CALCIUM ION UPTAKE BY CULTURES OF SKIN FIBROBLASTS FROM NORMAL SUBJECTS AND FROM PATIENTS WITH SYSTEMIC CONNECTIVE TISSUE DISORDERS

M. D. Grozdova, A. F. Panasyuk, and I. L. Sharlova

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The uptake of radioactive calcium (^{45}Ca) and the effect of adrenalin on this process were studied in suspensions of fibroblasts from monolayer cultures of skin fibroblasts from healthy persons and from patients with systemic scleroderma and rheumatic fever. A considerable increase in calcium uptake was found in systemic scleroderma compared with normal and with rheumatic fever. Adrenalin, in concentrations of $2 \cdot 10^{-7}$ - $2 \cdot 10^{-5}$ M, increased the rate of calcium accumulation by normal fibroblasts but had the opposite action in scleroderma. The results point to a disturbance of the function of the fibroblast membrane in systemic scleroderma, and this may lie at the basis of changes in the metabolic activity of these cells.

KEY WORDS: calcium uptake; systemic scleroderma; fibroblast; adrenalin; rheumatic fever.

In some systemic connective tissue disorders in man the fibroblasts have the power of increased collagen biosynthesis [2, 8, 14]. However, there are virtually no data in the literature on the biochemical mechanisms which lie at the basis of activation of the function of fibroblasts in these diseases.

Considering the role of Ca^{2+} ions as regulators of the level of cell functional activity, an investigation was undertaken to determine the accumulation of ^{45}Ca by skin fibroblasts from normal human subjects and patients with rheumatic fever and with systemic scleroderma (in vitro) and to study the effect of adrenalin on this process.

EXPERIMENTAL METHOD

Cultures of fibroblasts were obtained from biopsy specimens of the skin from the forearm of patients with systemic scleroderma and rheumatic fever and from healthy donors aged from 14 to 52 years. The cells were cultured in medium of the following composition: Eagle's medium 50%, 0.5% lactalbumin hydrolysate 30%, inactivated bovine serum 20%, penicillin and streptomycin 50 units/ml of each. The fibroblasts of the cultures were studied between the 2nd and 6th subcultures on the seventh to eighth day after the last subculture during the stationary phase of growth. To prepare the cell suspension, the culture was treated with 0.25% trypsin for 5 min, suspended in growth medium, after which the sample was centrifuged for 10 min at 200g and the cells were resuspended in medium No. 199 containing 1% bovine serum. The final concentration of fibroblasts was 1.0×10^6 cells/ml. The protein concentration in the sample was determined by Lowry's method [9].

To determine Ca^{2+} ions, ^{45}Ca was added to 1 ml of the fibroblast suspension in a dose of 1.4×10^6-2.3×10^6 cpm (the final Ca^{2+} concentration was 1.2-2.1 mM) and the sample was incubated at 37°C with constant mechanical shaking for 5 min. After incubation, ethyleneglycolbis(β-aminoethyl)-N,N'-tetraacetate (EGTA) in Tris-HCl buffer, pH 7.4, and ruthenium red were added (the final concentrations were 2 mM and 12 μM respectively). Preliminary analysis showed that addition of EGTA and ruthenium red inhibited the uptake of ^{45}Ca by the fibroblasts almost completely. Isotope bound nonspecifically to the outer surface of the cells could thus be washed off and, in that way, the intracellular ^{45}Ca only could be determined. The samples were then centrifuged in the cold and the residue was washed off 3 times (with subsequent centrifugation) with cold Hanks' solution made up without Ca^{2+} ions and containing EGTA and ruthenium red in the above-mentioned concentrations. The dried residue was hydrolyzed in concentrated $HClO_4$ (2 h at 70°C) and the radioactivity of the hydrolyzate (0.1 ml) was

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TABLE 1. Uptake of ^{45}Ca by Skin Fibroblasts from Normal Donors and Patients with Systemic Connective Tissue Disorders (M ± m)

Characteristics of cultures				Rate of uptake of ^{45}Ca, pmoles/min/10^6 cells			
				Ca^{2+} concentration in medium 1.2 mM		Ca^{2+} concentration in medium 2.1 mM	
experimental conditions and No.	time from beginning of explantation, days	subculture	day	without additions	adrenalin 2.2×10^{-5} M	without additions	adrenalin 2.2×10^{-5} M
Control							
1	159	2	8			21	40
1[a]	175	4	7			42	45
2	184	4	7			19	34
3	73	2	7			109	114
4	60	2	8	53	60	100	—
5	54	2	7			57	—
6	54	2	8			46	47
7	60	2	8			32	38
		Mean				53±12	53±12
Systemic scleroderma							
1	64	1	8			153	121
2	156	4	7	135	88		
3	162	2	7			97	45
4	159	3	8			91	25
5	105	4	7			104	38
6	94	4	8			136	56
		Mean				116±12	68±18
Rheumatic fever							
1	122	3	7	24	—	35	—
2	120	2	8			36	34
3	131	4	7			61	46
4	116	2	7			19	71
5	196	2	8	16	—		
5[a]	218	5	8	22	25	19	—
6	82	2	8			79	—
6[a]	92	4	7	38	69		
7	55	2	7			122	99
		Mean		26±5		53±14	63±14

