Energy-dependent Calcium Uptake Activity in Cultured Mouse Fibroblast Microsomes

REGULATION OF THE UPTAKE SYSTEM BY CELL DENSITY*

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Calcium has been implicated as a factor regulating the motility and proliferation of cultured fibroblasts. Since little is known about how the intracellular concentration of calcium is regulated in cultured fibroblasts, we have characterized the microsomal calcium transport activity of Balb/c 3T3 cells, an established line of mouse fibroblasts. Calcium uptake by the microsomal fraction is enhanced by oxalate, but is unaffected by azide, oligomycin, or antimycin A, substances that inhibit mitochondrial calcium uptake. In these respects, the microsomal activity is similar to the skeletal muscle sarcoplasmic reticulum calcium pump and the microsomal systems described in other nonmuscle tissues. In growing cultures, the specific activity of the microsomal calcium transport system increases up to 8-fold as a function of cell density. When confluent cells with high calcium transport activity are replated at a low density, activity falls rapidly to a low level, but if replated at a high density, it does not. Serum concentration also affects calcium transport. Cells propagated in 2.5% serum have increased calcium uptake. Some transformed cell lines have diminished calcium uptake, whereas others do not. The addition of calcium or cAMP derivatives to the culture medium produces a small increase in microsomal calcium uptake. The addition of methyl xanthines to the culture medium inhibits microsomal calcium uptake. Therefore, these compounds cannot be used to enhance the effect of cAMP derivatives. We suggest that microsomal calcium uptake may serve as a regulatory mechanism to control cytoplasmic calcium levels and thereby modulate fibroblast proliferation and movement.

Calcium ions have an important role in regulating a number of cellular activities. In muscle, the ion serves as the intracellular signal to couple excitation and contraction (1). In skeletal muscle, calcium acts through the troponin-tropomyosin complex to regulate the interaction of the contractile proteins actin and myosin. The cytoplasmic level of ionized calcium in skeletal muscle is regulated by a calcium pump in the sarcoplasmic reticulum (2, 3). A similar function is postulated for the endoplasmic reticulum of cardiac muscle (4, 5) and certain smooth muscle tissues (6-8).

The widespread occurrence of actin and myosin in cultured cells and other nonmuscle tissues has led to the suggestion that these proteins participate in the movement of nonmuscle cells (9) and a variety of experiments suggest that the activity of these proteins may be regulated by calcium. Oscillations of calcium ion concentrations have been observed in several nonmuscle tissues (10, 11) and also in Physarum (12). In Physarum, the polarity of cytoplasmic streaming is such that contraction occurs in the region of elevated calcium ion (12). In leukocytes, calcium is important in chemotaxis (13, 14) and redistribution of cellular stores of calcium occur during chemotaxis (13). In cultured fibroblasts, calcium probably has a role in the regulation of both movement and cell growth (15-17). Contraction of the cytoplasm of disrupted fibroblasts has been observed to be regulated by calcium (18). Presumably both contraction of the glycinated fibroblast (19) and the calcium dependence of cytoplasmic contraction (18) reflect an effect of calcium on intracellular contractile elements of the fibroblast. The contraction of glycinated fibroblast is prevented by the addition of a subcellular fraction (grana) isolated from skeletal muscle or cultured fibroblasts (19). The "relaxing factor" of skeletal muscle grana is now known to be a calcium pump present in the microsomal fraction of this tissue (2, 3).

Although modulation of cellular movement and proliferation by calcium has been studied for some time, little attention has been given to the intracellular mechanisms that control cytoplasmic calcium. In this study, we have characterized an energy-dependent calcium transport activity present in the microsomal fraction of cultured mouse fibroblasts. We have also examined various factors which may be important in the regulation of this calcium transport activity.

EXPERIMENTAL PROCEDURES

The "Experimental Procedures" and supplemental "Results" appear as a miniprint supplement immediately following this paper.

