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SHORT COMMUNICATION

A comparison of the ATPase activity of the glial cell fraction and the neuronal perikaryal fraction isolated in bulk from rat cerebral cortex[1]

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SINCE SKOU (1957) first described a Na–K-activated, Mg-dependent, and ouabain-sensitive adenosinetriphosphatase in crab nerve, considerable evidence has accumulated to implicate this enzyme in the transport of Na and K (see reviews by SKOU, 1965; and ALBERS, 1967). The activity of this ATPase is high in membrane preparations from various organs, the highest activity being that of membranes from nervous tissues (BONTING, SIMON and HAWKINS, 1961). The enzyme is intimately associated with the membranes, but its solubilization and stabilization in solution have recently been accomplished (MEDZIHRADSKY, KLINE and HOKIN, 1967). Also, the regional distribution of Na–K ATPase has been mapped in the brain of the rabbit (HARMONY, URBÁ-HOLMGREN and URBAY, 1967) and the rhesus monkey (FAHN and CÔTÉ, 1968).

In view of the cellular heterogeneity of brain and of unsettled questions concerning the role of glial cells and of their interrelations with neurons, we have compared the activities of Na–K ATPase and total ATPase of the neuronal perikaryal fraction and glial cell fraction obtained from cerebral cortex as two separate populations by a bulk-isolation procedure. In addition, we determined the intracellular distribution of these two enzymic activities in neuronal perikarya by the use of standard differential centrifugation techniques.

EXPERIMENTAL PROCEDURES

Isolation and subcellular fractionation procedures. The neuronal perikaryal fraction and the glial cell fraction were prepared from the cerebral cortex of 18–20-day-old male Sprague–Dawley rats by a procedure which involves sieving of the tissue through decreasing pore sizes of nylon cloth followed by centrifugation in sucrose–Ficoll gradients, as described in detail elsewhere (JOHNSON and SELLINGER, 1971). The appearance of these fractions under phase-contrast microscopy is illustrated in Fig. 1.

The neuronal perikaryal fraction was subfractionated by a procedure developed for cerebral cortex (SELLINGER, NORDRUM and IDOYAGA-VARGAS, 1971) which yields myelin, nuclear, heavy and light mitochondrial, microsomal and soluble cell sap fractions.

Assay of enzymatic activity. The ATPase activities were determined in neuronal and glial homogenates as well as in the neuronal subcellular fractions. However, since the activities were barely detectable in the nuclear and soluble cell sap fractions, they have not been reported here.

After isolation, the fractions were immediately frozen and kept at −70°C until analysis. Immediately before analysis, they were thawed and diluted with 50 mM imidazole-HCl buffer (pH 7·0). The concentration of protein in the diluted samples ranged from 0·01 to 0·34 mg/ml. Six 50-μl portions of each fraction were pipetted into tubes (of 5·5 mm inside diameter) kept on ice. To four of these tubes, 50 μl of the incubation medium, prepared 'double strength', were added to give a final concentration (mM) of: NaCl, 90; KCl, 20; $MgCl_2$, 3; ATP, 3; and imidazole buffer (pH 7·0), 50; two tubes also received ouabain to a final concentration of 0·1 mM. Ice-cold 0·6 M-$HClO_4$ (400 μl) was added to two of the ouabain-free tubes. The tubes not treated with $HClO_4$ were incubated for 40 min at 37°C in a shaker-bath; they were then placed in ice and the proteins were precipitated with $HClO_4$ as already described for the assay blanks. After centrifugation (1700 g, 10 min), 250 μl of a freshly prepared solution of 144 mM-$FeSO_4$ in 8·15 mM-NH_4-molybdate-0·58 M H_2SO_4 were added to 250 μl portions of the protein-free supernatant fluids. After 5 min at room temperature, the absorbance at 700 nm was determined against distilled water blanks. Standards in the linear range of 0·5 to 5 μg of P_i (K_2HPO_4) were included. P_i liberated in the absence of ouabain and corrected for the zero-time

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inactivated assay blanks represented the total ATP-hydrolysing activity. The activity of the Na–K ATPase was calculated by subtracting the activity remaining in the presence of ouabain (Mg ATPase) from the total activity.

