Effects of the calcium ionophore A 23187 on low-density lipoprotein processing and lipid metabolism in cultured human fibroblasts

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Pretreatment of cultured human fibroblasts with the calcium ionophore A 23187 resulted in a decrease in low-density lipoprotein internalization. This effect was dose-dependent and did not occur in a medium devoid of calcium. About 2-fold reduction was observed with 10^{-5} M A 23187. In contrast, the low-density lipoprotein binding was only slightly affected. The incorporation of [^{14}C]acetate and [^{14}C]oleate into all classes of lipids (sterol, triacylglycerols and phospholipids) was strikingly reduced by ionophore pretreatment.

Calcium ionophore *A 23187* *LDL internalization* *Lipid synthesis* *(Human fibroblast)*

1. INTRODUCTION

The low-density lipoprotein processing by cells is mainly achieved by a specific receptor-mediated endocytosis, which results in a decrease of both sterol synthesis and low-density lipoprotein (LDL) receptor expression [1–3]. The LDL-receptor recycling appears to be inhibited by monovalent cation ionophores such as nigericin or monensin, which results in the disappearance of the receptors from the cell surface [4,5], but so far there have been no reports on an effect of calcium ionophores on LDL processing by cells. The divalent cation ionophore A 23187 [6] has been widely used for the study of calcium-dependent phenomena such as arachidonate release from platelets [7], human chorionic gonadotropin secretion by trophoblastic cells [8], or steroid hormone biosynthesis [9]. On the other hand, calcium ions are known to be involved in the control of membrane processes such as capping of lectins [10] or lateral diffusion of surface antigens [11]. Hence, we investigated the effect of the calcium ionophore A 23187 on LDL and lipid metabolism in cultured human fibroblasts.

2. MATERIALS AND METHODS

2.1. *Cell culture*

MRC5 (human fetal lung) fibroblasts purchased from Biomérieux were cultivated in 30 mm Petri dishes containing Ham F10 medium supplemented with 10% foetal calf serum (Gibco), at 37°C, in a humidified 5% CO_2 atmosphere. Experiments were performed on confluent cells.

2.2. *LDL preparation and labeling*

The LDL was prepared from human blood as described [12], and labeled according to [13], using INa (Amersham). The specific radioactivity of the LDL was about 250 cpm/ng.

2.3. *Effect of A 23187 on LDL binding and internalization*

Prior to experiments, cells were incubated for 24 h in Ham F10 medium supplemented with 2% Ultroser G (Industrie Biologique Française), a serum substitute devoid of lipoproteins, for maximal expression of LDL receptors, and standardisation of experiments. For studies concerning the effect of A 23187 in relation to the drug concentra-

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tion, cells were washed 3 times with the same medium, then incubated for 1 h at 37°C in Ham F10 medium + Ultroser (2%) + 10 mM Hepes buffer (pH 7.4), in the absence or presence of A 23187 (Sigma) at final concentrations varying from 10^{-6} to 2×10^{-5} M. The ionophore was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the medium was 1%, which does not impair LDL binding or internalization. Controls were performed with DMSO alone at the same concentrations. To study the effect of the drug in relation to the time of preincubation, cells were preincubated for 30 min–4 h with or without the drug (10^{-5} M final concentration) in the same medium. For experiments designed to check the calcium dependence of the phenomenon, cells were first incubated for 30 min in Minimum Essential Medium (MEM) free of calcium and magnesium but supplemented with 1 mM EGTA, then washed 3 times with calcium-free MEM (Gibco), and further incubated for 30 min in the same medium without EGTA, containing either DMSO alone, or 10^{-5} M A 23187 in DMSO, in the presence or absence of calcium at concentrations varying from 0.05 to 5 mM. After preincubation under specified conditions, the ^{125}I-labelled LDL (5 μg/ml) was added, and the cells further incubated for 1 h, either at 4°C (binding) or 37°C (binding + internalization). Cells were then washed 5 times with 2 ml of an isotonic Tris–NaCl buffer (pH 7.4), harvested with a rubber policeman, centrifuged at 4°C, and the radioactivity measured on the pellet with a Packard instrument. Protein determination was done on the pellet according to [14]. Results are expressed in percentage of controls (mean of at least 3 experiments).

