigated after a 24 h pretreatment of human monocyte-derived macrophages. Both the effectors decreased in a dose-dependent manner the enzyme activity recovered in the culture medium. The decrease in lipoprotein lipase activity appeared to be related to reduced enzyme synthesis without apparent modification of its stability and half-life and was conversely associated with an increase of lysosomal acid hydrolase activities. This effect was reversible on removal of the nucleotide. The present findings suggest that cyclic AMP may play a role in lipoprotein lipase expression in human macrophages and therefore may participate in the regulation of lipoprotein uptake by these cells, which are strongly implicated in the atherogenic process.

Lipoprotein lipase; cyclic AMP; Enzyme synthesis; (Human monocyte-derived macrophage)

1. INTRODUCTION

The monocytic origin of foam cells found in atherosclerotic lesions has been demonstrated recently [1]. The activity of lipoprotein lipase (LPL), the key enzyme of triglyceride-rich lipoprotein degradation is altered in these lesions [2]. Human monocyte-derived macrophages (H-MDM) synthesize and secrete LPL during their differentiation in vitro. Most of the synthesized enzyme (about 80%) is found in the culture medium [3–6]. Cyclic 3′:5′-adenosine monophosphate (cAMP), which is the intracellular messenger of the action of several hormones, has been shown to play an important role in LPL regulation in several tissues or cell lines [7–11]. Thus it was of interest to determine whether the regulation of LPL in H-MDM involves cAMP.

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In the present study, we describe a dose-dependent inhibition of LPL synthesis by dibutyryl cyclic AMP (DbcAMP) and theophylline in H-MDM without apparent modification of the enzyme characteristics.

2. MATERIALS AND METHODS

2.1. *Monocyte-derived macrophage culture*

Human monocytes from healthy subjects were isolated from buffy coat obtained after erythrocyte sedimentation using Dextran T 500 (Sigma) (1% final concentration). The leukocytes were washed once with Ca^{2+} and Mg^{2+} free phosphate buffered saline, pH 7.4 (PBS), layered over Lymphoprep (Nyegaard, Oslo, Norway, d = 1.007 g/ml) and centrifuged for 15 min at 400 × g at 18°C. Mononuclear cells were resuspended in RPMI 1640 serum-free medium (10^7 cells/ml) and washed three times. Cells were plated on 35 mm gelatin-autologous plasma-coated Petri dishes following

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the technique described by Freundlich and Avdalovic [12]. 1 ml mononuclear cell suspension was added to each dish and incubated for 90 min at 37°C. Non-adherent cells were removed and the dishes washed three times with serum-free medium. The monolayer was reincubated with RPMI 1640 containing antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml) and either 20% autologous serum or 4% Ultroser G (IBF, France). Incubations with effectors were started 2 days after plating and cells were harvested after a first 24 h preincubation period.

At the end of incubation time, the medium was collected, frozen at −20°C and assayed for LPL activity within 1 week. 1 ml of a 1:1 mixture of 10 mM EDTA in Ca^{2+},Mg^{2+}-free PBS with RPMI 1640, 20% autologous serum was added for 15 min to remove adherent cells. The cells were washed twice with Ca^{2+},Mg^{2+} PBS. The pellets were resuspended in the appropriate buffer, homogenized by sonication and protein determination was performed by the method of Lowry et al. [13].

2.2. *Protein and LPL synthesis*

Cells were preincubated as described above. At the end of this period, medium was discarded and replaced with fresh medium containing 10 μCi/ml [^{3}H]leucine (CEA, France) and further incubation was performed for 3 h. Medium and cells were harvested as described above. Aliquots of medium were submitted to Sephadex G 50 chromatography in 4 M guanidinium chloride, 0.2 M leucine buffer to measure total secreted protein synthesis. Heparin-Sepharose chromatography was performed following the technique of Speake et al. [14] on a column equilibrated with 5 mM sodium barbital, pH 6.5, 20% glycerol, 0.1% (v/v) Triton X-100, and 50 mM NaCl. LPL was eluted by 5 mM sodium barbital, pH 6.5, 20% glycerol, 0.1% (v/v) Triton X-100 and 2 M NaCl.

