Vol. 117, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1983 Pages 313-318

SODIUM ION MODULATES COLLAGEN TYPES IN HUMAN FIBROBLASTIC CELLS IN CULTURE

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Received October 7, 1983

SUMMARY : Collagen synthesis in human fibroblasts was increased by the presence of 0.1M excess NaCl in the culture medium. Type analysis of the collagen produced by the cells showed the increase in the ratio of type III collagen to the total collagen. Similar NaCl effects were observed in several cell lines derived from different tissues.

Sodium ion but not chlorine ion seems to be essential to the modulation of collagen production by the cells, because addition of CH_3COONa induced similar effects but addition of KCl instead of NaCl resulted in specific inhibition of the collagen production.

Many biological phenomena are accompanied by modulation of collagen metabolism in cells (1). These phenomena include epithelial-mesenchymal interactions (2), cell-matrix interactions (1,3) transformation of cells by viruses (4) and increment of cell density in culture (5). Serum factor also modulates collagen metabolism (6). During the course of the study on the regulation mechanisms of collagen metabolism in human fibroblasts, we found that changes of concentration of Na^+ in the culture medium modulated collagen types in the cells. Since sodium ion is a major ion species present in body fluids and in culture media used for tissue culture experiments, the phenomenon may be related to basic cellular metabolism.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium, fetal bovine serum and Fungizone were purchased from GIBCO, NY. Penicillin G and dihydrostreptomycin were generously donated by Toyo Jozo, Co., Ltd., Shizuoka. β-aminopropionitrile fumarate was obtained from Tokyo Kasei Co., Ltd., Tokyo, and sodium ascorbate was from Wako Junyaku Co., Ltd., Osaka. Pepsin (porcine gastric mucosa) was from Boehringer Mannheim GmbH. Purified Cl. histolyticum collagenase was obtained from Amano Pharmaceutical Co., Ltd., Nagoya, and further purified by gel chromatography (7). L-[2,3-^{3}H]Proline was purchased from New England Nuclear, Boston, MA. and purified by chromatography on a column of LCR-2 with 1M HCl before use (8).

0006-291X/83 $1.50

Cell culture and metabolic labeling

Human skin fibroblasts were obtained from biopsy specimens of normal adults or patients with progressive systemic sclerosis (9) or with osteogenesis imperfecta. Human stomach muscle cells were obtained from the outgrowth of non-cancerous muscle layer in the specimen of stomach cancer. The cells seem to be mainly composed of smooth muscle cells on the basis of their morphology and of relatively high production of type III collagen.

Cells were plated onto 35 mm or 60 mm dishes and grown in Dulbecco's modified Eagle's medium supplemented with Fungizone (250 μg/l), penicillin G, dihydrostreptomycin (50mg/l) and with 10% fetal bovine serum until confluent phase. Then cells were incubated with the fresh medium in the presence of 50μCi/ml of chromatographically purified [^{3}H]proline, 0.1mM sodium ascorbate, 0.5mM β-aminopropionitrile fumarate and of the desired concentrations of Na^+ or K^+ for 1 to 48 h. Concentrations of Na^+ and K^+ were adjusted by addition of NaCl, CH_3COONa and KCl, respectively. In some experiments, cells at log phase were used and were also incubated with [^{3}H]proline in the absence of fetal bovine serum.

Analysis of collagen and non-collagenous proteins with bacterial collagenase

After the incubation of cells with [^{3}H]proline, the medium and the cell layer were combined and sonicated. Aliquots were heated for 10 min in boiling water, dialyzed against 0.05M acetic acid and used for the analysis of radioactive collagen and non-collagenous protein with purified Cl. histolyticum collagenase as described previously (10).

Analysis of total hydroxyproline

An aliquot of the combined fraction of cell layer and medium was lyophilized and hydrolyzed with constant-boiling HCl and total hydroxyproline was quantitated with an amino acid analyzer (8).

Type analysis of collagen

Cells were cultured and labeled with L-[2,3-^{3}H]proline for 24h. Collagenous protein was isolated, purified after pepsin digestion and subjected to sodium dodecylsulfate- 4.5% polyacrylamide slab gel electrophoresis in the presence and absence of dithiothreitol (10). After fluorescence autoradiography, the amount of collagenous components was determined by densitometry (11).

