$(Na^+ + K^+)$-Activated ATPase and K^+-Activated *p*-Nitrophenyl-Phosphatase Activities of the Nervous System Cells in Tissue Culture

Active transport of cellular sodium outward and potassium inward is thought to be due to a membrane-bound $(Na^+ + K^+)$-activated, ouabain-inhibitable ATPase system (reviews[1–4]). Of several tissues, the highest activity of $(Na^+ + K^+)$-ATPase has been found in membranes from the nervous system[5]. The regional distributions of $(Na^+ + K^+)$-ATPase activity in the brain of rabbit[6], rhesus monkey[7] and mouse[8] have been established. For cellular distribution, different approaches were necessary in view of the complexity of brain tissue and limitations in cell separation techniques. ATPase activity has been studied on neurons and glia obtained by freehand dissection[9] and on fractions 'enriched' in either glial or neuronal cells after carrying out tissue sieving and density gradient centrifugation[10,11]. Methods for large scale preparation of the central nervous system cells produce a large amount of cell damage and provide highly heterogeneous neuronal and glial cell fractions with mutual contamination by cell processes and membrane structures[12].

The use of subcutaneously grown rodent astrocytes permitted the study of $(Na^+ + K^+)$-ATPase activity of these cells uncontaminated by neuronal cell bodies, processes and synaptic membranes, or by other glial cell types[13]. Recently developed cell lines with biochemical characteristics of differentiated glia[14] and neuronal cells[15] offered an opportunity for study of the sodium pump enzyme system. We wish to report here the $(Na^+ + K^+)$-ATPase activity in several mouse neuroblastoma clones[15,16], NN hamster astroblasts[17], C6 glioblastoma cells[14] and in astroblasts from the new-born rat brain in primary culture[18]. We also report the existence of a K^+-activated, ouabain-inhibited *p*-nitrophenylphosphatase activity in these cell lines. Identity of the K^+-activated phosphatase activity with the dephosphorylation step in the $(Na^+ + K^+)$-ATPase reaction has previously been suggested (see[2,4]). While this work was in progress, KIMELBERG[19] reported on $(Na^+ + K^+)$-ATPase activity in mouse neuroblastoma 2A and rat glioma C6 cells.

Methods. Cell cultures. The NN cell line[17] and C6 cell line[14] were obtained from North American Biologicals Inc. and American Type Culture Collection, respectively. Clones N18, S21 and N1E-115 were a gift from Dr. M. NIRENBERG (Laboratory of Biochemical Genetics, National Heart and Lung Institute, NIH, Bethesda, Md.). Clonal cell line M1 was developed and characterized in our laboratory. Cells were grown in monolayer culture in Falcon tissue culture flasks with a growth area of 75 cm^2 in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum (Gibco, Grand Island, N.Y.) and 50 units of sodium penicillin G and 25 µg of streptomycin sulfate per ml. The cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Astrocytes from brain of new-born rats were cultured, as previously described[18], and studied after 20 days of cultivation. Cells were grown to stationary phase before use. Tests for infestation with mycoplasma were negative.

Enzyme assay. Enzymatic activity was determined in homogenates prepared as follows. Before harvesting the cells, the culture medium was decanted and the cultures were washed 3 times with buffered isotonic saline containing 1 m*M* Na_2 EDTA. The cells were removed from the plastic by scraping with a rubber policeman. The suspended cells were collected by centrifugation and homogenized in double distilled water in a Potter-Elvehjem homogenizer with a teflon pestle.

$(Na^+ + K^+)$-ATPase and K^+-activated *p*-nitrophenylphosphatase activities were determined as described previously[8]. Incubation medium for the ATPase activity contained about 300 µg of cell protein, 30 m*M* *Tris*-HCl buffer (pH 7.4), 3 m*M* ATP, 3 m*M* $MgCl_2$, 100 m*M* NaCl and 20 m*M* KCl. One set of triplicates contained 1 m*M* ouabain. The $(Na^+ + K^+)$-ATPase activity was calculated by subtracting the activity in ouabain-containing medium from that without ouabain.

The assay of *p*-nitrophenylphosphatase activity was carried out in a medium containing about 100 µg of cell protein, 30 m*M* *Tris*-HCl buffer (pH 7.8), 3 m*M* *p*-nitrophenylphosphate, 3 m*M* $MgCl_2$ and 10 m*M* KCl. One set of triplicates received ouabain to a final concentration of 1 m*M*. K^+-activated *p*-nitrophenylphosphatase activity was estimated as the difference between the total activity and the activity in the presence of 1 m*M* ouabain. Protein was determined by a modification of the method of LOWRY et al.[20].

Results and discussion. Four mouse neuroblastoma clones have been studied. With respect to neurotransmitter synthesis[16], N1E-115 and M1 clones were defined as adrenergic, S21 as cholinergic and N18 as inactive cells. $(Na^+ + K^+)$-ATPase activity of neuroblastoma cells (Table) ranged from 0.44 ± 0.02 to 0.62 ± 0.04 µmol/mg cell protein/h, in the N18 and M1 clones, respectively. Glial cell lines, neoplastic-C6 and neonatal hamster astroblasts-NN had higher $(Na^+ + K^+)$-ATPase activity than neuroblasts in culture. Astroblasts from new-born rat brain in primary culture had about 4–5 times higher $(Na^+ + K^+)$-ATPase activity than neuroblastoma clones and 2.5–3 times higher than NN and C6 cell line.

