Upsala J Med Sci 86: 137–142, 1981

Autophosphorylation at the Outer Surface of Different Human Cells in Culture

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INTRODUCTION

A lot of work has been performed on the various effects of exogenous ATP on intact animal cells. In most cases these effects are ascribed to changes in permeability induced by ATP. The effects as regards permeability changes are usually highly specific and are not obtained by nonspecific chelating agents nor by other nucleoside triphosphates (12, 24). Dramatic effects on efflux and sometimes influx of potassium and some other ions were obtained on different cells when exposing them for short times with low concentrations of external ATP (8,34). Effects by exogenous ATP on the water and ionic contents of the cells of kidney tubules have also been reported (32). Among the various effects of low concentrations of exogenous ATP on the intact cells the inhibition of the insulin- stimulated glucose oxidation might also be mentioned (9).

Small concentrations of ATP have been effective in all instances mentioned above in agreement with the existence of small amounts of ATP in the extracellular space. The question arises whether any of the effects of exogenous ATP have physiological significance. Several possibilities for providing extracellular ATP have been advanced. One possibility might be the production of ATP in the surface membrane, since several cells have shown the capability to form extracellular ATP in in vitro experimental systems (2,3). Evidence also exists that ATP may be translocated from the cytosol to the exterior of the cell (32,34). The existence of nucleotide pyrophosphatases (10), ectoATPase (1,2,28) and ATP requiring protein kinases (4,27,31) on the surface of animal cells almost makes it obligatory that some mechanism for providing external ATP should exist.

Many of the permeability changes induced by low concentrations of extracellular ATP might be attributed to a protein kinase catalyzed phosphorylation of endogenous acceptors at the outer surface of the cell (15). Plasma membrane bound protein kinase phosphorylating endogenous membrane proteins at the cell surface was first described in Ehrlich cells (5,26,31). Using exogenous

(γ^{32}P) ATP as substrate and sometimes histone and phosvitin as exogenous acceptors (27,29,30), it was apparent that the reaction took place at the outer surface of these cells and resulted in phosphorylation of proteinbound seryl- and threonyl- residues. Such reactions were not further stimulated by cyclic AMP or cyclic GMP (6,30). The cyclic nucleotides were instead inhibitory in case of using prepared plasma membrane vesicles instead of intact Ehrlich cells for the incubation experiments (31). Other inhibitors for the reaction were Ca^{2+} and adenosine (27,31). Exogenous GTP was as efficient as ATP as a phosphoryl group donor in the Ehrlich cell experimental system while CTP, UTP and inorganic pyrophosphate were practically unable to replace ATP (4).

^{32}P-labeled phosphorylserine and phosphorylthreonine were also isolated from a plasma membrane fraction recovered from Ehrlich cells that had been prelabeled with (^{32}P) orthophosphate (Table V in reference 7). These studies on Ehrlich cells have now been extended to comprise several human cells in culture such as glia, glioma cells, peripheral lymphocytes and five different hematopoietic cell lines.

METHODS

Separation of lymphocytes from peripheral blood and description of cell lines and tissue conditions as well as preparation of cells for incubation experiments have been described elsewhere (1). The incubation procedure and analytical methods in previous reports were followed (1,31). The (γ^{32}P)ATP concentration in the present work was always 25 μM and incubations were carried out at 37°C.

RESULTS AND DISCUSSION

Some of the characteristics of the human hematopoietic cell lines are given in Table 1. All these human cells had the capability to phosphorylate the surface membrane to different degree indicating the presence of endogenous protein kinase as well as acceptor proteins at the surface of these cells. (^{32}P) phosphoryl serine was always in excess of (^{32}P) phosphorylthreonine by a factor of 2.5-5.5 (Table 2). Normal lymphocytes and lymphoblastoid cells displayed low protein kinase activities at their cell surfaces and comparable to those of human glia and glioma cells. The myeloma and leukemia cell lines on the contrary displayed high activities. The lymphoblastoid cells, that showed low protein kinase activity, were not further stimulated by 1×10^{-5}M cyclic AMP while a 48.3% stimulation by the cyclic nucleotide was obtained with the lymphocytic leukemia cells (Table 2).

TABLE 1 Characteristics of human hematopoietic cell lines

Cell line	Cell type	Cell size (diam.)(μ)	Cell surface architecture (a),(c)	Reactivity with anti-actin	Motility (a),(b),(c)	Immunoglobulin production	Reference
Normal peripheral lymphocytes	Normal lymphocytes	8.0	Mixed population of B lymphocytes	Not examined	Not examined	Surface immunoglobulin(SIg)	
U-698 M	Lymphocytic	9.1	Short villi and blebs	Membrane located, low intensity of IFL	Mobile villi and some extensions of cytoplasm. No directional locomotion	SIg	Nilsson & Sundström 1974
K-562	Leukemia myeloid	13.5	Not examined by scanning, electron microscopy	Not tested	No directional locomotion	No production	Lozzio & Lozzio 1975
U-266 B1	Myeloma	11.2	No villi. Blebs covering the entire surface	No staining	Rapid surface blebbing. No directional locomotion	Secretion	Nilsson et al 1970
RPMI 8226	Myeloma	10.5	No villi. Blebs covering the entire surface	Not examined	Rapid surface blebbing. No directional locomotion	Secretion	Matsuoka et al 1968
U-61 M	Lymphoblastoid	12.2	Long and short villi mostly confined to the uropode. Interchangeable cell shape and surface morphology	Strong IFL located to surface villi	a)motility confined to surface villi b) active direction at locomotion	con-SIg +	Pontén 1967

