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Neurons from Rat Forebrain and Cerebellum. Isolation and Biochemical Characterization

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Isolation of neurons from rat forebrain and cerebellum has been performed by a method including trypsin incubation and tissue disaggregation by filtration through successive nylon meshes with a different pore size. Phase contrast microscopy shows highly purified cell preparations. Lactate dehydrogenase, acetylcholinesterase and (Na^+-K^+) ATPase activities indicate that neurons possess a high cytosolic content and show a good preservation of the plasmatic membrane.

Key words: Neurons isolation, Lactate dehydrogenase, Acetylcholinesterase, (Na^+-K^+) ATPase.

Many procedures have been published for the isolation of neurons from the Central Nervous System (1, 7, 9, 11, 13, 14). All bulk isolation procedures yield neuronal cell bodies with different degrees of purification. Since damage of cells constitues a serious limitation for these techniques (12), biochemical characterization of isolated neurons is necessary to establish their membrane integrity and metabolic pool content.

In this work nerve cell bodies from rat forebrain and cerebellum, with little contamination by other elements, were obtained by a method including trypsin incubation and tissue disaggregation by filtration through successive nylon meshes with progressively fine pore size. Lactate dehydrogenase, acetylcholinesterase and (Na^+-K^+) ATPase activities of isolated neurons are discussed in relation to their cellular integrity.

Materials and Methods

Wistar rats weighing from 150-200 g were used.

Cell isolation procedure. Rat brains were removed from decapitated rats, and forebrain and cerebellum were processed separately. The tissue was finely chopped with a razor blade, washed 4-5 times with isolation medium (100 mM KCl, 0.1 mM

K-EDTA, 10 % [w/v] Ficoll in potassium buffer 10 mM at pH = 7.4), weighed and incubated with trypsin and trypsin inhibitor as described by FAROOQ *et al.* (5). Incubated tissue was resuspended in the isolation medium, (5 ml medium per g of wet tissue), and the suspension filtered successively through nylon meshes with progressively pore size of 420, 190, 100 and 53 μm as described by NAGATA *et al.* (12). Filtered suspension was diluted by adding an equal volume of isolation medium but containing 20 % (w/v) of Ficoll, obtaining the homogenate fraction (H). The homogenate was centrifuged at 8,000 *g* for 30 min, the supernatant discarded and the resultant cell rich pellet (P) was gently resuspended in isolation medium containing 20 % (w/v) of Ficoll and layered in a discontinuous gradient containing 5 ml each of isolation medium with 30 and 25 % (w/v) of Ficoll. Centrifugation was carried out at 8,000 *g* for 5 min and the fractions corresponding to each Ficoll layer (F-20, F-25 and F-30) and the pellet (neuronal cell bodies) were removed with a Pasteur pipet.

The cellular composition of the fractions was determined by phase contrast microscopy.

For chemical analysis the fractions were resuspended in isolation medium, concentrated by centrifugation and stored at −20° C. For (Na^+-K^+) ATPase estimations, the fractions were washed 2-3 times with a free-phosphate medium consisting of 0.32 M sucrose and 10 mM Tris-HCl buffer at pH = 7.4.

Enzyme assays. Acetylcholinesterase was determined by the method of ELLMAN *et al.* (4). Lactate dehydrogenase was assayed as described by CLARK and NICKLAS (2). (Na^+-K^+)-ATPase activity was assayed at 35° C for 4 min in the absence and presence of 1 mM ouabain in an incubation medium containing 2 mM ATP-Tris, 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$ and 50 mM Tris-MES buffer at pH = 7.4. The phosphate formed during incubation was measured according to the method of SERRANO (15). From the difference between (Na^+-K^+)-ATPase activities, in the absence and presence of ouabain, (Na^+-K^+)-ATPase ouabain-sensitive was calculated.

Proteins were determined by the method of BIURET (6) with bovine plasma albumin as standard.

Results

Morphology of the isolated neurons. Phase contrast microscopy of the isolated fractions showed that the only fraction containing pure neurons was that obtained from the pellet of the discontinuous Ficoll gradient. Fractions F-20, F-25 and F-30 contained some neurons but with a great contamination by capillaries and glial cells. Neuronal cell bodies from forebrain (fig. 1A), showed a highly purified cell preparation consisting of typical perikarya neurons. Neither glial contamination nor capillary fragments were observed in this fraction. The only contaminating elements were occasional free nuclei. Micrographs 1B and 1C present the same fractions in more detail. Figure 1C shows that isolated neuronal cell bodies contain relatively large nuclei with distinct single nucleoli and abundant citoplasm. The micrograph of the neuronal cell bodies from the cerebellum shows a highly purified cell preparation of granular cells (fig. 1D). No contamination by other elements was observed.

