Vol. 84, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 30, 1978 Pages 922-927

CYSTIC FIBROSIS SERUM PROMOTES [^{45}Ca] UPTAKE BY NORMAL HUMAN LEUKOCYTES

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Received September 11,1978

SUMMARY

A two-fold increase in [^{45}Ca] labeling was observed when normal human leukocytes were incubated in the presence of serum obtained from patients with cystic fibrosis. The degree of [^{45}Ca] labeling of leukocytes isolated from patients with cystic fibrosis was also significantly greater than that observed for leukocytes that had been isolated from normal individuals.

INTRODUCTION

A perturbation in the cellular transport of calcium may be responsible for the ciliary dyskinetic effect of cystic fibrosis (CF) serum on cilia beating in the rabbit trachea explant cilia bioassay system (1,2). Since the effect of CF serum on calcium transport in normal human cells has not been examined and a defect in calcium transport may be involved in the etiology of CF (3), we decided to evaluate the effect of CF serum on the cellular uptake of [^{45}Ca] using normal human leukocytes. [^{45}Ca] uptake by CF leukocytes was also examined since Davis *et al.* recently demonstrated that CF leukocytes produce less cyclic AMP when stimulated with β-agonists than do normal leukocytes (4) and cyclic AMP production in some types of cells is inhibited by high levels of intracellular ionized calcium (5).

0006-291X/78/0844-0922$01,00/0

METHODS

Leukocytes were isolated from 1 ml of whole heparinized blood, drawn by venipuncture from three CF patients and three normal subjects as described by Davis *et al.* (4). Serum was obtained from 4 ml of whole blood, drawn by venipuncture from three CF patients and three normal subjects using a plastic syringe. All blood samples were drawn after obtaining informed signed consent. The three CF patients were recruited from the Shreveport Cystic Fibrosis Treatment Center. DP, an 11 year old male was taking sulfisoxazole (55 mg/kg/day) and sodium dicloxacillin (72 mg/kg/day). BS, a 19 year old male was taking sodium oxacillin (179 mg/kg/day), cephalexin (36 mg/kg/day) and tetracycline (18 mg/kg/day). LG, a 14 year old male was taking sodium cloxacillin (117 mg/kg/day) and tetracycline (29 mg/kg/day). Normal subjects were recruited from among the staff and faculty of the LSU Medical Center in Shreveport. MWB was a 31 year old male, PKL was a 25 year old female and LTG was a 38 year old male. None of the three normal subjects had taken any medication within 48 hours prior to the time that blood was drawn.

Whole blood was allowed to clot at room temperature for 30 min. in a 15 ml plastic centrifuge tube and then centrifuged at 1,800 x *g* for 5 min. One ml of serum was removed from each sample and stored at room temperature for approximately 30 min. in a plastic test tube. After isolation, leukocytes were washed once in 4 ml of 0.9% NaCl (w/v) (centrifugation at 500 x *g* for 5 min.) and then resuspended in 4 ml of 0.9% NaCl (w/v) which contained 2.5 mMolar $CaCl_2$. Each suspension of leukocytes was then split to yield two 2 ml fractions which were placed in 15 ml conical plastic centrifuge tubes. Then 0.5 ml of normal or CF serum was added to each tube followed by 30 µl of [^{45}Ca]Cl_2 (0.5 mCi) obtained from New England Nuclear, Boston, MA (17 mCi/mg). Each tube was vortexed for 5 sec. and allowed to sit at room temperature for 1 hour. Every 5 min., the contents of each tube was gently mixed. The time period between the drawing of blood for leukocyte isolation and the end of the incubation was approximately 2 hours.

After the incubation, leukocytes were isolated by centrifugation at 1,000 x *g* for 5 min. and then washed one time in 4 ml of 0.9% NaCl (w/v) which contained 2.5 mMolar $CaCl_2$. Then 5 ml of liquid scintillation cocktail developed by Anderson and McClure (6) for the solubilization of cultured cells was added to each tube and the cells were vortexed until completely dissolved. Beta emissions from [^{45}Ca] were counted using a Packard Tri-Carb Model 3375 three channel liquid scintillation counter. Quenching was determined using the external standard channels ratio method but corrections for quenching were not made since all samples displayed essentially the same degree of quenching.

RESULTS

Data presented in Table 1 demonstrate that CF serum significantly stimulates the uptake of [^{45}Ca] by normal human leukocytes. Data presented in Table 2 indicate that even in the absence of CF serum, CF leukocytes take up significantly more

Table 1.

Effect of CF Serum on [^{45}Ca] Uptake By Normal Human Leukocytes

Normal Leukocytes and Normal Serum	Normal Leukocytes and CF Serum	Difference
(cpm)	(cpm)	(cpm)
34,345 (MWB-MWB)	109,014 (MWB-DP)	74,669
36,889 (PKL-PKL)	75,500 (PKL-BS)	38,611
23,950 (LTG-LTG)	54,526 (LTG-LG)	30,576
31,728 ± 3,958	79,680 ± 15,867*	47,952 ± 13,558†

Leukocytes obtained from three normal human subjects were incubated in 2 ml of 0.9% NaCl containing 2.5 mMolar $CaCl_2$ with 0.5 mCi [^{45}Ca] plus 0.5 ml of serum obtained from the same normal subject or 0.5 ml of serum obtained from a CF patient as described in the text. Initials in parenthesis designate individuals from whom leukocyte and serum samples were obtained. The bottom row of data represent the mean ± S.E.M. for each set of data.