a) Nilsson & Pontén b) Nilsson, K 1971 c) Fagreus et al 1975

Table 2. Autophosphorylation at the cell surface of different human cell lines. Results given in picomoles (^{32}P) phosphoryl groups transferred x min^{-1} x 10^8 cells^{-1}. (γ ^{32}P) ATP conc.: 25μM.

Human cell type	Phosphorylserine	Phosphorylthreonine
glioma cells	0.33	0.06
normal lymphocytes	0.95	0.20
lymphoblastoid (line U-61M)[x]	1.10	0.34
glia cells	1.49	0.37
myeloma cells (line RPMI 8226)	7.40	2.43
leukemia, myeloid (line K-562)	20.6	6.38
leukemia, lymphocytic (line U-698M)[x]	55.5	21.9
myeloma cells (line U-266)	87.1	22.0
[x]Results in the presence of $1x10^{-5}$ M cyclic AMP in incubation medium:		
lymphoblastoid (line U-61M)	1.05	0.33
leukemia, lymphocytic (line U-698M)	82.3	30.2

The reason for the rather large differences in protein kinase activity at the cell surface is not known at present. Since all the cells studied also have an ecto ATPase activity (1,2) one might argue that the cells with high ATPase activity would display low protein kinase activities due to concomitant ATP splitting activity thereby decreasing the substrate concentration. A reciprocal relationship was indeed discernible for some of the cell lines so that cells like RPMI 8226 and U 61M with high ectoATPase activities (1) showed relatively low proteinkinase activities and cells like U 698 with a low ectoATPase activity showed a rather high protein kinase activity. Exceptional to that rule was cell line U 266 B1 that showed high activities for both ectoATPase and endogenous proteinkinase at the cell surface. Other aspects to be considered are possible steric hindrances at the cell surface for the hydrophilic ATP molecule preventing a close contact between the active center of the surface-located protein kinase and the substrate. Also, a possible phosphoprotein phosphatase reaction at the cell surface has not been taken into account and differences between cells can not only be explained by the protein kinase activity but also by a possible dephosphorylation at the cell surface.

The present findings on several human cell lines imply a more general significans for phosphorylation reactions at the cell surface. The mode of regulating membrane protein kinases and the function of such phosphorylated membrane components pose intriguing questions for future studies on the mechanism of plasma membrane regulatory functions.

REFERENCES

1. Ågren, K.G., Nilsson, K & Ronquist, G.: Mg^{2+} - and Ca^{2+} -stimulated ATP-ase at the outer surface of human peripheral lymphocytes and hematopoietic cell lines. Correlation between enzyme activity and immunoglobulin secretion. Acta Physiol Scand 103:320-330, 1976.
2. Ågren, K.G., Pontén, J., Ronquist, G. & Westermark, B.: Demonstration of an ATPase at the cell surface of intact normal and neoplastic human cells in culture.J Cell Physiol 78:171-176, 1971.
3. Ågren, K.G. & Ronquist, G.: Formation of extracellular adenosine triphosphate by tumor cells. Acta Physiol Scand 75:124-128, 1969.
4. Ågren, K.G. & Ronquist, G.: Isolation of ^{32}P-labelled phosphorylserine and phosphorylthreonine from Ehrlich mouse-ascites tumor cells, suspended in an isotonic medium containing ^{32}P-labelled nucleoside triphosphate or inorganic pyrophosphate. Acta Chem Scand 25: 2931-2934, 1971.
5. Ågren, K.G. & Ronquist, G.: Isolation of ^{32}P-labelled phosphorylserine from Ehrlich mouse-ascites tumor cells containing ^{32}P-labelled adenosine triphosphate. Acta Physiol Scand 79:125, 1970.
6. Ågren, K.G. & Ronquist, G.: Phosphorylation of endogenous membrane proteins by endogenous protein kinase at the outer surface of Ehrlich cells. Upsala J Med Sci 81:129-134, 1976.
7. Ågren, K.G. & Ronquist, G.: Phosphoryl group transfer from Ehrlich cells prelabelled in vivo with (^{32}P)-orthophosphate and (^{14}C)-glucose to unlabelled glioma cells. Acta Physiol Scand 103:320-330, 1978.
8. Aiton, J.F. & Lamb, J.F.: The transient effect of ATP on K movements on HeLa cells. J Physiol 248:14 P, 1975.
9. Chang, K.-J. & Cuatrecasas, P.: Adenosine triphosphate-dependent inhibition of insulin -stimulated glucose transport in fat cells. Possible role of membrane phosphorylation. J Biol. Chem 249:3170-3180, 1974.
10. Evans, W.H.: Nucleotide pyrophosphatase, a sialoglucoprotein located on the hepatocyte surface. Nature 250:391-393, 1974.
11. Fagreus, A.K. Nilsson, K., Lidman, K.& Norberg, R.: Reactivity of smooth muscle antibodies, surface ultrastructure and mobility of human hematopoietic cell lines. J Natl Cancer Inst 55:780-783, 1975.
12. Hempling, H.G., Stewart, C.C. & Gasic, G.: The effect of exogenous ATP on the electrolyte content of TA_3 ascites tumor cells. J Cell Physiol 73:133-140, 1969.
13. Kinzel, V. & Mueller, G.C.: Phosphorylation of surface proteins of HeLa cells using an exogenous protein kinase and ^{32}P ATP. Biochim Biophys Acta 322:337-351, 1973.
14. Lozzio, C.B. & Lozzio, B.: Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. Blood 45:321-333, 1975.
15. Mastro, A.M. & Rozengurt, E.: Endogenous protein kinase in outer plasma membrane of cultured 3T3 cells. J Biol Chem 251:7899-7909, 1976.
16. Matsuoka, Y., Takahasi, M., Yagi, Y., Moore, G.E. & Pressman, D.: Synthesis and secretion of immunoglobulins by established cell lines from human hematopoetic origin. J Immunol 101:1111-1120, 1968.
17. Nilsson. K.: Characteristics of established human myeloma and lymphoblastoid cell lines in long term culture. A comparative study. Int. J Cancer 7: 380-396, 1971 a.
18. Nilsson, K.: High frequency-establishment of human immunoglobulin producing lymphoblastoid lines from normal and malignant lymphoid tissue and pheripheral blood. Int J Cancer 8: 432-442, 1971 b.
19. Nilsson, K.: Synthesis and secretion of IgE by an established human myeloma cell line. Clin. Exp Immunol 9: 785-793, 1971 c.
20. Nilsson, K., Bennich, H., Johansson, S.G.O. & Pontén, J.: Established immunoglobulin producing myeloma (IgE) and lymphoblastoid (IgG) cell lines from an IgE myeloma patient. Clin Exp Immunol 7: 477-489, 1970.