2.4. *Effect of A 23187 on lipid synthesis*

Experiments were performed with sodium [1-^{14}C]acetate (48 mCi/mmol, Amersham), or [1-^{14}C]oleic acid (52 mCi/mmol, Amersham). Prior to experiments, cells were cultured for 24 h either in Ham F10 medium + 2% Ultroser (for acetate incorporation), or in Ham F10 medium + 10% foetal calf serum (for oleate incorporation). Cells were then preincubated for 1 h with either A 23187 (final concentration 10^{-5} M) dissolved in DMSO (1% final concentration), or DMSO alone (controls). The precursor ([^{14}C]acetate, 10 μCi/ml or [^{14}C]oleate, 0.5 μCi/ml) was then added and a further 4-h incubation performed. Cells were then extensively washed, and lipid analysis performed by thin-layer chromatography according to [15]. Results are expressed in pmol precursor incorporated/mg cellular proteins (mean of 3 experimental values ± SD).

3. RESULTS AND DISCUSSION

Fig.1 displays the effect of A 23187 in relation to the drug concentration (1 h preincubation of cells with the drug, then 1 h incubation with ^{125}I-labelled LDL at either 4 or 37°C). LDL internalization was decreased in a dose-dependent manner, whereas LDL binding was only slightly affected. The maximal effect on LDL internalization was obtained at 10^{-5} M (about 2-fold decrease compared to controls), and plateaued at higher concentrations.

Fig.2 displays the effects of the drug (10^{-5} M) in relation to the preincubation time. Observe that

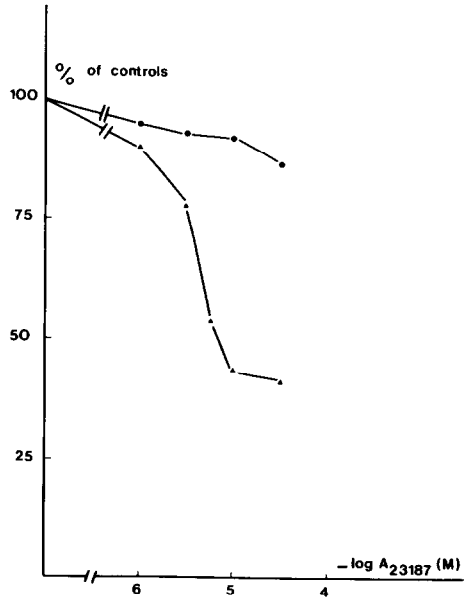

Fig.1. Effects of the ionophore A 23187 on LDL binding and internalization by cultured human fibroblasts, in relation to the drug concentration. For experimental conditions, see section 2. Results are expressed in % of control values (DMSO alone). Each point is the mean of at least 3 experimental values. (●) Binding (100% = 82.5 ± 12.6 ng ^{125}I-LDL/mg cell protein); (▼) internalization (100% = 532 ± 46 ng ^{125}I-LDL/mg cell protein).