2.3. *Assay of lipoprotein lipase activity*

The LPL assay system contained 0.1 ml of the culture medium and 0.05 ml of the substrate ([^{14}C]triolein/lecithin emulsion) prepared according to [16]. Incubations were carried out at 37°C for 45 min. The reaction was stopped by addition of methanol/chloroform/heptane (1.4:1.25:1, v/v) and the fatty acid extraction performed according to Belfrage and Vaughan [17]. Enzyme activity was expressed as nmol free fatty acid released/mg cell protein per min.

2.4. *Lysosomal acid hydrolase assay*

β-Galactosidase, hexosaminidase and β-glucuronidase activities were determined by a fluorometric assay in the culture medium and in the cell using the corresponding 4-methylumbelliferyl derivatives as substrate [15]. 20 μl of substrate in appropriate buffer were mixed with either 20 μl of medium or 10 μl of cell homogenate and incubations were carried out for 2 h at 37°C and stopped by adding 2 ml of 0.5 M Na_2CO_3, pH 11. Fluorescence was monitored on a Perkin-Elmer fluorometer at 360 nm excitation and 448 nm emission.

3. RESULTS

Preliminary studies showed that Ultroser G was almost as efficient as autologous serum in stimulating LPL release by macrophages (75–80% of serum values) and this serum substitute was therefore used in our experiments to standardize culture conditions.

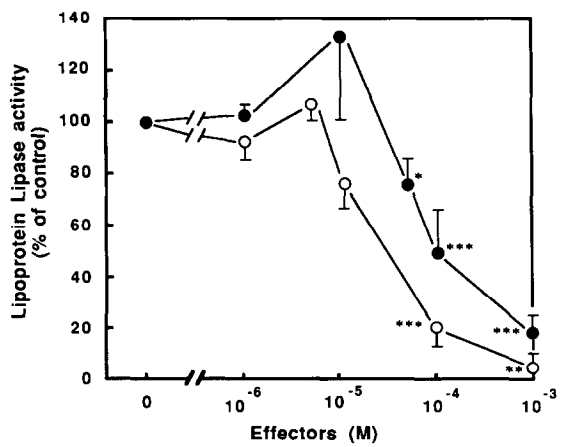

Fig.1. Effect of DbcAMP (○) and theophylline (●) on LPL activity secreted by H-MDM. Cells were pretreated with the effectors during a first 24 h period in RPMI 1640 supplemented with 4% Ultroser G and harvested after a second 24 h period in the same conditions. LPL was assayed in the medium as described in section 2. Each point is the mean ± SE of four experiments in duplicate (* $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$ versus control).

As shown in fig.1, the preincubation with either DbcAMP or theophylline produced, above 10^{-5} M, a dose-dependent decrease in LPL activity recovered in the culture medium. Low concentrations of the effectors (about 10^{-5} M) caused a slight rise in LPL activity. The activity released in the medium over a 24 h period was more significant 4 days after plating than at day 8. However, the incubation of macrophages in the presence of 10^{-4} M theophylline led to a similar decrease in LPL activity at both different stages of macrophages differentiation (not shown).

In order to determine precisely the mechanism involved in the decrease in LPL activity found in the medium of treated macrophages, we measured the enzyme synthesis using [^{3}H]leucine labelling and heparin-Sepharose separation. Treated cells showed a lowered [^{3}H]leucine incorporation in 2 M NaCl eluted fractions as well in the medium as in the cell lysate where the enzyme was not detectable (table 1). The ability of macrophages to synthesize and secrete proteins was conversely slightly enhanced in DbcAMP-treated cells as were the measured lysosomal acid hydrolase activities ($\times$ 1.6, $\times$ 1.5, $\times$ 1.8 in medium and $\times$ 1.6, $\times$ 1.2, $\times$ 2 in cells for β-galactosidase, hexosaminidase and β-glucuronidase, respectively) (table 1). In addition, the secretion rate is diminished in treated cells as compared to control (fig.2). Although the inhibition was lower than after 24 h of treatment, the difference became significant within 2 h after medium change and increased all along the 8 h of incubation.