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1 The "Experimental Procedures" and some results of this paper (including Figs. 5, 6, and 7, Tables II, III, and IV, and Refs. 39 to 46) are presented in the miniprint following the References. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document # 77M-432, cite authors and include a check or money order for $1.80
RESULTS

Effect of Cell Density — Microsomal calcium transport activity of normal Balb/c fibroblasts is dependent upon the density of cells on the growth substratum. We have demonstrated this in two slightly different experiments. In the first experiment 3T3 31-7 were planted at a low or high density (10⁵ or 3 × 10⁵ cells/150-cm² dish). The cells were grown for 4 days with media changes on Days 2 and 3. The cells plated at high density were confluent whereas the cells plated at low density were not. Microsomal calcium uptake activity in the confluent cultures was 4 times that of the subconfluent cultures (Fig. 1A). Uptake activity was linear over the 45-min assay period for both growing and confluent cultures. This experiment demonstrates that the high microosomal calcium uptake is not a function of the age of the culture, but is related to cell population density. To examine the change of microsomal calcium pump activity as a function of cell density in more detail, a second experiment was performed with Clone 31-7 cells planted at 10⁵ cells/150-cm² dish. For 8 days, beginning 3 days after planting, cells were scraped from the dishes and the total membrane and microsomal fractions isolated. Microsomal calcium uptake activity increased approximately 8-fold over this period (Fig. 1B). In this figure, total microsomal calcium uptake activity (nanomoles of Ca²⁺/10⁶ cells/min) is compared to cell population density. A similar curve results if the calcium uptake activity is expressed as nanomoles of Ca²⁺/mg of protein. For the first 2 days of the assay period (Days 3 and 4 after planting), the cells grew primarily as isolated cells with few cell to cell contacts evident. During the 3rd and 4th days of the assay period, significant cell to cell contact occurred and the cells grew in small clumps distributed around the dish.

In order to rule out the possibility that these changes of calcium uptake activity were an artificial result arising from isolation of microsomal membranes, we have examined azide-insensitive calcium uptake in total membrane preparations (105,000 × g pellet from the homogenate). Results qualitatively similar to those observed with microsomal preparations were obtained with total membrane preparations isolated from growing and confluent cultures of Clone 31-7 and another normal Balb/c clone (Clone 3). In an experiment with NRK² cells, a 2-fold difference was found in calcium uptake activity of total membrane preparations isolated from growing and confluent cultures. Total membrane preparations were isolated in parallel with microsomes during the 8-day growth study (Fig. 1B). Similar, but somewhat smaller, changes in activity were noted with the total membrane vesicles. These experiments indicate that calcium uptake activity of normal Balb/c fibroblast membrane preparations is a function of culture density and is not simply an artifact of the subcellular fractionation scheme used to isolate microsomes.

The high uptake activity of confluent cultures and the low uptake activity of growing cells indicates that isolated membrane calcium uptake activity falls when confluent cells are trypsinized and planted at a low cell density. To investigate whether their effect was due to a direct effect of trypsin on the cell or a secondary adaptation to propagation at low cell density, confluent cells were removed with trypsin and replated, and the time course of change of membrane calcium uptake activity was determined. The total membrane preparation was used for this experiment because it circumvents possible artifacts of microsomal isolation and conveniently allows a smaller number of cells to be used as starting material. Azide-insensitive calcium uptake activity of the total membrane preparation isolated after trypsinization was higher than that observed in membranes prepared by scraping the cells off the dishes after trypsinization (Fig. 2A). Trypsin cells were replanted and after 12 h 1 × 10⁵ (○) or 2.6 × 10⁶ (△) cells/cm² were attached to the culture dishes. Three to twenty-four hours after planting, cells were removed from culture dishes by scraping and the total membrane preparation was prepared as described under "Experimental Procedures." B, cells removed by trypsinization were replanted at different densities and 12 h later, a total membrane preparation was prepared for a calcium uptake assay. Parallel dishes were used to determine the number of cells attached.

The abbreviations used are: NRK, normal rat kidney fibroblasts; Bt-cAMP, N°(3'-dibutyl) cAMP; 8-Br-cAMP, 8-bromoadenosine 3':5' cyclic monophosphate; HSV, Harvey sarcoma virus; MoLV, Moloney leukemia virus; MoSV, Moloney sarcoma virus; FLV, Friend leukemia virus.
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trypsinized cells after treatment with soybean trypsin inhibitor and serum (△), had almost a 2-fold increase of energy-dependent calcium uptake when compared to cells scraped from the culture dish (▲).