Enzymatic content of isolated neurons. In the present work, lactate dehydrogenase activity was determined as a parameter of cytosolic content and the activity increase observed in the presence of Triton-X-100 was used as an index of cellular integrity.

Lactate dehydrogenase activity of neuronal cell bodies from both forebrain

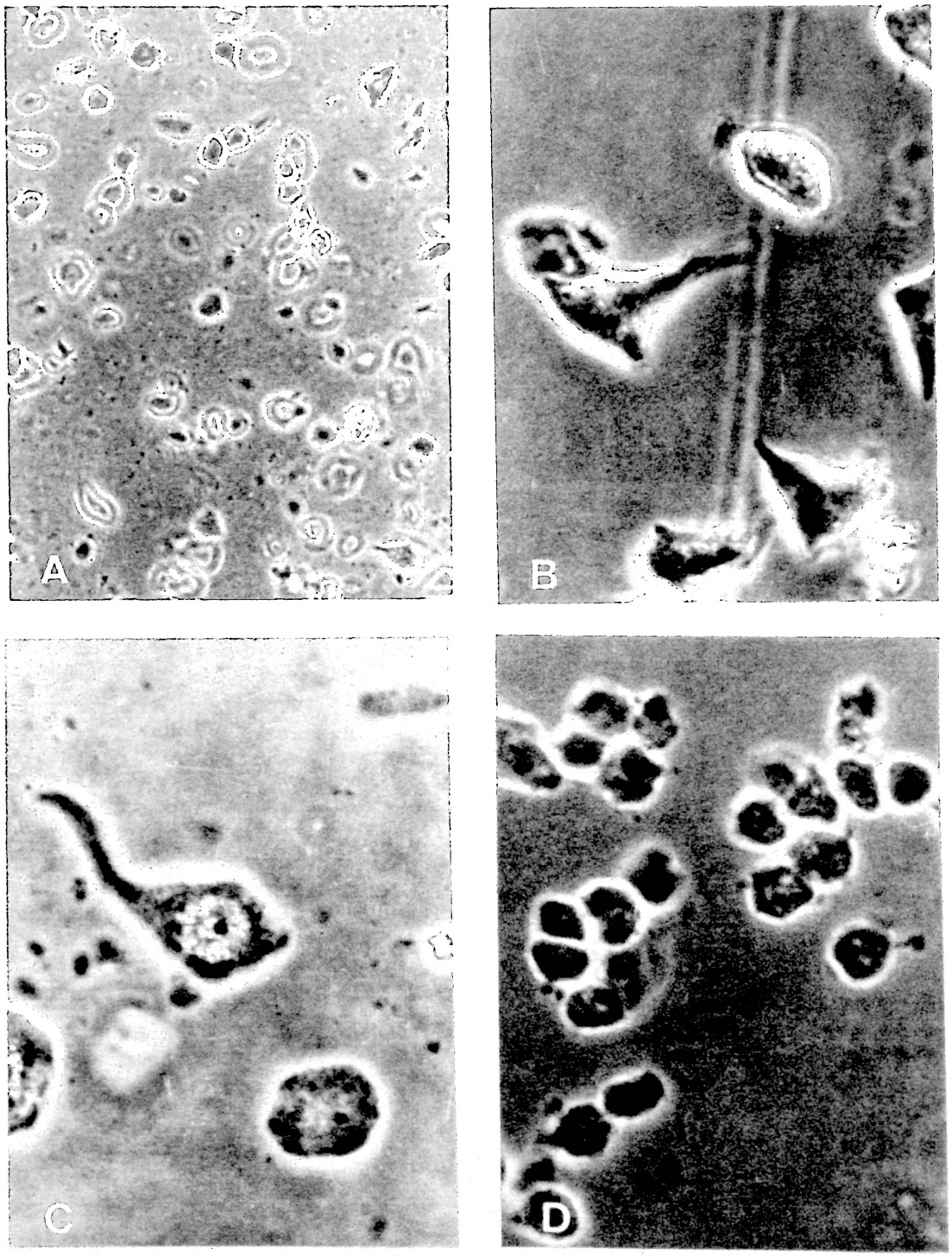

Fig. 1. *Phase contrast micrographs of neurons isolated from rat forebrain and cerebellum at different magnifications.*
Neurons from forebrain A, (× 230), B y C (× 770). Neurons from cerebellum D (× 1,100).