Protein was determined according to LOWRY, ROSEBROUGH, FARR and RANDALL (1951) by an adaptation found to be linear in the range of 0·3 to 5 μg (MEDZIHRADSKY, BEDNARCZYK and NANDHASRI, unpublished data): briefly, portions of the diluted fractions were adjusted with distilled water to 50 μl in tubes of 5·5 mm inside diameter. To the tubes were added 5 μl of 3·5 N-NaOH, 140 μl of the alkaline $CuSO_4$-sodium tartrate solution and 14 μl of 1 N-Folin-reagent. Appropriate blanks were included for each sample dilution to correct for the contributions of sucrose and imidazole to the absorbance at 750 nm.

RESULTS

The specific activities of both types of ATPases were considerably higher in the glial cell fraction than in the neuronal perikaryal fraction (Table 1). This difference was greater for the Na–K ATPase, hence the G/N ratio (Table 1) for this type of ATPase was higher than for the ouabain-insensitive ATPase (6·63 *vs.* 4·46).

TABLE 1.—THE SPECIFIC ACTIVITIES OF ATPASES IN THE NEURONAL PERIKARYAL FRACTION AND THE GLIAL CELL FRACTION ISOLATED FROM CEREBRAL CORTEX OF 18-DAY-OLD RATS

Fraction	(A) Total ATPase	(B) ATPase insensitive to ouabain (Mg ATPase)	Na–K ATPase Activity*	Na–K ATPase % of total ATPase
	(μmoles of P_i/mg of proteins/h)			
Neuronal perikaryal fraction (N)	11·66; 12·36	8·32; 7·58	3·34; 4·78	28·4; 38·7
	6·24; 11·55	3·01; 6·03	3·32; 5·52	51·8; 47·8
	7·77; 11·47	5·96; 8·15	1·81; 3·32	23·3; 28·9
	7·37; 10·55	5·05; 7·60	2·32; 2·95	31·5; 28·0
	8·47; 8·44	4·67; 6·73	3·80; 1·71	44·9; 20·3
Mean:	9·58	6·31	3·28	34·1
Glial cell fraction (G)	69·01	30·41	38·60	55·9
	60·36	37·36	23·00	38·1
	23·91	13·58	10·33	43·2
	49·67	37·31	12·36	24·9
	46·50	22·30	24·20	52·0
Mean:	49·88	28·19	21·69	43·5
Ratio: (G/N)	5·21	4·46	6·61	—
Whole cortex homogenate	10·97	6·40	4·57	41·7
	12·08	6·83	5·25	43·5
Mean:	11·52	6·61	4·91	42·6

For each of the ten experiments on the neuronal perikaryal fraction and the five experiments on the glial cell fraction, the fractions were isolated from a pool of ten cerebral cortices (6·7 g) of 18-day-old rats by the procedure outlined in the text. The 'homogenate of whole cortex' refers to the cortical suspension after passage of the tissue mince through the nylon sieves. The individual values are given rather than means ± S.D. to emphasize the constancy of the relationships between the Na–K ATPase and the total ATPase despite the rather wide fluctuations of the absolute values of specific activity in individual samples.

* Difference, Column A – Column B.