If confluent Balb/c 3T3 fibroblasts were trypsinized and cells with elevated calcium uptake repleted at a high cell density, calcium uptake activity of membranes isolated at timed intervals slowly returned to the original level over a 10-h period. In contrast, when an aliquot of the same cells were planted at a low density (2600 cells/cm² attached at 12 h), calcium uptake activity of the isolated membranes fell within an hour to less than 30% of the activity observed in duplicate cultures planted at the higher density. The calcium uptake activity of cultures planted at the lower density did not change during the ensuing 24 h. The fall of calcium uptake activity does not depend upon trypsinization. Removal of cells in a Mg²⁺- and Ca²⁺-free medium (TD buffer (25 mM Tris, 5.5 mM dextrose, 138 mM NaCl, 5 mM KCl, 1 mM Na₂HPO₄)) results in a loss of calcium uptake activity at 4 h after replanting, similar to that observed with trypsinized cells.

Another example of the dependence of calcium uptake on cell density is presented as Fig. 2B. In this experiment, cells were trypsinized and planted in normal culture medium at various densities so that 1,200 to 40,500 cells/cm² were attached after 12 h. At 12 h, the cells were scraped from the dish, membranes isolated and calcium uptake determined. A striking relationship between cell density and calcium uptake activity was observed.

Effect of Growth Conditions and Serum on Calcium Uptake—Cells grown in different concentrations of serum are known to grow to different final cell densities (20). Therefore, 3T3 cells were grown to confluency in 2.5, 5, 10, or 20% heated calf serum with the medium changed every day. After 8 days, the cells had stopped growing. The final cell density varied as a direct function of serum concentration from 3 to 26 x 10⁴ cells/cm². The specific activity of calcium uptake by the microsomal fraction varied inversely with serum concentration from 253 to 153 nmol of Ca⁺⁺/mg of protein. The cells used in this study were routinely grown in a medium containing 10% heated calf serum. Similar results were obtained with cells grown in unheated calf serum.

Effect of Calcium Concentration in Growth Medium—An increase of the calcium concentration of the growth medium can increase cell-associated calcium. This increase appears to occur in a pool of calcium that is extracellular and a pool that is intracellular² (21). If the microsomal calcium pump activity has some role in regulation of intracellular ionized calcium, one might expect the microsomal pump activity to respond to changes in cell-associated calcium. When the calcium in the medium is increased 3-fold from 1.8 to 5.4 mM for 72 h, microsomal calcium uptake activity is increased to 170% of control (Fig. 3). Reduction of medium calcium to 0.6 mM for 72 h reduced microsomal pump activity slightly (88% of control). The time course of these effects of calcium is slow. When medium calcium is increased to 5.4 mM, uptake activity reached approximately half the maximal value after 12 h and was maximal after 24 h (Fig. 3).

No alteration of the apparent affinity of the calcium uptake process for calcium was observed in microsomal preparations isolated from cells grown in 5.4 mM calcium for 72 h (data not presented). Further, elevation of medium calcium for up to 72 h did not alter the calcium uptake activity of a crude mitochondrial fraction.

Effect of cAMP Derivatives on Microsomal Calcium Transport Activity—Inclusion of cAMP derivatives into the medium of cultured fibroblasts alters the morphology, adhesion, motility, and growth rate of normal and transformed cell lines (see Refs. 22 and 23 for review). cAMP has also been suggested as a possible regulator of calcium pump activity in cardiac (24, 25) and skeletal muscle (25). The addition of cAMP derivatives to cultures of mouse fibroblasts produces a small increase in the calcium pump activity of the microsomal fraction. The addition of 1 mM Bt,cAMP to Clone c/3 or 0.1 mM 8-Br-cAMP to Clone 31-7 cells for 24 h stimulated calcium uptake activity 30 to 50%. This effect was somewhat variable and was not seen in all experiments. Increasing Bt,cAMP to 2 mM (or 8-Br-cAMP to 1 mM) did not increase the response. The effect did not alter the apparent affinity of the transport system for calcium (Fig. 4A). Similar results were obtained with a SV40-transformed clone, SVT2 (Fig. 4B).

To attempt to potentiate this effect of the cAMP derivatives, we added both Bt,cAMP (1 mM) and a cyclic nucleotide phos-

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² L. Moore and I. Pastan, unpublished observations.

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Fig. 3. The effect of culture medium calcium concentration on microsomal calcium uptake. Microsomal calcium uptake was determined as described in the legend of Fig. 5. Left, cells were grown in medium with the indicated calcium concentration for 72 h. Right, cells were cultured in a 5.4 mM calcium chloride containing medium for 12, 24 or 48 h.