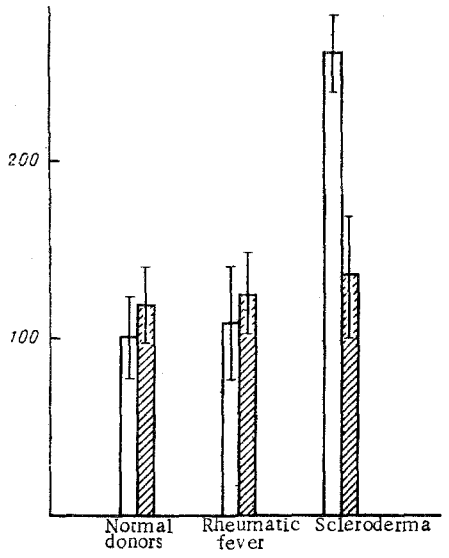

Fig. 1. Effect of adrenalin on ^{45}Ca uptake by fibroblasts. Unshaded columns indicate ^{45}Ca uptake without addition of adrenalin, shaded columns – in the presence of $2 \cdot 10^{-5}$ M adrenalin. Ordinate, uptake of ^{45}Ca by fibroblasts (in %; uptake of ^{45}Ca in healthy donors taken as 100%).

determined in a scintillation mixture: 3 ml methylcellosolve and 6 ml toluene, containing 0.4% 2,5-diphenyloxazole (PPO).

EXPERIMENTAL RESULTS

Eight cultures of skin fibroblasts from 7 healthy donors, 6 cultures from 6 patients with systemic scleroderma, and 9 cultures from 7 patients with rheumatic fever were investigated.

Comparison of the rate of uptake of ^{45}Ca in the same donor at the second and fourth subcultures in the case of normal fibroblasts and the second and fifth subcultures in the case of rheumatic fever, showed no difference in the uptake of ^{45}Ca.

As Table 1 shows, under normal conditions the ^{45}Ca uptake varied from 19 to 109 pmoles/min/10^6 cells; the protein content in the sample varied from 300 to 439 μg. Only one reference could be found in the literature to the study of Ca^{2+} transport in fibroblasts (from chick embryos), in which the Ca^{2+} uptake was 5-7 moles/mg protein/15 min of incubation (the conditions of incubation differed from those used in the present investigation) [12].

A marked increase in the ^{45}Ca uptake into fibroblasts was discovered in systemic scleroderma (Table 1); in this case the protein content in the samples varied from 322 to 376 $\mu g/10^6$ cells and was indistinguishable from normal. A tendency toward an increase in the uptake of ^{45}Ca also was observed in cultures of skin fibroblasts from some patients with rheumatic fever (Table 1).

The activation of collagen synthesis in the skin fibroblasts in systemic scleroderma was thus combined with a sharp increase in Ca^{2+} incorporation. The latter, in turn, was evidence of a marked increase in permeability of the outer cell membranes of the fibroblasts and a possible change in the velocities of a whole range of metabolic processes (glycolysis, oxidative phosphorylation, etc.).

In the cells of many tissues the permeability of the cell membranes with respect to Ca^{2+} is known to be controlled by cyclic AMP [1]. Meanwhile, no effect of accumulation of cyclic AMP in the cells on Ca^{2+} transport has been found in bone cells [5] and lymphocytes [11, 15]. This problem has not been studied in relation to fibroblasts. Attempts accordingly were made to study the effect of adrenalin as an agent raising the cyclic AMP level in cultures of fibroblasts [4] on Ca^{2+} transport in fibroblasts in health and disease. As Fig. 1 shows addition of adrenalin in a concentration of $2 \cdot 10^{-5}$ M stimulated ^{45}Ca uptake into normal fibroblasts. In systemic scleroderma the opposite effect was found. Adrenalin in concentrations of $2 \cdot 10^{-6}$ and $2 \cdot 10^{-7}$ also had a stimulating action on ^{45}Ca uptake in normal donors but an inhibitory action in systemic scleroderma. In rheumatic fever, adrenalin had a stimulating action in most experiments.

Preliminary results of experiments with cultures of skin fibroblasts from patients with rheumatoid arthritis showed that the Ca^{2+} uptake was below or equal to normal, and that adrenalin inhibited this process.

Changes in the response of the cells to adrenalin cannot at present be explained. The abnormal effect of hormones on enzymic processes, as we know, may be the result of the appearance of isozymes that are controlled differently from normally. However, there are no corresponding data with respect to fibroblasts from patients with collagen diseases. It must however be pointed out that factors such as changes in the lipid (especially fatty acid) composition of the cell membrane [7, 10] and a modification of its conformation [13] may play an important role in the modulation of adenylate cyclase activity and Ca^{2+} binding. Lasting changes, maintained during subculture, in the structure and function of the fibroblast membrane must in turn have a significant role in regulating the action of certain enzyme associations [10].

The results thus point to a marked increase in permeability of the cell membranes of fibroblasts with respect to Ca^{2+} in systemic scleroderma and the abnormal response of these cells to hormonal action.

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