21. Nilsson, K., Ghetie, V. & Sjöquist, J.: A human lymphoid cell line with an IgG-like membrane component. Eur. J Immunol 5:518-526, 1975.
22. Nilsson, K. & Pontén, J.: Classification and biological nature of established human hematopoietic cell lines. Int J Cancer 15: 321-341, 1975 a,
23. Nilsson, K. & Sundström, C.: Establishment and characteristics of two unique cell lines from patients with lymphosarcoma. Int J Cancer 13: 808- 823, 1974.
24. Palek, J., Curby, W.A. & Lionetti, F.J.: Effects of calcium and adenosine triphosphate on the volume of red cell ghosts. Am J Physiol 220: 19-26, 1971.
25. Pontén, J.: Spontaneous lymphoblastoid transformation of long-term cell cultures from human malignant lymphoma. Int J Cancer 2: 311-325, 1967.
26. Ronquist, G. & Ågren, K.G.: Isolation of ^{32}P-labelled phosphoryl threonine from Ehrlich mouse-ascites tumor cells suspended in an isotonic medium containing ^{32}P-labelled adenosine triphosphate. Acta Chem Scand 24: 728, 1970.
27. Ronquist, G. & Ågren, K.G.: Isolation of ^{32}P-labelled phosphorylserine and phosphorylthreonine from Ehrlich mouse-ascites cells suspended in different isotonic media containing ^{32}P-labelled adenosine triphosphate. Acta Chem Scand B28:1169-1174, 1974.
28. Ronquist, G. & Ågren, K.G.: A Mg^{2+} - and Ca^{2+} -stimulated adenosine triphosphatase at the outer surface of Ehrlich ascites tumor cells. Cancer Res 35:1402-1406, 1975.
29. Ronquist, G. & Ågren, K.G.: Phosphoryl transfer by endogenous protein kinase at the Ehrlich cell surface into extrinsic acceptor proteins. Upsala J Med Sci: 79: 138-142, 1974.
30. Ronquist, G.. Ågren, K.G., Eklund, S. & Wernstedt, C.: Cyclic 3´, 5´-GMP independent protein kinase at the outer surface of intact Ehrlich cells. Upsala J Med Sci 82:1-5, 1977.
31. Ronquist, G., Ågren, K.G. & Lindquist, I.: Phosphorylation of endogenous proteins by endogenous protein kinase of density gradient- purified plasma membrane vesicles of Ehrlich cells. Acta Physiol Scand 105:404-413, 1979.
32. Rorive, G. & Kleinzeller, A.: The effect of ATP and Ca^{2+} on the cell volume in isolated kidney tubules. Biochem Biophys Acta 274:226-239, 1972.
33. Shlatz, L. & Marinetti, G.V.: Protein kinase mediated phosphorylation of the rat liver plasma membrane. Biochim Biophys Res Commun 45: 51-56, 1971.
34. Trams, E.G.: Evidence for ATP action on the cell surface. Nature 252: 480-482, 1974.