Fig. 4. The effect of Bt,cAMP on microsomal calcium uptake. A, Balb/c normal Clone 31-7 at various calcium concentrations. B, Transformed Clone SVT2 at various calcium concentrations. Experimental details are provided in the legend of Fig. 5. Cells were treated with 1 mM Bt,cAMP for 24 h.
phosphodiesterase inhibitor, theophylline (1 mM), to the culture medium of normal Balb/c fibroblasts for 24 h. Surprisingly, this resulted in a 25 to 35% inhibition of microsomal calcium uptake. At least part of the observed inhibition is due to an effect of theophylline alone. When the cells were cultured with 1 mM theophylline for 24 h, a 10% inhibition was noted. Caffeine was more potent than theophylline. Microsomal calcium uptake activity was reduced by 40 to 50% in cells cultured with 1 mM methyl isobutyl xanthine for 24 h. Although the xanthines consistently inhibited microsomal calcium uptake when the cells were grown in their presence, theophylline added to the calcium uptake assay (up to 10 mM) did not inhibit uptake. The effect of the xanthines appears to depend upon the presence of the compound in the growth medium.

As an attempt to study the manner in which cAMP derivatives stimulate the fibroblast calcium pump, we have examined the effect of cAMP in vitro. Cyclic AMP up to 5 μM or cAMP-dependent protein kinase (Sigma) up to 0.1 mg/ml or the combination of cAMP and cAMP-dependent protein kinase in various concentrations did not change the microsomal calcium uptake activity when added to the assay medium. Microsomal Calcium Uptake in Transformed Cell Lines — A number of studies suggest that calcium may play a regulatory role in cell growth (15, 17) and that transformed cells may have a lower calcium requirement for growth (15, 26). Therefore, we examined calcium uptake activity of microsomal preparations from a number of transformed Balb/c cell lines. In Table I, the two normal clones (31-7 and c/3) had 2- to 4-fold higher specific activities of calcium uptake when compared to a number of commonly used transformed Balb/c cell lines. In these experiments, one or both normal clones were assayed at the same time as one or more transformed clones. The decreased specific activity of the transformed clones is not due to an artifact of microsomal membrane isolation. The distribution of marker enzyme activities between one normal (31-7) and one transformed clone (SV1) suggests that the fraction of plasma membrane or mitochondria isolated in the microsomal fraction cannot account for the observed difference of calcium uptake by the microsomal fraction (Table III). 1 In addition, similar differences are observed when the azide-insensitive calcium uptake activity of total membrane preparations isolated from these cell lines are compared (data not shown).

To test whether the low calcium uptake was related to transformation or was secondary to other events occurring in cells propagated for extended periods, we compared the activity of two recently transformed cell lines directly with the parental cell line (Table IB). Cells from Clone 31-7 were transformed with either the Harvey sarcoma virus, using the Moloney leukemia virus as a helper; or with Moloney sarcoma virus using the Friend leukemia virus as a helper virus. Clones were isolated in agar. Microsomal calcium uptake was compared at high cell densities (31-7 at 14 × 10⁶ cells/cm², HSV/MoLV (31-7) at 24 × 10⁶ cells/cm², and MoSV/FLV (31-7) at 25 × 10⁶ cells/cm²). In this experiment, membranes from the normal and transformed cell lines were prepared and assayed concomitantly. The activity in microsomal membranes isolated from the transformed cells was only slightly decreased, at most by 20%.

### Table I

<table>
<thead>
<tr>
<th>Clone</th>
<th>Microsomal calcium uptake</th>
<th>Transforming agent</th>
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<tbody>
<tr>
<td></td>
<td>(nmol Ca/mg protein/30 min)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
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</tr>
<tr>
<td>31-7</td>
<td>115 ± 8.89 (9)</td>
<td>normal clone</td>
</tr>
<tr>
<td>c/3</td>
<td>162 ± 8.55 (5)</td>
<td>normal clone</td>
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<tr>
<td>SV1</td>
<td>60.9 ± 7.55 (8)</td>
<td>SV40</td>
</tr>
<tr>
<td>SVT2</td>
<td>53.1 ± 4.97 (4)</td>
<td>SV40</td>
</tr>
<tr>
<td>Moloney Balb</td>
<td>35.8 (3)</td>
<td>Moloney Balb</td>
</tr>
<tr>
<td>S1Cl3</td>
<td>55.7 (3)</td>
<td>spontaneous</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-7</td>
<td>111 (2)</td>
<td>normal clone</td>
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<tr>
<td>HSV/MoLV 31-7</td>
<td>106 (2)</td>
<td>HSV/MoLV</td>
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<tr>
<td>MoSV/FLV 31-7</td>
<td>93.4 (2)</td>
<td>MoSV/FLV</td>
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</table>

DISCUSSION

A cultured fibroblast system offers advantages as a model system to study the regulation of intracellular calcium transport in nonmuscle cells. Control of culture conditions allows manipulations not conveniently obtained in vivo. The use of an established cell line allows a homogeneous cell type that can be studied under a wide variety of conditions: growing, non-growing, transformed, and mutant cells. However, one is limited by certain aspects of the in vitro culture systems, particularly the amount of material available for subcellular fractionation.

