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# Acid hydrolases in neuronal and glial enriched fractions of rat brain

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SUMMARY

Fractions enriched in neuronal and glial cells from rat brain were assayed for several lysosomal acid hydrolases, namely, β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23), β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21), α-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22), α-mannosidase (α-D-mannoside mannohydrolase, EC 3.2.1.24), *N*-acetyl-β-glucosaminidase (β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30), *N*-acetyl-β-galactosaminidase, acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2), β-glucuronidase (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31), arylsulfatase (aryl-sulfate sulfohydrolase, EC 3.1.6.1), glucocerebrosidase and galactocerebrosidase. The data do not show any significant difference in the distribution of these activites between the two cell types.

Following the development of the lysosomal concept for rat liver[1] several authors[2–8] have demonstrated the presence of enzymes in brain that are particulate, exhibit structural latency and show acid pH optima for their activity. The literature on neural lysosomes has been extensively reviewed[9–12]. Conventional techniques of differential centrifugation employed for their isolation from peripheral tissues when applied to brain tissue have failed to yield cerebral "lysosomes" in a morphologically homogeneous state. This is presumably due to the anatomical complexity as well as diversity of cell types present in the brain tissue and to the presence of myelin. Sellinger and Hiatt[13] provided evidence for the presence of two populations of lysosomes based on a comparison of enzyme activity in different regions of the rat brain. Application of quantitative histochemical methods[14,15] for the assay of lysosomal enzymes in individual neuronal body, neuropil, granular and molecular layer of cerebellum of various

Abbreviation: 4-MU, 4-methylumbelliferyl.

species led Hirsch[16,17] to suggest a relative abundance of lysosomes in neuronal perikarya as compared to axons, dendrites and glia cells. The present report is a comparative investigation of the levels of several lysosomal acid hydrolases in isolated "neuronal" and "glial" enriched cell fractions from rat brain.

4-Methylumbelliferyl (4-MU) derivatives of β-galactoside, α-galactoside, β-glucoside, α-mannoside, 2-acetamido-2-deoxy-β-glucoside, 2-acetamido-2-deoxy-β-galactoside, β-glucuronide, 4-MU-phosphate and 4-MU-sulfate were obtained from Pierce Chemical Co., Rockford, Ill. [1-$^{14}$C]Glucosyl-*N*-palmitoyl-DL-erythrosphingosine (glucocerebroside) (232 cpm/nmole), was synthesized as previously described[18] and [6-$^{3}$H]galactosyl ceramide (galactocerebroside) (20 018 cpm/nmole) according to a published procedure[19].

The "neuronal" and "glial" enriched fractions were prepared from 15–20-day-old rat brains as previously described[20] according to the methods of Norton and Poduslo[21], the time of trypsinisation of brain tissue being reduced to 45 min. An aliquot of the total cell suspension in hexose–albumin–phosphate buffer was removed prior to gradient separation and centrifuged at 700 × *g* for 10 min. This sample represents the sum of all cell types and is referred to as the pre-gradient pellet. The remainder of the original suspension was processed as usual to yield "neurons" and "glia". The neurons, glia and pre-gradient pellet were washed with phosphate buffer to remove the hexose and albumin, taken in a suitable volume of distilled water and sonicated for 30 s at Step 5 in a Sonifier Cell Disruptor Model W185 (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) equipped with a Semi-microtip. After removing aliquots for the determination of proteins[22], each of the sonicates was assayed for several hydrolytic enzymes employing the following conditions which were found to be optimum in this laboratory for these preparations: *β-galactosidase* (β-D-galactoside galactohydrolase, EC 3.2.1.23), 200–250 μg protein, 0.05 μmole of 4-MU-β-D-galactoside, 5 μmoles of citrate–phosphate buffer, pH 3.4; *β-glucosidase* (β-D-glucoside glucohydrolase, EC 3.2.1.21), 200–250 μg protein, 0.05 μmole of 4-MU-β-D-glucoside, 5 μmoles of citrate–phosphate buffer, pH 4.6; *α-galactosidase* (α-D-galactoside galactohydrolase, EC 3.2.1.22), 200–250 μg protein, 0.05 μmole of 4-MU-α-D-galactoside, 5 μmoles of citrate–phosphate buffer, pH 4.6; *α-mannosidase* (α-D-mannoside mannohydrolase, EC 3.2.1.24), 200–250 μg protein, 0.05 μmole of 4-MU-α-D-mannoside, 5 μmoles of citrate–phosphate buffer, pH 5.2; *β-N-acetylglucosaminidase* (β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30), 15–25 μg protein, 0.5 μmole of 4-MU-2-acetamido-2-deoxy-β-D-glucoside, 5 μmoles of citrate–phosphate buffer, pH 5.2; *β-N-acetylgalactosaminidase*, 100–120 μg protein, 0.05 μmole of 4-MU-2-acetamido-2-deoxy-β-D-galactoside, 5 μmoles of citrate–phosphate buffer pH 5.2; *acid phosphatase* (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2), 15–25 μg protein, 0.5 μmole of 4-MU-phosphate, 5 μmoles of acetate buffer, pH 4.8; *β-glucuronidase* (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31), 200–250 μg protein, 0.05 μmole of 4-MU-β-D-glucuronide, 5 μmoles of acetate buffer, pH 4.0; *arylsulfatase* (aryl-sulfate sulfohydrolase, EC 3.1.6.1), 200–300 μg protein, 0.05 μmole of 4-MU-sulfate, 5 μmoles of acetate buffer, pH 5.6; and water in a final volume of 0.2 ml. The