RESULTS AND DISCUSSION

When cells from various origins were cultured in the medium containing 0.1M excess NaCl, all the cells responded to NaCl addition by increased production of collagen (Table 1). Similar response to NaCl was also found in the cells at log phase and the cells incubated in the absence of fetal bovine serum.

It is reported that some collagen polypeptides are degraded inside the cells during synthesis and/or intracellular transport (12), suggesting that the observed increase in collagen production described above could be explained by decreased degradation of newly synthesized collagen. Total radioactive hydroxyproline, which accounts for both high molecular weight (non-dialyzable) and low molecular weight

Table 1. Effects of excess NaCl in the culture medium on the collagen metabolism of cells from various origins[a)]

Cell	Ratio of radioactivity (DMEM-10 + 0.1M NaCl/DMEM-10) in		Type III collagen / Types I + III collagen	
	Collagenase sensitive protein	Total Hydroxyproline	DMEM-10 (%)	DMEM-10+0.1M NaCl (%)
Human skin fibroblasts				
Normal	2.7 ± 0.5	2.2 ± 0.6	9.0 ± 2.0	17.1 ± 2.5
PSS[b)]	1.8 ± 0.1	1.7 ± 0.4	5.5 ± 0.8	11.9 ± 0.3
OI[c)]	1.8 ± 0.2	1.5 ± 0.2	12.1 ± 0.2	16.0 ± 0.4
Human stomach muscle cells	1.5 ± 0.1	1.5 ± 0.2	23.5 ± 1.9	34.7 ± 0.4

a) Cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (DMEM-10) with or without 0.1M excess NaCl in the medium in the presence of chromatographically purified [^{3}H]proline for 24 h. Analytical procedures are described under Materials and Methods. The values are the mean (±s.e.m.) of two to four experiments.

b) Progressive Systemic Sclerosis

c) Osteogenesis Imperfecta

(dialyzable) collagenous materials, however, was increased by the addition of 0.1M excess NaCl (Table 1). Therefore, the activation of collagen production by NaCl observed here was not explained by the decreased degradation of collagen.

Type analysis of the collagen showed that the relative rate of type III collagen production was increased with increasing NaCl concentration, even though the relative content of type III collagen to total collagen varied from cells to cells examined (Table 1).

In order to find whether Na^+ or Cl^- is effective to the change of collagen metabolism, we added, 0.1M excess CH_3COONa to the medium and found the increase of both collagen production (Fig. 1) and relative content of type III collagen (data not shown). On the other hand, collagen production was decreased to one fifth of the control value by the presence of 0.1M excess KCl in the medium (Fig. 1). These results clearly show that the increase of Na^+ concentration is responsible for the observed modulation of collagen metabolism in the

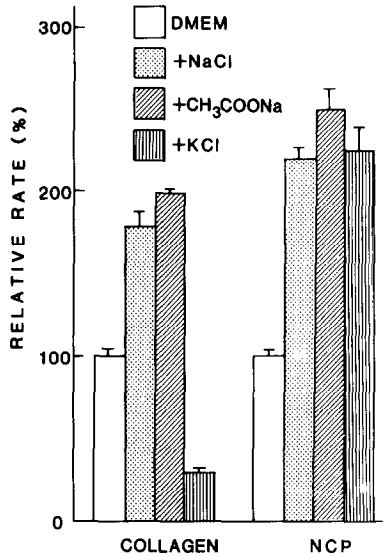

Fig. 1 Effect of excess NaCl, CH_3COONa or KCl in the culture medium on the production of collagen and non-collagenous protein. Normal human skin fibroblasts were labeled with [^{3}H]proline for 24 h in the absence (DMEM) or presence of 0.1 M excess concentration of NaCl (+NaCl), CH_3COONa ($+CH_3COONa$) or KCl (+KCl). Production of collagen and non-collagenous protein (NCP) were analyzed as described under "Materials and Methods". Data shown are the mean (±s.e.m.) of quadruplicate determinations.

fibroblasts. Decreased production of collagen in the presence of 0.1M excess KCl is not due to the decrease in cell viability, because production of non-collagenous protein was increased under the culture conditions employed here (Fig. 1). Activation of cell membrane transport of radioactive proline by Na^+ (13) might induce apparent activation of collagen production but the cell response to the excess NaCl in cell membrane transport did not correlate with the response in collagen production (unpublished data) and also increased cell membrane transport can not explain the modulation of collagen types observed here.