Table 1

Effect of DbcAMP on incorporation of [^{3}H]leucine into total secreted proteins and LPL in macrophages

	Control	DbcAMP (10^{-4} M)
Total secreted proteins	74745 (100%)	99553 (133%)
LPL		
Medium	2194 (100%)	757 (35%)
Cells	393 (100%)	ND
β-Galactosidase		
Medium	19.4 ± 1.1	31.7 ± 3.5[b]
Cells	1.1 ± 0.1	1.8 ± 0.2[c]
Hexosaminidase		
Medium	387.9 ± 26.9	590.6 ± 31.9[d]
Cells	31.2 ± 3.3	36.1 ± 0.9[a]
β-Glucuronidase		
Medium	0.6 ± 0.3	1.1 ± 0.1[a]
Cells	1.0 ± 0.1	2.1 ± 0.1[d]

Results are expressed in cpm/mg cell protein and represent the mean of two separate experiments (ND, not detectable). Lysosomal acid hydrolase activities in control and DbcAMP-treated cells are expressed in arbitrary fluorescence units perμg cell proteins (mean ± SE of six determinations). [a] $p < 0.05$, [b] $p < 0.02$, [c] $p < 0.01$, [d] $p < 0.001$ versus control

In order to rule out the possibility of a general toxic effect of drugs on cultured cells, 24 h DbcAMP pretreated dishes were incubated in fresh medium in the presence or absence of the nucleotide. Dishes pretreated with DbcAMP were washed and reincubated in fresh medium devoid of effector. After an about 4 h lag period, the macrophages partially recovered their ability to secrete LPL in the medium. 24 h after DbcAMP removal, secretion returned to levels similar to those expressed in cells which have not been exposed to the nucleotide (fig.3). Furthermore, no difference could be detected either in the number of cells remaining at the end of the incubation time

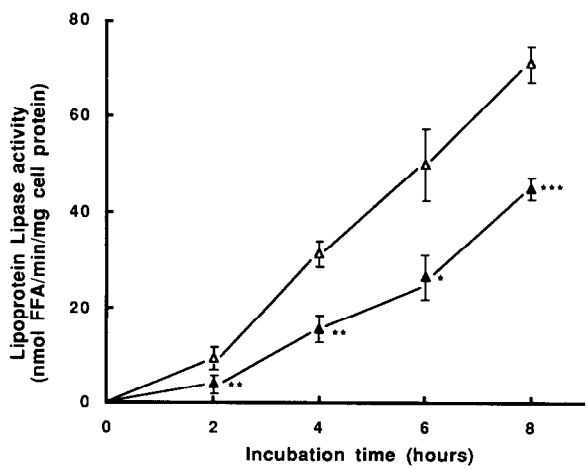

Fig.2. Effect of DbcAMP (10^{-4} M) on LPL activity secreted in the medium of cultured H-MDM. Cells were preincubated 24 h with (▲) or without (△) DbcAMP. After medium change, cells were reincubated in the same conditions for 8 h and the medium was collected at the times indicated. LPL activity was assayed as described in section 2. Results are the mean ± SE of four determinations (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control).

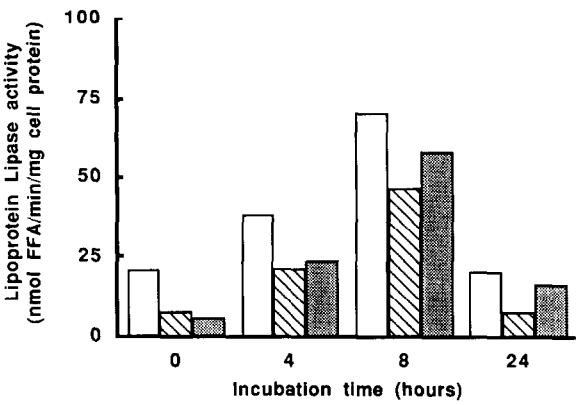

Fig.3. Effect of removal of DbcAMP on LPL activity secreted by H-MDM. Cells were preincubated during 24 h in the presence of DbcAMP (10^{-4} M). After removal of the medium, cells were reincubated in medium with (▨) or without (▩) DbcAMP. Control cells (□) were incubated in the same conditions without the nucleotide. Aliquots of medium were collected during the 24 h of further incubation and LPL activity was assayed as described in section 2. Results are the mean of duplicate dishes.

