EFFECT OF DIBUTYRYL-cAMP ON THE CONTENT OF CHOLESTEROL ESTERS IN CELLS OF THE ATHEROSCLEROTIC HUMAN AORTA

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KEY WORDS: atherosclerosis; aortic cell culture; cholesterol esters; dibutyryl-cAMP.

In atherosclerosis of the human aorta considerable accumulation of lipids is observed in the vessel wall, most of them consisting of cholesterol esters [4, 6]. The increase in lipid content may be the result of their arrival from the plasma or of a disturbance of intracellular lipid metabolism [7]. Lipid metabolism in some types of cells is known to be controlled by the cyclic nucleotide system, and cAMP is able to intensify the utilization of lipids, including cholesterol esters [2, 3].

In the investigation described below the effect of the dibutyryl derivative of cAMP (db-cAMP) on the content of free and esterified cholesterol was studied in cultures of intimal cells isolated from unaffected regions of the vessel and from regions with atherosclerotic changes.

EXPERIMENTAL METHOD

Experiments were carried out on primary cultures of aortic intimal cells. The vessels were removed under aseptic conditions from men aged 40-60 years, 1.5-3 h after sudden death. Atherosclerotic lesions were divided into four types on the basis of Smith's classification [6]: outwardly unaffected regions, regions with fatty infiltration, lipid streaks, and atherosclerotic plaques. The intima were separated mechanically and subjected to enzymic dispersion with collagenase and elastase, as described previously [5]. The isolated cells were seeded in plastic petri dishes 35 mm in diameter (from Corning, USA) with a density of 10^4 cells/cm^2. The cells were cultured for 9 days in 1.5 ml of medium 199 containing 10% embryonic calf serum, 2.5 μg/ml fungisone, 100 μg/ml kanamycin, and 2 mM glutamine (all reagents from Gibco, USA). The cells were cultured at 37°C in an atmosphere of 5% CO_2 and 95% air, saturated with water vapor. The medium was changed every 3 days. On the 10th day, N^6,O^2-dibutyryl cyclic adenosine-3',5'-monophosphate (db-cAMP), N^6,O^2-dibutyryl cyclic guanosine-3',5'-monophosphate (db-cGMP), from Sigma (USA), or sodium butyrate (from Merck, West Germany) was added to the cultures. Addition of these agents was repeated 24 h later. After 24 days the cultures were washed twice with Dulbecco's isotonic phosphate buffer and the cells were suspended with 0.25% trypsin and 1 mM EDTA (from Gibco, USA). The resulting suspension was washed by centrifugation (200g, 10 min) and resuspended in isotonic phosphate buffer. Lipids were extracted from the cells by the method in [1]. The content of free and esterified cholesterol was determined by means of kits from BDH (England). The experimental results were subjected to statistical analysis. The significance of differences was evaluated by Student's t test.

EXPERIMENTAL RESULTS

Most cells (50-90%) in cultures of intimal cells isolated from regions of atherosclerotic changes contained lipid inclusions. The content of free and esterified cholesterol in these cells was several times higher than in cells from unaffected regions of the vessel (Table 1). A primary culture of cells from the atherosclerotic aorta can thus provide a convenient model with which to study the action of agents on the intracellular lipid content.

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TABLE 1. Effect of db-cAMP (0.1 mM), db-cGMP (0.1 mM), and Sodium Butyrate (0.2 mM) on Content of Cholesterol Esters in Intimal Cells Isolated from Unaffected and Atherosclerotic Regions of the Human Aorta (M ± m)

Experimental conditions	Content of cholesterol esters, μg /10⁵ cells			
	control	db-cAMP	db-cGMP	sodium butyrate
Aorta 1				
normal	3,8±0,4 (6)	3,2±0,6 (3)	6,8±0,6 (3)	—
fatty infiltration	10,6±1,0**(6)	5,6±0,6*(3)	9,0±0,6 (3)	—
Aorta 2				
normal	2,2±0,4 (6)	2,4±0,4 (3)	3,8±0,6 (3)	2,4±0,6 (3)
lipid streak	20,4±1,8**(6)	11,4±1,6*(3)	14,6±1,0 (3)	20,0±1,6 (3)
Aorta 3				
normal	6,5±0,8 (3)	5,7±0,5 (3)	6,9±0,6 (3)	6,0±0,3 (3)
plaque	26,5±3,0**(3)	19,5±1,1*(3)	29,8±4,2 (3)	27,7±2,7 (3)

Legend. Number of determinations shown in parentheses. *) Difference from control significant ($P < 0.05$), **) difference from cultures obtained from unaffected regions of aorta significant ($P < 0.01$).