Pawlowski (14) claimed that collagen synthesis is inhibited under the hypertonic culture conditions, when cells are cultured for a short period of incubation time (less than 1h). We also found that collagen production by human skin fibroblasts in 0.1M excess NaCl medium was lower than that in the control system (without supplement of NaCl)

during the initial 5h incubation, but became higher by 12h of incubation. Therefore, the activation of collagen production seems to be a kind of cell reaction in response to changes of their environmental conditions. Pawlowski also claimed the increase of the ratio of α1(I) to α2 (14). According to our experiments presented here, this result should be explained by the increase of type III collagen, because α1(I) and α1(III) could not be separated by the method he employed and also the change was only found in the tissues which contain type III collagen, such as muscle and skin.

Increase of the ratio of type III collagen to the total collagen was also found with viral transformation of mouse 3T3 cells (4). Increase of cell growth rate are observed in association with viral transformation but activation of cell growth was not found by the addition of NaCl (unpublished data). These findings suggest that increased rate of type III collagen synthesis is not related to cell growth and also indicate that the result obtained here is not the secondary effect caused by the increase of cell density (5).

The mechanism of the increase of type III collagen synthesis is not clear at present, but this phenomenon is much similar to the change found in the early phase of tissue differentiation and tissue fibrosis such as atherosclerosis and liver cirrhosis (15). One possible situation which occur to cells in vivo will be under elevated blood pressure, where effective Na^+ concentration may increase.

ACKNOWLEDGEMENTS

The authors wish to express our deep gratitude to Professor Makoto Nakao, Tokyo Medical and Dental University, for valuable discussion and to Professor Takuo Fujita, Kobe University Medical School, for the supply of osteogenesis imperfecta cells.

This work was supported in part by Grants-in-Aid for Scientific Research and for the Special Research Project on Connective Tissue Diseases from the Ministry of Education Science and Culture of Japan and from the Intractable Disease Division, Public Health Bureau, the Ministry of Health and Welfare (Project Team for the Reserach on Connective Tissue Disorder Research Committee and Scleroderma Research Committee) of Japan and from the Yamanouchi Foundation for Research on Metabolic Disoders.

REFERENCES

1. Bornstein, P., and Sage, H. (1980) Annu. Rev. Biochem. 49, 957-1003
2. Hata, R., and Slavkin, H.C. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 2790-2794
3. Gibson, G.J., Schor, S.L., and Grant, M.E. (1982) J. Cell Biol. 93, 767-774
4. Hata, R., and Peterkofsky, G. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 2933-2937
5. Abe, S., Steinmann, B.U., Wahl, L.M., and Martin, G.R. (1979) Nature 442-444
6. Narayanan, A.S., and Page, R.C. (1977) FEBS Lett. 80, 221-224
7. Peterkofsky, B., and Diegelmann, R. (1971) Biochemistry 10, 988-994
8. Hata, R., and Nagai, Y. (1982) Seikagaku 54, 650, in Japanese
9. Ninomiya, Y., Hata, R., Nagai, Y., Tajima, S., Nishikawa, T., and Hatano, H. (1982) Biomed. Res. 3, 70-82
10. Hata, R., Ninomiya, Y., Nagai, Y., and Tsukada, Y. (1980) Biochemistry 19, 169-176
11. Lasky, R.A., and Mills, A.D. (1975) Eur. J. Biochem. 56, 335-341
12. Bienkowski, R.S., Baum, B.J., and Crystal, R.G. (1978) Nataure 276, 413-416
13. Guidotti, G.G., Borghetti, A.F., and Gazzola, G.C. (1978) Biochim. Biophys. Acta 515, 329-366
14. Pawlowski, P.J. (1982) Biochemistry 21, 34-38
15. Prockop, D.J., Kivirikko, K.I., Tuderman, L., and Guzmann, N.A. (1979) New Eng. J. Med. 301, 77-85