or in total cell protein concentration (protein: control, 64.4 ± 11.7; theophylline, 66.5 ± 14.7; DbcAMP, 65.3 ± 6.0 μg/dish; cell counts ($\times 10^5$): control, 3.2 ± 0.2; theophylline, 2.8 ± 0.7; DbcAMP, 3.2 ± 0.2 cells/dish).

To determine whether this decrease in LPL activity could also be related to an associated alteration of the enzyme stability in treated cells, we studied the decay of LPL activity in a cell-free system. There was no significant difference in the inactivation rate of enzyme isolated from control and 24 h DbcAMP preincubated cells. The apparent half-life of the enzyme in such conditions was 122 and 129 min for control and DbcAMP-treated cells, respectively (control: $y = 0.146 + 35.386$, $r = 0.987$; DbcAMP: $y = 0.076 + 19.576$, $r = 0.985$).

4. DISCUSSION

Serum has been shown to be essential for LPL expression in human monocyte-derived macrophages [6]. The use of the synthetic serum Ultroser G, which did not affect LPL secretion or cell viability, allowed one to minimize variations in cell culture conditions occurring with serum. Furthermore, the absence of lipoproteins in this serum substitute demonstrated that these compounds are not required for enzyme expression.

The decrease in LPL activity with high concentrations of DbcAMP or theophylline is in good agreement with the findings for adipose tissue in vitro either by using theophylline [7] or under hormonal stimulation [8]. In contrast, heart LPL has been shown by several authors to be enhanced by chemical or hormonal rise in cAMP intracellular level [9,10].

The biphasic response of LPL to DbcAMP we found was also noted by Palmer et al. [9] in perfused rat heart. A similar paradoxical effect of cyclic AMP has been shown for macrophage differentiation and inhibition of cytotoxicity [18,19]. This biphasic effect could be dependent on the two different cyclic AMP-dependent protein kinases which are expressed in human macrophages [20].

The fact that the decrease in LPL activity was reversed by removing effectors and that the nucleotide did not significantly affect the number of adherent cells or cell protein concentration suggested that this decrease was not related to a non-specific toxic effect. The lowering of LPL activity by DbcAMP or theophylline appears to be mainly due to a decrease in enzyme synthesis without significant alteration of LPL stability as assessed by in vitro half-life determination. Conversely, some other secreted proteins seemed to be enhanced by this stimulation.

A similar lowering effect on LPL activity in macrophages was obtained by LPS stimulation [21]. However this effector led also to a dramatic decrease of lysosomal acid hydrolase activities in medium and cell lysate [22]. In contrast, lysosomal acid hydrolase activities, which were assayed, were enhanced by DbcAMP at concentrations which decreased LPL without modification of their ratio. The same results were obtained with theophylline (not shown). This fact is in favour of a complex regulation of macrophage LPL depending on the kind of stimulation and therefore on the specialisation acquired by the cell (i.e. cytotoxicity which is increased with LPS [23] and decreased by cAMP [19]).

Incubation of macrophages with VLDL or chylomicrons led to lipoprotein uptake by the β-VLDL receptor which is present at the cell surface. Although not a prerequisite step in particle capta-

tion, the LPL-mediated degradation into remnant particles favors the enhancement of cholesterol cellular content [24,25]. cAMP has been shown to be specifically increased in atherosclerotic lesions of rabbits fed a high cholesterol diet [26]. Recently, Jonasson et al. [27] demonstrated that LPL is not immunologically detectable in the lipid core of human atherosclerotic plaques. Conversely, β-glucuronidase is enhanced in human atherosclerotic aortas [28]. Our findings that cAMP dramatically decreases LPL synthesis and secretion by H-MDM is consistent with these observations. Thus the cAMP-mediated decrease of macrophage LPL could play a role in lipoprotein uptake and in foam-cell formation in atherosclerotic lesions.