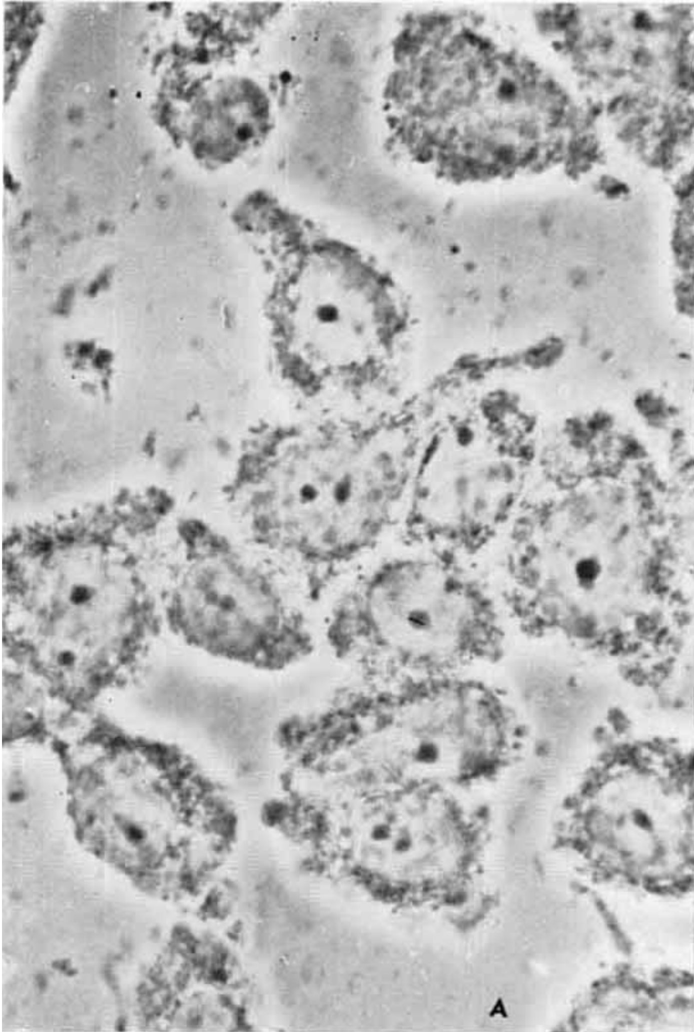

FIG. 1. –A: Phase-contrast micrograph of a preparation of nerve cell bodies isolated from the cerebral cortex of 18-day-old rats (× 1000). For a low power view of such a preparation see Fig. 1A in the paper by JOHNSON and SELLINGER (1971).

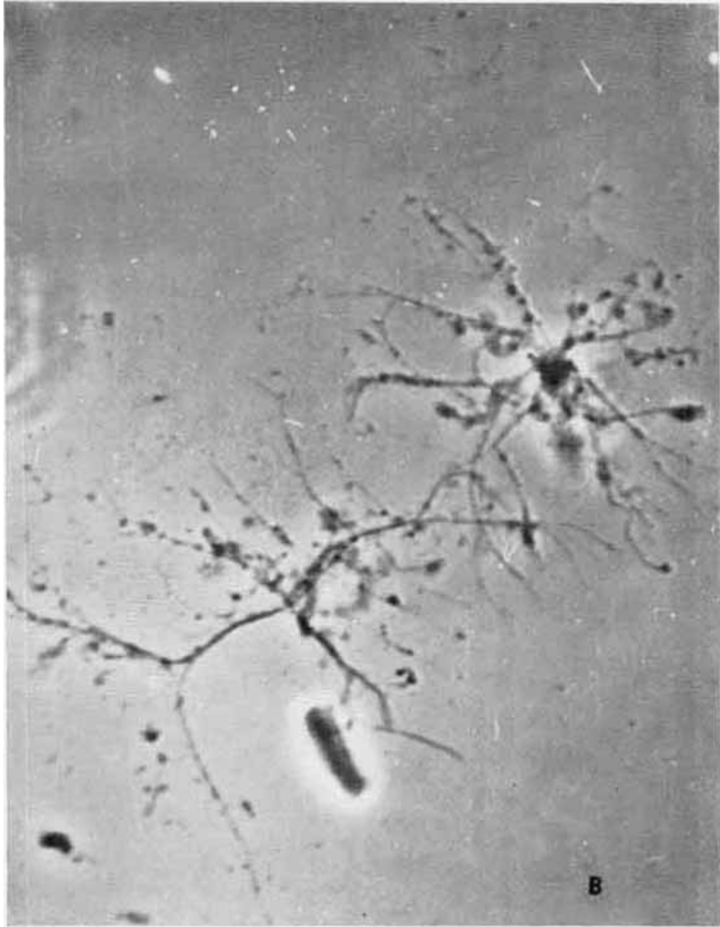

Fig. 1.—B: Phase-contrast micrograph of two glial cells isolated from the cerebral cortex of 18-day-old rats (× 1000).