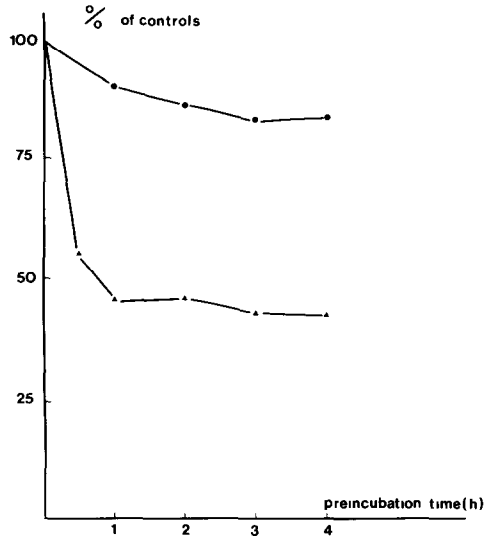

Fig.2. Effects of the ionophore A 23187 on LDL binding and internalization, in relation to the preincubation time of cells with the drug. For experimental conditions, see section 2. Results are expressed in % of control values (DMSO alone). Each point is the mean of 3 experimental values. (●) Binding (100% = 89.7 ± 11.5 ng ^{125}I-LDL/mg cell protein); (▼) internalization (100% = 546 ± 29 ng ^{125}I-LDL/mg of cell protein).

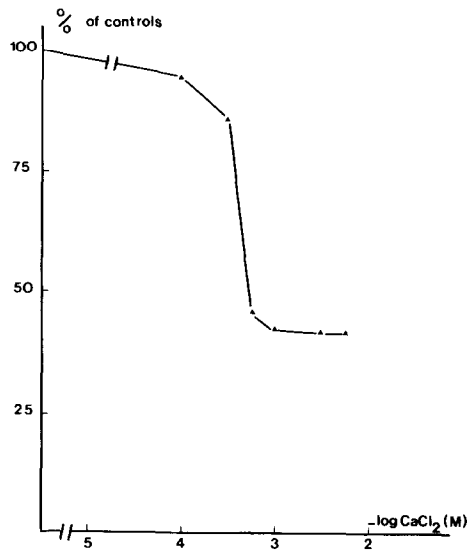

Fig.3. Influence of the calcium concentration of the medium on the effect of A 23187 on LDL internalization. For experimental conditions, see section 2. Results are expressed in % of control values. Each point is the mean of 3 experimental values. 100% = 562 ± 37 ng ^{125}I-LDL/mg cell protein.

the maximal decrease in LDL internalization was obtained within 30 min of preincubation. In contrast, the LDL binding was only slightly affected up to 4 h preincubation.

Fig.3 displays the influence of the calcium concentration in the incubation medium on the effect of A 23187 on LDL internalization. Note that ionophore A 23187 had no effect on LDL internalization in a medium completely devoid of calcium. The maximal effect was obtained with 0.5 mM calcium. This clearly demonstrates that the decrease in LDL internalization induced by A 23187 was related to the calcium influx into cells and not to a possible effect of the drug itself. This last hypothesis had especially to be ruled out, as A 23187 is a hydrophobic compound, which could interfere with membranes and thus could alter the LDL receptor-mediated endocytosis, as has been previously found for other hydrophobic drugs [16,17].

It can be seen in table 1 that a 2-h preincubation of the cells with 10^{-5} M A 23187 induced a dramatic decrease in [^{14}C]acetate incorporation into all classes of lipids studied (sterols, triacylglycerols and phospholipids). This effect is more marked for sterols and phospholipids, which have a faster turnover than triacylglycerols. Moreover, table 1 shows that the pretreatment of the cells with A 23187 also resulted in a marked decrease of [^{14}C]oleic acid incorporation into cholesteryl esters and triacylglycerols. It must be noted that in all experiments, cell viability was not significantly affected, and that if in some cases A 23187 induced a significant detachment of the cells from the support, only attached cells were analyzed for precursor incorporation into lipids or for LDL binding and internalization.