*$P < 0.05$ using a one-tailed two sample unpaired student's t-test.
†$P < 0.025$ using a one-tailed two sample paired student's t-test.

[^{45}Ca] than do normal leukocytes. The uptake of [^{45}Ca] by CF leukocytes in the presence of CF serum was similar to that observed for the normal leukocytes when they were exposed to CF serum (compare data in Tables 1 and 2).

DISCUSSION

Current evidence suggests that a factor present in CF serum may alter cellular metabolism via a disturbance in the cellular transport of calcium (1,2). Since exocrine gland secretory activity is tightly regulated by intracellular calcium levels (7), a defect in the cellular transport of calcium in CF patients could be responsible for producing the exocrine gland dysfunction that is routinely observed in these patients (8). The data pre-

Table 2.

Uptake of [^{45}Ca] By CF Leukocytes

Normal Leukocytes and Normal Serum	CF Leukocytes and Normal Serum	CF Leukocytes and CF Serum
(cpm)	(cpm)	(cpm)
34,345 (MWB-MWB)	85,678 (DP-MWB)	100,381 (DP-DP)
36,889 (PKL-PKL)	69,236 (BS-LTG)	76,437 (BS-BS)
23,950 (LTG-LTG)	65,644 (LG-PKL)	64,969 (LG-LG)
31,728 ± 3,958	73,519 ± 6,167*	80,596 ± 10,432†

Leukocytes obtained from three normal human subjects or three CF patients were incubated in 2 ml of 0.9% NaCl containing 2.5 mMolar $CaCl_2$ with 0.5 mCi [^{45}Ca] plus 0.5 ml of serum obtained from normal subjects or CF patients as described in the text. Initials in parenthesis designate individuals from whom leukocytes and serum samples were obtained. The bottom row of data represent the mean ± S.E.M. for each set of data.

*$P < 0.01$ using a one-tailed two sample unpaired student's *t*-test.
†$P < 0.025$ using a one-tailed two sample unpaired student's *t*-test.

sented in this paper clearly demonstrate that something in CF serum can promote the uptake of [^{45}Ca] by normal human cells. The data also demonstrate that [^{45}Ca] uptake by CF leukocytes, even in the absence of CF serum, is significantly greater than that observed for normal leukocytes. This observation is in agreement with data presented by Shapiro *et al.* which demonstrate that CF fibroblasts take up significantly more [^{45}Ca] than do normal fibroblasts (9).

If this greater uptake of [^{45}Ca] by CF leukocytes is indicative of a greater intracellular pool of both bound and unbound calcium, then cyclic AMP production by adenyl cyclase might be expected to be depressed in CF leukocytes if ionized intracellu-

lar calcium inhibits adenyl cyclase activity in leukocytes as it does in some other types of cells (5). Davis *et al.* recently demonstrated that cyclic AMP production in CF leukocytes was significantly depressed compared to normal leukocytes when stimulated with β-agonists (4).

Most CF patients are routinely placed on oral antibiotics to prevent bacterial infection of the pulmonary system which represents the major cause of death among these patients. Some antibiotics may act as ionophores. However, the antibiotics taken by the patients selected for this study are not currently known to exert their antimicrobial activities via an ionophoreic effect according to literature supplied by the manufacturers and also according to recent reviews on the mechanism of action of the penecillin-type antibiotics (10), the tetracyclines (11) and the sulfonamides (12). In addition, Davis *et al.* demonstrated that the antibiotics tetracycline and cloxacillin are not responsible for depressing the cyclic AMP response of CF leukocytes to β-agonist stimulation (4). Bogart *et al.* did not indicate which antibiotics their patients were taking when blood samples were obtained for cilia bioassay studies (1,2). Shapiro *et al.* did not indicate if their patients were taking antibiotics when skin biopsies were obtained (9), but antibiotic levels in skin fibroblasts would probably be very low and during the cell culturing process, antibiotic effects on the cultured cells would probably be insignificant.

Additional experiments are being conducted to provide more information on this apparent defect in the cellular transport of calcium in CF patients. An attempt will be made to try to determine if the ciliary dyskinesia factor isolated from CF serum by Wilson and Fundenberg (13) is the agent responsible for producing

this [^{45}Ca] uptake effect of CF serum on normal human leukocytes. If a defect in the cellular transport of calcium does indeed occur in CF patients, then this calcium transport defect may be responsible for inducing exocrine gland dysfunction in these patients.

ACKNOWLEDGEMENTS

This work was supported by a grant obtained from the Edward R. Stiles Trust Fund.

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