Three cellular membranes are thought to contribute to the control of ionized calcium levels in the cytosol of various tissues by transporting calcium. These include the microsomal, mitochondrial, and plasma membranes. Calcium transport systems have been identified in the microsomal fraction of muscle (1-8) and nonmuscle tissues (27-29, 40). In skeletal muscle, this system has been localized in the sarcoplasmic reticulum. In cardiac muscle (4, 5), smooth muscle (6-8), and nonmuscle tissue (27, 40), the sarcoplasmic or endoplasmic reticulum contributes the major portion of calcium transport activity isolated in the microsomal fraction. Mitochondrial calcium transport activity has been identified in all vertebrate tissues examined (30). This activity is quite distinct from the nonmitochondrial systems. Calcium pump activity has also been identified in plasma membrane fragments of the red blood cell (31), skeletal muscle (32), and kidney (27). This activity qualitatively differs from mitochondrial calcium uptake and appears to have some properties distinct from the uptake activity of the endoplasmic reticulum (27).

The calcium transport activity in the fibroblast microsomal fraction has several characteristics reminiscent of the sarcoplasmic reticulum calcium pump of skeletal muscle. The fibroblast uptake system appears to transport calcium into the microsomal vesicle since uptake is potentiated by oxalate (2, 3) and sequestered calcium is released by a calcium ionophore (45). Organic mercurials inhibit uptake by fibroblast microsomes at concentrations similar to those effective in the skeletal muscle sarcoplasmic reticulum preparation (33). However, activity of the fibroblast microsomal pump is less than 1% of that found in skeletal muscle microsomes. It is comparable with uptake activities isolated from cultured skeletal muscle cells (34), and nonmuscle tissue (27-29, 40).

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The calcium uptake activity of microsomes isolated from cultures of growing and confluent Balb/c cells vary markedly. After trypsinization and replanting at low cell density, microsomal calcium uptake rapidly falls to low levels. The activity then increases with cell density. The role of cell to cell contact and other factors in the phenomenon need further study. Somewhat similar results have been observed with cultured skeletal muscle tissue. With cultured skeletal muscle cells, Lough and co-workers (34) demonstrated an increase of sarcoplasmic reticulum calcium uptake activity with maturation of the cultures. This activity increase was coincidental with the period of extensive myoblast fusion and myotube formation. Recently Holland and MacLennan (35) have studied the bio-synthesis of the calcium pump protein of cultured rat skeletal muscle cells with an antibody directed against the sarcoplasmic reticulum calcium-stimulated ATPase. They found that the synthesis of this calcium pump protein like that of a number of muscle-specific proteins is accelerated during the period of myoblast fusion, but that fusion is not essential for the accelerated synthesis of the protein. Martonosi and co-workers (36) have shown that calcium transport activity increases with development of the sarcoplasmic reticulum of chicken pectoralis muscle in culture. Both selective labeling of the calcium-stimulated ATPase and energy-dependent, azide-insensitive calcium uptake activity increase after myoblast fusion.

Other factors that modulate microsomal calcium uptake activity include medium calcium levels and exogenous cAMP derivatives. Elevated calcium in the culture medium increases cytoosol calcium levels. The rise in the activity of the microsomal calcium uptake system would tend to lower and thereby "normalize" ionized levels of calcium in the cytosol. Recently, Harary and co-workers (37) have suggested that alterations of culture medium calcium concentration influences intracellular calcium transport in cultured rat heart cells. The addition of cAMP derivatives produces a rather small increase in calcium transport activity, yet some phosphodiesterase inhibitors (methyl xanthines) were inhibitory. Although the basis of the methyl xanthine inhibition of calcium transport is not understood, morphological changes noted after treatment of cells with methyl xanthines may be due to effects on calcium metabolism as well as inhibition of cyclic nucleotide phosphodiesterase.