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REFERENCES

[1] Aquel, N.M., Ball, R.Y., Waldmann, H. and Mitchinson, M.J. (1984) Atherosclerosis 53, 265–271.
[2] Vijayakumar, S.T., Leelamma, S. and Kurup, P.A. (1975) Atherosclerosis 21, 1–14.
[3] Mahoney, E.M., Khoo, J.C. and Steinberg, D. (1982) Proc. Natl. Acad. Sci. USA 79, 1639–1642.
[4] Wang-Iverson, P., Ungar, A., Bliumis, J., Bukberg, P.R., Gibson, J.C. and Brown, W.V. (1982) Biochem. Biophys. Res. Commun. 104, 923–928.
[5] Chait, A., Iverius, P.H. and Brunzell, J.D. (1982) J. Clin. Invest. 69, 490–493.
[6] Stray, N., Letnes, H. and Blomhoff, J.P. (1985) Scand. J. Gastroenterol. 20 (suppl.107), 67–72.
[7] Bourdeaux, A.M., Rebourcet, M.C., Nordmann, J., Nordmann, R. and Giudicelli, Y. (1982) Biochem. Biophys. Res. Commun. 107, 59–67.
[8] Ashby, P. and Robinson, D.S. (1980) Biochem. J. 188, 185–192.
[9] Palmer, W.K., Caruso, R.A. and Oscai, L.B. (1981) Biochem. J. 198, 159–166.
[10] Friedman, G., Chajek-Shaul, T., Stein, O. and Stein, Y. (1983) Biochim. Biophys. Acta 752, 106–117.
[11] Melmed, R.N., Friedman, G., Chajek-Shaul, T., Stein, O. and Stein, Y. (1983) Biochim. Biophys. Acta 762, 58–66.
[12] Freundlich, B. and Avdalovic, N. (1983) J. Immunol. Methods 62, 31–37.
[13] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[14] Speake, B.K., Parkinson, C. and Robinson, D.S. (1985) Horm. Metab. Res. 17, 637–640.
[15] Robins, E., Hirsch, H.E. and Emmons, S.S. (1968) J. Biol. Chem. 243, 4246–4252.
[16] Nillson-Ehle, P. and Shotz, M.C. (1976) J. Lipid Res. 17, 536–541.
[17] Belfrage, P. and Vaughan, M. (1969) J. Lipid Res. 10, 341–344.
[18] Olsson, I.L., Breitman, T. and Galdo, R.C. (1982) Cancer Res. 42, 3928.
[19] Schultz, R.M., Pavlidis, N.A., Stoychkov, J.N. and Chirigos, M.A. (1979) Cell. Immunol. 42, 71–78.
[20] Wenger, G.D. and O'Dorisio, M.S. (1985) J. Immunol. 134, 1836–1843.
[21] Goldberg, D.I. and Khoo, J.C. (1987) Biochem. Biophys. Res. Commun. 142, 1–6.
[22] Riches, D.W. and Henson, P.M. (1986) J. Cell Biol. 102, 1606–1614.
[23] Cameron, D.J. and Churchill, W.H. (1980) J. Immunol. 123, 708–711.
[24] Lindqvist, P., Ostlund-Lindqvist, A.M., Witztum, J.L., Steinberg, D. and Little, J.A. (1983) J. Biol. Chem. 258, 9086–9092.
[25] Ostlund-Lindqvist, A.M., Gustafson, S., Lindqvist, P., Witztum, J.L. and Little, J.A. (1983) Arteriosclerosis 3, 433–440.
[26] Augustyn, J.M. and Ziegler, F.D. (1975) Science 187, 449–450.
[27] Jonasson, L., Bondjers, G. and Hansson, G.K. (1987) J. Lipid Res. 28, 437–445.
[28] Miller, B.F. and Kothari, H.V. (1969) Exp. Mol. Pathol. 10, 288–294.