Table I. *Enzymatic content of neurons from forebrain and cerebellum.*
Enzymatic activities were expressed as nmol × min^{-1} × mg^{-1} of protein. Results are the average of at least three determinations ± S.D.

Fraction	Lactate dehydrogenase				Acetylcholinesterase		$(Na^+ - K^+)$ - ATPase			
	Forebrain		Cerebellum		Forebrain	Cerebellum	Forebrain		Cerebellum	
	No Triton	Triton	No Triton	Triton			Ouabain sensitive	Ouabain insensitive	Ouabain sensitive	Ouabain insensitive
Homogenate (H)	178 ± 21	700 ± 60	148 ± 39	370 ± 60	54 ± 2	51 ± 15	140 ± 20	102 ± 30	43 ± 7	131 ± 13
Cell rich pellet (P)	42 ± 9	287 ± 25	21 ± 5	92 ± 30	52 ± 5	64 ± 7	140 ± 15	93 ± 25	46 ± 12	148 ± 28
F-20	60 ± 12	382 ± 43	32 ± 12	190 ± 40	60 ± 9	79 ± 20	140 ± 40	112 ± 19	78 ± 5	137 ± 9
F-25	22 ± 5	124 ± 18	6 ± 3	39 ± 15	72 ± 9	87 ± 10	101 ± 19	173 ± 35	74 ± 14	142 ± 37
F-30	11 ± 5	51 ± 6	9 ± 5	29 ± 9	73 ± 7	187 ± 17	43 ± 10	159 ± 17	39 ± 11	171 ± 23
Neuronal cell bodies	15 ± 4	61 ± 10	18 ± 6	62 ± 12	73 ± 6	95 ± 10	55 ± 10	188 ± 9	44 ± 11	190 ± 9

and cerebellum increased four and three times respectively in the presence of Triton (table I). This increment was quite similar to that found in their respective homogenates. Lactate dehydrogenase activity of neuronal cell bodies derived from both forebrain and cerebellum was similar.

Acetylcholinesterase specific activity of neurons from both forebrain and cerebellum was higher than that found in their respective homogenates. (Na^+-K^+)-ATPase ouabain sensitive activity of the neurons derived from forebrain was about three times lower than that found in the homogenate. On the other hand, this difference was not observed in the same fractions derived from cerebellum.

Discussion

In the present work, nerve cell bodies isolated in bulk from rat forebrain and cerebellum appeared to be one of the least contaminated preparations so far reported (figure 1 A, B, C and D). These neurons were used for the estimation of their cytosolic content and ATPase and acetylcholinesterase activities in order to prove the suitability of this preparation for the study of aspects related to neurotransmission.

Te results concerning the lactate dehydrogenase activity of the neuronal cell bodies from forebrain and cerebellum, when compared to those in their respective homogenates, seem to indicate that the neuron isolation method involves a loss of cytosolic content. However this enzymatic activity per unit of protein in the neurons from both forebrain and cerebellum was higher than that reported by WEILER *et al.* (16), who found a specific activity of 29 nmol $\times$ min^{-1} $\times$ mg^{-1} of protein in albino rabbit neurons. In addition, the cellular integrity was preserved since this enzymatic activity in the presence of Triton increased four and three times in the neurons from forebrain and cerebellum respectively.

Isolated neurons possess an acetylcholinesterase activity higher than that reported by KUENZLE *et al.* (9) and NAGATA *et al.* (12), who found an enzymatic activity of 45 and 30 nmol $\times$ min^{-1} $\times$ mg^{-1} of protein respectively in rat neurons. (Na^+-K^+)-ATPase, ouabain-sensitive activity, in both forebrain and cerebellum neurons was higher than that prepared from Deiters nucleus (3-8) and cerebral cortex (10-12).

From the present results we may conclude that trypsin incubation before tissue disaggregation does not seem to affect the acetylcholinesterase and (Na^+-K^+)-ATPase activities of isolated neurons. On the contrary, the incubation with trypsin allows the preparation of neurons with a good membrane preservation and a high cytosolic content. However, future studies would be required to prove that the metabolic integrity of these cells has been preserved.

Resumen

Se aíslan neuronas de cerebro anterior y cerebelo de rata por un método que incluye la incubación con tripsina y la disgregación del tejido por filtración a través de mallas de nylon de diferente tamaño de poro. La microscopía de contraste de fases muestra una preparación celular altamente purificada. Las actividades de lactato deshidrogenasa, acetilcolinesterasa y ATPasa-(Na^+-K^+) dependiente indican que las neuronas obtenidas presentan un alto contenido citosólico y una buena preservación de la membrana plasmática.

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