LOWERING THE INTRACELLULAR LIPID CONCENTRATION IN THE ATHEROSCLEROTIC HUMAN AORTA BY COMPOUNDS RAISING THE INTRACELLULAR cAMP LEVEL

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One of the chief manifestations of atherosclerosis at the cellular level is marked accumulation of intracellular lipids, predominantly cholesterol esters [9]. Lipid metabolism in certain types of cells is known to be controlled by the cyclic nucleotide system, and an increase in the intracellular cyclic adenosine-3',5'-monophosphate (cAMP) level is known to reduce the content of lipids, including cholesterol esters [3, 4]. It can accordingly be postulated that an increase in the cAMP content in cells of atherosclerotic arteries would reduce their degree of lipid saturation.

To test this hypothesis cells of unaffected and atherosclerotic human aortas were used in primary culture. Methylisobutylxanthine, which inhibits phosphodiesterase, the enzyme of cAMP hydrolysis, cholera toxin (CT), which stimulates adenylate cyclase, the enzyme of cAMP synthesis, and the dibutyryl derivative of cAMP which, unlike native cAMP, can penetrate into cells, were added to the culture. These agents are known to raise the cAMP level in mammalian cells [1, 5].

EXPERIMENTAL METHOD

Cells of the intimal layer of the human aorta were isolated by dispersion with collagenase and elastase and were cultured as described previously [7]. On the 10th day of culture N^6,O^2-dibutyryl-3',5'-cyclic AMP (DB-cAMP), 1-methyl-3-isobutylxanthine (MIX), and CT (all reagents were from Sigma, USA) were added. After 24 h the test agents were added a second time to the culture medium. The lipid composition of the cells was analyzed after 48 h of culture of the cells with the agents. Lipids were extracted from the cells by the method in [2]. To analyze losses, 0.01 μCi of [^{14}C]cholesterol (Amersham Corporation, England) was added to the extracting mixture. Phospholipids were determined as in [10]. Neutral lipids were fractionated by thin-layer chromatography in a solvent system of N-hexane—diethyl ether—acetic acid (73:25:2) and determined quantitatively by scanning densitometry [8]. The significance of differences was determined by Student's t test.

EXPERIMENTAL RESULTS

Compared with cells obtained from unaffected areas, cells cultured from lipid streaks and atherosclerotic plaques contained more phospholipids, triglycerides, and free and esterified cholesterol (Table 1) — the main classes of lipids, which accumulate in atherosclerotic arteries [6, 9].

MIX, DB-cAMP, and CT lowered the triglyceride and cholesterol ester levels in cells obtained from zones with atherosclerotic lesions by 33-50% (Table 2). These agents left the concentrations of free cholesterol and phospholipids unchanged. No change in the lipid content in cells obtained from unaffected areas of the vessel was found under the influence of these agents in any of the cases studied.

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TABLE 1. Content of Main Classes of Lipids in Intimal Cells Cultured from Unaffected and Atherosclerotic Areas of Human Aorta (M ± m)

Type of lesion	Lipid content, $\mu g/10^5$ cells			
	phospholipids	cholesterol	triglycerides	cholesterol esters
Normal (n = 4)	43,5±4,0	4,7±0,4	4,3±0,7	5,1±0,6
Lipid streak (n = 3)	73,3±4,1*	9,2±1,0	13,3±1,8*	29,9±1,9*
Plaque (n = 3)	70,6±5,3*	9,3±0,9*	16,1±1,7*	34,7±6,0*

Legend. Here and in Table 2: *P < 0.05 compared with normal; n) number of vessels studied.

TABLE 2. Effect of DB-cAMP, Cholera Toxin, and Methylisobutylxanthine on Lipid Content in Intimal Cells Cultured From Unaffected and Atherosclerotic Areas of Human Aorta (M ± m)

Type of lesion	Lipids	Lipid content, percent of control		
		DB-cAMP (10^{-4} M)	CT (100 ng/ml)	MIX (10^{-4} M)
Normal (n = 4)	Phospholipids	108±4	103±11	97±7
	Cholesterol	98±5	106±3	95±5
	Triglycerides	107±4	113±8	95±4
	Cholesterol esters	95±4	96±11	87±2
Lipid streak (n = 3)	Phospholipids	99±4	91±8	98±11
	Cholesterol	100±5	111±12	91±4
	Triglycerides	82±10	47±9*	62±16*
	Cholesterol esters	59±5*	46±2*	48±4*
Plaque (n = 3)	Phospholipids	107±1	—	114±10
	Cholesterol	105±2	—	106±8
	Triglycerides	73±1*	—	52±8*
	Cholesterol esters	73±4*	—	61±13*

Legend. see Table 1.

The results of this investigation thus indicate that compounds capable of increasing the cAMP concentration at the same time reduce the concentrations of cholesterol esters and triglycerides — lipids which accumulate in the atherosclerotic human aorta — in the affected intimal cells. Accordingly, compounds which raise the intracellular cAMP level can evidently be regarded as potential therapeutic agents capable of reducing the degree of lipoidosis.

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