The effects of the calcium ionophore A 23187 on LDL and lipid metabolism in cultured fibroblasts have not previously been reported. The only ionophores which have been studied at the present time for their interaction with LDL processing by cells are monensin and nigericin [4,5]. These drugs are monovalent cationic ionophores, and abolish the Na^+/K^+ gradient. They have been demonstrated to decrease rapidly the number of

Table 1

Effect of ionophore A 23187 on the incorporation of [^{14}C]acetate and [^{14}C]oleate into lipids

Lipid	Drug	[1-^{14}C]Acetate incorporation (pmol/mg protein)	[1-^{14}C]Oleate incorporation (pmol/mg protein)
Sterols	Control	870 ± 140 (100%)	0
	A 23187	131 ± 16 (15%)	0
Cholesterylesters	Control	n.d.	1364 ± 190 (100%)
	A 23187	n.d.	380 ± 68 (28%)
Triacylglycerols	Control	1245 ± 206 (100%)	8355 ± 916 (100%)
	A 23187	450 ± 74 (36%)	1921 ± 290 (22%)
Total phospholipids	Control	25460 ± 4580 (100%)	17352 ± 2260 (100%)
	A 23187	3055 ± 575 (12%)	5550 ± 870 (32%)

Prior to experiments, cells were cultured 24 h either in Ham F10 + 2% Ultroser (for further study of acetate incorporation), or Ham F10 + 10% fetal calf serum (for study of oleate incorporation). Ionophore (10^{-5} M final concentration), dissolved in DMSO (1% final concentration), or DMSO alone (controls) were then added for 1 h. After preincubation, the precursor was added ([^{14}C]acetate, 10 μCi/ml; [^{14}C]oleate, 0.5 μCi/ml), and further incubation performed during 4 h. Lipid analysis was performed by thin-layer chromatography. Results are expressed in pmol precursor incorporated/mg cellular protein ± SD (mean of 3 experimental values)

LDL receptors at the cell surface by interrupting receptor recycling [4]. From our results, the calcium ionophore A 23187 had a clearly different effect: there was no significant decrease in the number of LDL receptors at the cell surface, and only a slight increase for long incubation times (24 h). In contrast, LDL internalization was markedly inhibited. Note that cells treated with A 23187 exhibited, only in the presence of calcium, a rounded shape, which suggests alterations in the cytoskeleton. The effect of increasing intracellular calcium concentration on cell shape is well documented for the red cell [18–20]. Authors in [20] reported that in the presence of calcium + A 23187, the human erythrocyte morphology turns from discocyte to a rounded echinocyte morphology. This phenomenon was associated with a decrease in the intracellular ATP content [20]. The increase in the intracellular calcium concentration in red cells has also been found to increase the membrane rigidity [21] and to induce cross-linking of contractile proteins [22]. Such phenomena could be involved in the decrease of LDL endocytosis described here. Note that the morphological changes which we observed in cells treated with A 23187 were parallel with the effect of the ionophore on LDL endocytosis: neither a significant decrease in LDL internalization nor morphological alteration of cells were found for calcium concentrations lower than 0.2 mM. In some experiments in which the ionophore and the LDL were simultaneously introduced and the LDL internalization studied at 10, 20, and 30 min, we also observed a decrease in LDL internalization, but less marked than in experiments performed with preincubation (about 25–30% reduction, compared to a 2-fold decrease in cells preincubated 30 min with the ionophore, not shown). This could be due to the rapid internalization of the LDL in the first minutes, as the cells turned to a rounded shape only after 10–15 min of incubation with the ionophore.

The strong decrease in acetate and oleic acid incorporation into lipids induced by cell pretreatment with A 23187 remains difficult to explain. Authors in [9] reported a suppressive effect of A 23187 on the stimulatory action of luteinizing hormone on progesterone synthesis by ovarian cells in vitro. These authors also found an inhibition of protein synthesis by A 23187 in the

presence of calcium [9]. It can be supposed that in our experimental system, the inhibition of lipid synthesis by A 23187 could be related to the decrease in the intracellular ATP content [18], or/and to an effect of the ionophore on calcium-dependent processes involved in the regulation of lipid metabolism. Another hypothesis is that cross-linking of membrane proteins resulted in arresting cells and thus led to a decrease in all metabolism. In any case, this effect appears to be non-specific, as the synthesis of all the lipids studied was affected almost to the same extent.

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