CUMMINS and HYDÉN (1962) reported that maximal 'glial' (neuropil) ATPase could be determined in intact, unhomogenized preparations ('ecto'-localization), whereas the enzymic activity of neurons required rupture of the cells before their maximal value could be assayed. To ascertain that the differences here between the glial and the neuronal fraction ATPases were not consequences of varying degrees of membrane damage, both cellular preparations were thoroughly homogenized and/or subjected to repeated freezing and thawing prior to the assay. No increases of activity were detectable for the enzyme from either fraction.

The distribution of the total and Na–K ATPase activities was studied among the three subcellular fractions of the neuronal perikaryal fraction in which mitochondria, lysosomes and, presumably,

TABLE 2.—THE SPECIFIC ACTIVITIES OF ATPASES IN SUBCELLULAR FRACTIONS OF THE NEURONAL PERIKARYAL FRACTION OF CEREBRAL CORTEX FROM 20-DAY-OLD RATS

Fraction	(A) Total ATPase	(B) ATPase insensitive to ouabain (Mg ATPase)	Na–K ATPase	
	(μmoles of P_i/mg of protein/h)		Activity*	% of total ATPase
Homogenate†	9·58	6·31	3·27	34·1
'Light' mitochondrial	49·09	30·77	18·32	37·3
	53·97	23·81	30·16	55·9
	49·47	32·70	16·77	33·9
	71·12	47·60	23·52	33·1
	79·29	53·71	25·58	32·3
Mean:	60·58	37·71	22·87	37·8
'Heavy' mitochondrial	29·12	14·56	14·56	50·0
	27·35	14·18	13·17	48·1
	17·26	9·66	7·60	44·0
	42·05	25·23	16·82	40·0
	20·30	12·89	7·41	36·9
Mean:	27·21	15·30	11·91	43·8
Microsomal	17·99	9·65	8·34	46·4
	15·37	8·47	6·90	44·9
	12·97	6·92	6·05	46·6
	21·34	13·97	7·37	34·4
Mean:	16·91	9·75	7·16	42·3

The neuronal perikaryal fraction was isolated from the cerebral cortices of ten rats per experiment. 'Homogenate' refers to a suspension obtained by homogenizing the nerve cell bodies in ice-cold, 0·25 M-sucrose in a Teflon-glass Potter–Elvehjem tissue grinder at 1300 rev./min for about 30 sec. The centrifugation schedule was: 10 min at 1000 *g*, followed by 8 min at 1000 *g* to remove the nuclear fraction. The supernatant fluid was centrifuged at 25,000 *g* and the sediment was washed once under the same conditions. The resulting pellet was suspended in 0·25 M-sucrose and layered onto 15 ml of 0·9 M-sucrose which, in turn, had been layered on top of 10 ml of 1·3 M-sucrose. Centrifugation at 63,500 *g* for 30 min separated myelin, floating on the 0·9 M-sucrose, from a particulate fraction recovered from the 0·9 M—1·3 M-sucrose interface ('light mitochondrial fraction'). The 'heavy mitochondrial fraction' was the material found in the pellet. For separation of the microsomal fraction, the supernatant fluid remaining after the second 25,000 *g* step was centrifuged for 45 min at 269,000 *g*. Analyses showed 58 per cent of the perikaryal RNA to be in this pellet.

* Difference, Column A — Column B.

† Data from Table 1.

plasma membrane fragments abound (Table 2). The specific enzymic activity in the fractions exceeded that obtained for the original homogenate in each case, the enrichment being maximal in the 'light' mitochondrial fraction and minimal in the microsomal fraction. ATPase activity was below our limits of detection (5 nmoles of P_i/h/sample) in the nuclear and soluble cell sap fractions. Subfractionation of the glial cell fraction could not be carried out because of insufficient yields of material.