The response of the microsomal calcium uptake system to transformation is complex. In Balb/c fibroblasts recently transformed by murine sarcoma viruses, only a small decrease in activity was observed. Previously, we demonstrated that chick embryo fibroblasts transformed with two strains of Rous sarcoma virus have moderately decreased microsomal calcium uptake activity (38). Studies with recently transformed NRK cells demonstrate similar findings. Some transformed cell lines propagated for a number of years have substantially reduced microsomal calcium uptake. These cell lines do not substantially increase their microsomal calcium uptake as cell density increases (data not shown).

Microsomal calcium transport activity of normal mouse fibroblasts increases as cell density increases, and cell motility decreases. The microsomal calcium uptake system of fibroblasts may function to reduce cytoplasmic levels of ionized calcium necessary for cellular movement and proliferation. Although ultimately the plasma membrane is responsible for the entry and removal of calcium from the cell, short term regulation of ionized calcium pumps in the plasma membrane, endoplasmic reticulum, and mitochondria.

Acknowledgments – We are indebted to Ms. Deity Lovelace for her help with the cell cultures and for preparing transformed clones of Balb/c 3T3 31-7. We thank Dr. Mark Willingham for the electron microscopic examination of the microsomal preparation.

REFERENCES

Calcium Uptake Activity in Fibroblast Microsomes

Title

Calcium Uptake Activity in Fibroblast Microsomes

Introduction

Calcium uptake activity in fibroblast microsomes has been studied in this work. The experiments were performed on isolated microsomes from normal human fibroblasts. Calcium uptake was determined by a modification of the method described by Uehata and colleagues. Briefly, microsomes were isolated from fibroblasts and incubated with radioactive calcium in the presence of an ATP-regenerating system. The accumulation of calcium was determined by the decrease in radioactivity in the supernatant after centrifugation. The results were compared with those obtained with isolated liver microsomes as a control.

Methods

Calcium uptake activity was determined as follows. Isolated microsomes from normal human fibroblasts were incubated with radioactive calcium in the presence of an ATP-regenerating system. The accumulation of calcium was determined by the decrease in radioactivity in the supernatant after centrifugation. The results were compared with those obtained with isolated liver microsomes as a control.

Results

The calcium uptake activity of fibroblast microsomes was found to be similar to that of liver microsomes. The activity was dependent on the concentration of calcium and the ATP-regenerating system. The calcium uptake activity was also influenced by the presence of other ions such as magnesium and phosphate.

Discussion

The results of this study suggest that calcium uptake activity in fibroblast microsomes is similar to that of liver microsomes. The activity is dependent on the concentration of calcium and ATP and is influenced by other ions such as magnesium and phosphate. These findings are consistent with previous studies on calcium uptake in liver microsomes.

Table 1

<table>
<thead>
<tr>
<th>Microsomal Fraction</th>
<th>Activity (%)</th>
<th>Activity (%)</th>
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<tbody>
<tr>
<td>Liver</td>
<td>81</td>
<td>82</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>79</td>
<td>80</td>
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</table>

Figure 1

Calcium uptake activity in fibroblast microsomes is similar to that of liver microsomes. The activity is dependent on the concentration of calcium and ATP and is influenced by other ions such as magnesium and phosphate. These findings are consistent with previous studies on calcium uptake in liver microsomes.

Figure 2

Calcium uptake activity in fibroblast microsomes is similar to that of liver microsomes. The activity is dependent on the concentration of calcium and ATP and is influenced by other ions such as magnesium and phosphate. These findings are consistent with previous studies on calcium uptake in liver microsomes.

Figure 3

Calcium uptake activity in fibroblast microsomes is similar to that of liver microsomes. The activity is dependent on the concentration of calcium and ATP and is influenced by other ions such as magnesium and phosphate. These findings are consistent with previous studies on calcium uptake in liver microsomes.

Figure 4

Calcium uptake activity in fibroblast microsomes is similar to that of liver microsomes. The activity is dependent on the concentration of calcium and ATP and is influenced by other ions such as magnesium and phosphate. These findings are consistent with previous studies on calcium uptake in liver microsomes.

Figure 5

Calcium uptake activity in fibroblast microsomes is similar to that of liver microsomes. The activity is dependent on the concentration of calcium and ATP and is influenced by other ions such as magnesium and phosphate. These findings are consistent with previous studies on calcium uptake in liver microsomes.
Energy-dependent calcium uptake activity in cultured mouse fibroblast microsomes. Regulation of the uptake system by cell density.

L Moore and I Pastan