Nitrophenylphosphatase activity which is stimulated by potassium and inhibited by ouabain has been found in

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ATPase and *p*-nitrophenylphosphatase activities of cultured glioma and neuroblastoma cells

	ATPase activity[a]		*p*-Nitrophenylphosphatase activity[b]	
	$(Na^+ + K^+)$-ATPase	Mg^{2+}-ATPase	K^+-*p*-nitrophenyl-phosphatase	Mg^{2+}-*p*-nitrophenyl-phosphatase
Mouse neuroblastoma N1E-115	0.55 ± 0.05	3.95 ± 0.13	0.14 ± 0.01	2.11 ± 0.05
Mouse neuroblastoma M1	0.62 ± 0.04	4.07 ± 0.08	0.13 ± 0.01	1.81 ± 0.06
Mouse neuroblastoma S21	0.47 ± 0.03	3.80 ± 0.10	0.12 ± 0.01	2.59 ± 0.07
Mouse neuroblastoma N18	0.44 ± 0.03	3.89 ± 0.08	0.12 ± 0.01	2.10 ± 0.09
Rat astrocytoma C6	0.67 ± 0.05	2.35 ± 0.10	0.17 ± 0.02	2.40 ± 0.08
Hamster astroblasts NN	0.89 ± 0.03	2.76 ± 0.09	0.17 ± 0.01	2.27 ± 0.12
New-born rat astroblasts	2.33 ± 0.06	14.35 ± 1.10	0.28 ± 0.03	3.10 ± 0.18

Each value is the mean of 7 experiments ± SEM. [a] As μmol Pi liberated/mg protein/h. [b] As μmol *p*-nitrophenol released/mg protein/ h.

neuroblastoma and glioblastoma cells (Table). Astroblasts in primary culture had a K^+-activated *p*-nitrophenylphosphatase activity significantly higher than neuroblastoma or glioma clonal cell line. As in brain[8, 21, 22] or some other tissues[2, 4], this phosphatase activity parallels the $(Na^+ + K^+)$-ATPase activity, being in a fairly constant ratio with the latter activity. $(Na^+ + K^+)$-ATPase/K^+-activated *p*-nitrophenylphosphatase ratio was 3.7–4.8 for neuroblastoma cells, 3.9–5.2 for glioma cells and about 8.3 for cultured new-born rat astrocytes.

The data presented show that neuroblastoma and cultured glioma cells have a significant sodium pump enzyme activity. Higher $(Na^+ + K^+)$-ATPase activity in glia have previously been suggested, though measurements of ATPase activity were done on neuronal pericaryal preparations obtained by a bulk isolation procedure and virtually without neuronal processes and synaptic membranes. The nerve ending particles have been shown to have high $(Na^+ + K^+)$-ATPase activity[23, 24].

The specific activity of the sodium pump enzyme of neuroblastoma and glioblastoma cells in culture is much lower than the enzymatic activity of brain tissue. Cultures of neuroblastoma and glioma cells were prepared from neoplastic and/or embryonic nervous system. The study of $(Na^+ + K^+)$-ATPase activity during development showed a very low activity at birth and abrupt and marked increase postnatally[25–27]. Thus, the lower $(Na^+ + K^+)$-ATPase activity of cultured cells may be explained by the immaturity of these cells. Higher $(Na^+ + K^+)$-ATPase activity of astroblasts in primary culture fits this concept. Clonal cell lines NN and C6 have been maintained for a long time in culture and probably are more dedifferentiated. Dibutyryl-cAMP induced differentiation increased $(Na^+ + K^+)$-ATPase activity in neuroblastoma cells[28].

K^+-activated phosphatase activity of mouse neuroblastoma clones was lower than in mouse brain but correlates with the $(Na^+ + K^+)$-ATPase activity in these cells. A fairly constant $(Na^+ + K^+)$-ATPase/K^+-activated *p*-nitrophenylphosphatase activity ratio has previously been found in mouse brain, even in regions with different enzymic activities[8]. The ratio between the two activities stays constant during the purification of the $(Na^+ + K^+)$-ATPase[21, 29, 30]. Correlation between the $(Na^+ + K^+)$-ATPase and K^+-activated *p*-nitrophenylphosphatase activities in cultured cells, like in brain or some other tissues, further supports the view that $(Na^+ + K^+)$-ATPase and K^+-activated *p*-nitrophenylphosphatase activities are the same enzyme system.

Résumé. Les activités ATPasique $(Na^+ + K^+)$-activée et *p*-nitrophénylphosphatasique K^+-activée ont été étudiées dans des cellules du neuroblastome de souris et dans des cellules gliales en culture. L'activité ATPasique $(Na^+ + K^+)$ est plus élevée au niveau des cellules gliales. L'activité *p*-nitrophénylphosphatasique K^+-activée est parallèle à celle de l'activité ATPasique $(Na^+ + K^+)$ dans un rapport relativement constant avec cette dernière activité enzymatique.

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