DISCUSSION

Previous studies in which ATPase was compared in single neurons dissected freehand and in 'equal volumes' of surrounding glia (CUMMINS and HYDÉN, 1962), as well as a recent study (HAMBERGER, BLOMSTRAND and LEHNINGER, 1970) in which similar ATPase activity levels have been reported in neuronal cell bodies and glial cells isolated in bulk from bovine brain cortex have yielded conflicting results. The ATPase activities in the cellular fractions obtained here from the cerebral cortex of the 18–20-day-old rat do not agree with the recent measurements of ATPase activity made on homogenates, neuronal cell bodies and glial cells isolated from adult bovine cerebral cortex (HAMBERGER *et al.*, 1970). The latter results revealed no difference between neuronal and glial fractions in levels of ATPase activities assayed either in the absence or presence of Na and K. It is unlikely that these discrepancies between our results and those obtained by HAMBERGER *et al.* (1970) reflect differences in age, since DALTON, HOMMES and LEBLOND (1968) have shown that glial cells reach a relatively constant density in the rat cerebral cortex as early as 23 days post-natally. Therefore, species differences and better preservation in the present work of glial processes (likely to be rich in ATPase activity) are possible explanations for the observed discrepancies.

Interestingly, however, in the study by HAMBERGER *et al.* (1970) the activity of the Na–K ATPase in the mitochondrial fraction of the glial cells was higher than that in the same fraction of neuronal cell bodies. ROSE (1968) has recently cited his own unpublished experiments in which he noted no differences in the properties of the neuronal and 'glial' (neuropil) ATPase. CUMMINS and HYDÉN (1962) working with microdissected individual neurons and an 'equal volume' of surrounding glial tissue, reported a G/N ratio for total ATPase of about 1·8, in comparison to our ratio of 5·21 (Table 1).

We think it likely that part of the observed differences in activity between the 'neuronal' and the 'glial' Na–K ATPase reflects the loss of axons, dendritic processes and, particularly, nerve terminals (KUROKAWA, SAKAMOTO and KATO, 1965; ABDEL-LATIF, BRODY and RAMAHI, 1967; APPEL, AUTILIO, FESTOFF and ESCUETA, 1969) during preparation of the neuronal cell bodies. This suggestion is supported by a specific activity for the neuronal Na–K ATPase lower than that of the initial homogenate of cerebral cortex (Table 1; 3·27 *vs.* 4·91). Apparently, the same reasoning does not apply to the ouabain-insensitive ATPase component, since its specific activity matched quite closely that of the initial tissue homogenate (Table 1; 6·31 *vs.* 6·61). Unpublished experiments (AZCURRA and SELLINGER) indicate that the preparation of glial cells used in our study is not contaminated by membranous synaptic elements, as judged by the total resistance of the enzymic activity measured with acetylthiocholine as substrate (ELLMAN, COURTNEY, ANDRES and FEATHERSTONE, 1961) towards 2×10^{-5} M BW-62-C-47, the specific inhibitor of 'true' synaptic acetylcholinesterase (BAYLISS and TODRICK, 1956; PETROPOULOS, VERNADAKIS and TIMIRAS, 1968).

In view of the high activity of the Na–K ATPase measured in the glial cell fraction, it is of interest that KOCH, RANCK and NEWMAN (1962) measured the electrolyte content in the lateral geniculate bodies of the cat before and after the retrograde degeneration of neurons. As a result of the neuronal degeneration the sodium content of the tissue, then composed mainly of neuroglia, increased markedly, whereas the potassium content showed no change.

Department of Biological Chemistry,
Upjohn Center for Clinical Pharmacology
and Mental Health Research Institute,
The University of Michigan Medical Center,
Ann Arbor,
Michigan 48104.

F. MEDZIHRADSKY
P. S. NANDHASRI
V. IDOYAGA-VARGAS
O. Z. SELLINGER

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