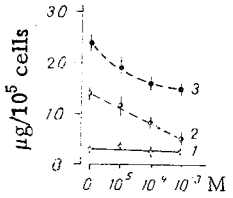

Fig. 1. Effect of db-cAMP on content of cholesterol esters in intimal cells isolated from unaffected regions of the aorta (1), from regions with fatty infiltration (2), and with lipid streaks (3), as a function of concentration. Each point reflects the results of three determinations.

In the present investigation the hydrophobic analog of cAMP, namely db-cAMP, which can pass through the plasma membrane of cells, was used. db-cAMP was used in a concentration of 10^{-4} M, that usually employed to study the effects of this agent in cell culture [8]. When added to the culture medium, db-cAMP did not change the intracellular content of free and esterified cholesterol in cells isolated from unaffected regions of the human aorta (Table 1). db-cAMP lowered the level of cholesterol esters in cells obtained from regions with fatty infiltration, lipid streaks, and atherosclerotic plaques. The effect under these circumstances depended on the db-cAMP concentration (Fig. 1). No significant changes in the free cholesterol content under the influence of db-cAMP were found in any of the cultures tested (the data are not given).

db-cAMP may be broken down in the cells with removal of a butyric acid residue, which may be responsible for the observed effect. Accordingly, the effect of sodium butyrate on the content of esterified cholesterol in the cells was studied. No change was found in the concentration of cholesterol esters as a result of the effect of this compound on the cells (Table 1).

Addition of db-cGMP to the cultures had no effect on the content of free and esterified cholesterol in cells isolated from affected regions of the aorta. These results point to the specificity of action of db-cAMP on the content of cholesterol esters in the cells.

This investigation thus showed that db-cAMP reduces the content of cholesterol esters in cells obtained from regions of the aorta with atherosclerotic changes. It can be tentatively suggested that cAMP derivatives and agents increasing the cAMP concentration in the cells may help to reduce the level of lipoidosis in the vessel *in vivo*.

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IDENTIFICATION OF SINUS NODE PACEMAKER CELLS OF THE RAT HEART BY INTRACELLULAR INJECTION OF LANTHANUM IONS

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The study of the structural basis for activity of different types of cardiomyocytes requires the use of methods that enable morphological and functional parameters of the same cell to be investigated. The localization of cells with a particular type of electrical activity is usually based on injection of solutions of dyes [3, 4], or cobalt [6] or lanthanum [7] ions through the recording electrode. The demands for a combination of electrophysiological and electron-microscopic methods of analysis of a single cell are best satisfied by the method suggested by Taylor et al. [7].

The aim of the present investigation was to study qualitative and quantitative characteristics of action potentials (AP) of sinus node pacemaker cells of the rat heart and also the suitability of the lanthanum labeling method for morphological identification of these cells.

EXPERIMENTAL METHOD

Male Wistar rats weighing 130-180 g were used. Under pentobarbital anesthesia (40 mg/kg) thoracotomy was performed and the heart was perfused with 10 ml of 0.01% trypan blue solution in Hanks' solution (pH 7.4) at 38°C. The right atrium was placed in a continuous-flow constant-temperature cuvette with modified Krebs–Ringer solution, equilibrated with a mixture of 5% CO_2 and 95% O_2 to pH 7.4 [8]. The rate of change of the medium was 5-10 ml/min. A glass microelectrode was filled with a mixture of 3 M KCl and 2% $LaCl_3$ and introduced into the sinus node by means of a KM-1 micromanipulator, under control of a stereoscopic microscope. The location of the sinus node was determined from the nodal artery, stained with trypan blue. The recording system consisted of a UPT-2 dc amplifier and dual-beam S8-2 storage oscilloscope. La^{+++} ions were injected after recording AP of the pacemaker cell by applying positive square pulses of current with a strength of 2-6 μA, frequency 5 Hz, and duration 100 msec to the microelectrode for 2 min. In this way from six to 15 pacemaker cells were labeled in each of the five animals studied. Parameters of AP of pacemaker cells obtained in this series were checked against traces of potentials recorded by microelectrodes filled with 3 M KCl only. The preparations were then fixed with 4% glutaraldehyde solution in Millonig's buffer at 4°C for 2 h, rinsed in buffer, the region of the sinus node was then isolated and post-fixed in 2% OsO_4 in Millonig's buffer, dehydrated, and embedded in Epon. Ultrathin sections, both stained with uranyl acetate and lead citrate, and also unstained, were examined in the Hitachi 11-E-2 electron microscope. The structure of the $LaCl_3$ tags was analyzed after drying the lanthanum chloride solution on a formvar support.

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