

ATPase ACTIVITY AT THE CELL SURFACE OF ASTROGLIA IN CULTURE

P. MANDEL, V. STEFANOVIC, J. CIESIELSKI-TRESKA and A. EBEL*

Centre de Neurochimie du CNRS, and Institut de Chimie Biologique, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cedex, France

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1. Introduction

The precise role of glial cells in brain metabolism is not yet understood. Since neurons and glia are intimately associated in the structure of the brain, studies on their functions and relationships at the cellular level need a methodology for separation of these two cell types and among glia, astrocytes from oligodendrocytes. Several methods for large scale separation of glia and neurons have been introduced but extensive cell damage and mutual contaminations by membrane structures and cell processes were pointed out [1]. Cell cultivation provides means for obtaining samples of neurons and glial cells separately. Cultures of neurons or glial cells are generally prepared from embryonic nervous system or are derived from neoplasms. The justification for the use of such a culture as a model of the normal central nervous system has been discussed previously [2].

Recently some cell lines with biochemical characteristics of differentiated glia have been developed [3, 4]. Their utility as models of glial cell investigations has been suggested. However we do not know how many characteristics of the original cell type they retain and how extensive may be the changes in gene expression in the transformed cell line. The present study was attempted to further biochemical characterization of some glial cell lines in culture by the study of an enzyme, the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity (ATP phosphohydrolase, EC 3.6.1.3), identified with the sodium pump. Three different cell lines of astroglia were studied: (1) astrocytes from newborn rat brain, in primary culture, (2) normal astroblasts from new-

born hamster brain, clonal cell line NN [5], (3) neoplastic astrocytoma cells, clonal cell line C₆, derived from glial tumors induced by repeated injections of *N*-nitrosomethylurea in Wistar rats [3]. A much higher ATPase activity at the external surface of astrocytes from rat brain in primary culture has been found compared to the stable clone lines. 'Ecto'-ATPase activity in NN and C₆ clonal cell lines does not differ significantly. An adaptation of cell metabolism to the culture conditions was suggested.

2. Methods

2.1. Cell cultivation

Clone C₆ (passage 37) and clone NN (passage 36) were obtained from American Type Culture Collection and North American Biologicals Inc., respectively. Cells were grown at 37°C in Falcon Petri dishes (6 cm ϕ) in 5 ml Dulbecco's modified Eagle medium supplemented with 10% foetal calf serum and antibiotics in an atmosphere of 5% CO₂–95% air. The medium was changed 3 and 5 days after plating and the cells were used on the sixth day. Astrocytes from brain of the newborn rats were cultivated essentially as previously described [6] and studied after 17 or 20 days of cultivation.

2.2. Assay of ATPase

Growth medium was carefully removed from Petri dishes and discarded and the cell monolayer was gently washed three times with a total of about 10 ml of incubation solution without ATP. Incubation medium contained 3 mM MgCl₂, 5 mM KCl, 30 mM Tris-HCl buffer (pH 7.4), 5.5 mM glucose, 225 mM sucrose and

* Chargée de Recherche au CNRS

0.1 M ouabain as indicated. Sodium in the incubation fluid was found to compete with potassium [7] and was not included in the medium used. Incubation was started by adding 3 mM Tris-ATP and conducted at 37°C for 2 min (astrocytes in primary culture) or 15 min (NN and C₆ clonal cell lines), in a final volume of 1.5 ml. After this period the medium was transferred to the ice cooled centrifuge tubes and the inorganic phosphate formed was determined according to the method of Gomori [8]. Appropriate controls without cells were incubated and analysed in order to correct for small amounts of P_i found in commercial preparations of ATP or deriving from its spontaneous hydrolysis. Another control with cells but without substrate was also included.

2.3. Protein determination

Protein was determined by a modification of the method of Lowry et al. [9]. Crystalline bovine serum albumin was used as standard.

2.4. Materials

Dulbecco's modified Eagle medium and foetal calf serum were purchased from Gibco, Grand Island (N.Y.). Tris-ATP, ouabain and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis (Mo.). All other chemicals were analytical grade.

3. Results

External surface ATP activity of three different cell lines of astroglia is presented in table 1. Normal astrocytes from newborn rat brain in primary cultures showed both a high Mg²⁺-dependent, and a ouabain sensitive 'ecto'-ATPase activity. The two indefinite clonal cell lines of astrocytes, neoplastic (C₆) and normal (NN), exhibited very low surface ATPase activity. A comparison shows that ATPase activity at the surface of astrocytes in primary culture is about 80 times greater than in the stable clones NN and C₆; no difference in the surface ATPase activity between NN and C₆ clonal lines has been found.

The existence of ATPase activity at the surface of intact normal and neoplastic human cells in culture was previously reported. Only the Mg²⁺-dependent

activity has been determined. Difference between activities in neoplastic and normal cells was observed. The observed phenomenon was ascribed to the neoplastic transformation of cells, concerning, in particular, membrane structure and function. The results of Agren et al. [10] are only partly supported in the present study and concern the low surface ATPase activity of neoplastic astroglia in culture. Another aspect of the low ATPase activity appeared from the fact that 'ecto'-ATPase activity of normal, untransformed newborn hamster astroglia, clone NN, does not differ from that of neoplastic astroglia. In order to gather some information about the differences of enzyme activity observed, some properties of the enzyme of NN and C₆ clone were studied and compared with those of normal astroglia in primary culture (table 2). Similar apparent K_m values for ATP and V_{max} of the enzyme of NN and C₆ clones were found. Astrocytes in primary culture had a higher V_{max} and proportionately a much higher K_m value for ATP than NN and C₆ clones. This finding indicates a greater affinity for substrate in C₆ and NN clones than in astrocytes in primary cultures. NN and C₆ clonal cell lines have been maintained for a long time in culture and the observed phenomenon is the most probably a metabolic adaptation of cells to the special culture conditions. Moreover, it appears that this higher affinity for substrate and low V_{max} is not specific for neoplastic transformation since similar values have been obtained in a clonal line, NN, or untransformed cells.

Normal glial cells in primary culture and both clonal cell lines, NN and C₆, showed a significant ouabain sensitive surface ATPase activity. Indirect evidence has been presented that ouabain sensitive 'ecto'-ATPase is involved in cation transport [7]. Direct analyses of cations performed on cells in cultures showed that K⁺ and not Na⁺ is the predominant intracellular cation of hamster and rat astrocytes grown in pure cultures [12]. The high concentration of K⁺ in cultured astrocytes and a significant ouabain sensitive activity of the 'ecto'-ATPase are compatible with the hypothesis originally put forward by Cummins and Hyden [13] that glial cells may serve as the regulators of the external K⁺ ions in the extracellular space surrounding neurons.

Our results draw also attention to the fact that specific changes may occur in cultivated cells and recommend caution in the extrapolation of data obtained in cell cultures to the in vivo situation.

Table 1
ATPase activity at the external surface of astroglia in culture
($\mu\text{mol P}_i$ liberated/ mg^{-1} prot./hr $^{-1}$)

Cell line	Without ouabain	0.1 mM ouabain
Newborn rat astrocytes	19.510 \pm 0.290	17.220 \pm 0.575*
Clonal line NN	0.248 \pm 0.007	0.224 \pm 0.006*
Clonal line C ₆	0.249 \pm 0.008	0.218 \pm 0.006*

Each value is the mean of 5–8 experiments \pm S.E.M.

* (without ouabain/0.1 mM ouabain) $p < 0.01$

Table 2
 K_m and V_{max} of surface ATPase activity of three astroglial
lines in culture

Cell line	K_m (mM)	V_{max} ($\mu\text{mol}/\text{mg}^{-1}/\text{hr}^{-1}$)
Newborn rat astrocytes	1.20	32.40
Clonal line NN	0.08	0.33
Clonal line C ₆	0.03	0.37

The basic medium and incubation conditions are outlined under Methods. ATP varied from 0.1–3.0 mM at a fixed Mg^{2+} concentration of 3 mM. Michaelis dissociation constants were calculated by statistical treatment of data plotted in the form of s/v against v according to Wilkinson [12] on an Olivetti Programa 602.

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