incubation mixtures were shaken at 37°C for 1 h and the reactions stopped by the addition of 0.2 ml of cold 10% trichloroacetic acid. The samples were mixed and 0.1 ml of 10% NaOH, 0.5 ml water and 1 ml of 0.25 M glycine–KOH buffer, pH 10.3, added. The 4-MU liberated in the reaction mixtures was quantitated fluorimetrically in an Aminco-Bowman Spectrofluorimeter with excitation at 366 nm and emission at 446 nm.

Ceramide glucosidase and ceramide galactosidase activities were assayed according to the procedure of Radin and Arora[23]. The reactions mixture contained 125 nmoles of [$^{14}$C] glucocerebroside or 12 nmoles of [$^{3}$H] galactocerebroside, 100 $\mu$moles of citrate–phosphate buffer, pH 5.0, and 0.5 to 1.0 mg protein in a total volume of 1 ml. The samples were incubated for 3 h at 37°C. The [1-$^{14}$C] glucose and [6-$^{3}$H] galactose liberated were counted in 15 ml Aquasol (New England Nuclear Corp.) in a Tri-Carb liquid scintillation spectrometer, Model 3380.

Experiments were undertaken to establish optimum pH for the assay of each hydrolytic enzyme, and identical results were obtained for both the neurons and the glia. In order to carry out the sulfatase assay, it was necessary to dialyze a small portion of the enzyme source for 2 h against distilled water prior to incubation. The specific activities of the various acid hydrolases in neurons, glia and the pre-gradient pellet are presented in Table I. It is evident that the levels of hydrolase activities are nearly identical in both neurons and glia. The range of activity for several of the enzymes employing different batches of cell preparation is 2–3-fold, however, marked differences were not observed in the individual activities between the two types of cell preparations. The variation in the activities of neurons and glia in different batches may be operationally inherent in their isolation procedure. No obvious differences were noted by visual inspection under phase contrast microscope. In general, the lower the yield of cells in a given preparation, the lower the activities.

It is also seen that except for $\beta$-glucuronidase all the activities are enriched in the cell preparation when compared to the pre-gradient pellet. The extent of enrichment of each specific enzyme over the pre-gradient pellet is similar between the two cell preparations. However, all these enzymes do not show the same degree of enrichment in the neurons and glia. Thus $\beta$-*N*-acetylglucosaminidase, acid phosphatase, $\beta$-*N*-acetylgalactosaminidase and $\alpha$-mannosidase showed higher relative enrichment in the two cell preparations when compared to the other acid hydrolases assayed. This might suggest that all the enzymes assayed are not strictly bound to a lysosomal structure and some of the more soluble ones may be leaking out due to damage to the cell during isolation. In this context, it should be mentioned that Marsh and Gourlay[24] have recently reported the presence of a nonlysosomal mannosidase in rat liver.

The activities of the acid hydrolases were quantitated in the pre-gradient pellet and the supernatant obtained after centrifuging the total cell suspension after trypsin treatment at 700 × *g* for 10 min. Several of these enzymes appeared to be equally distributed between the pellet and supernatant except arylsulfatase and $\beta$-glucuronidase which were present to the extent of 70% and 90%, respectively, in the supernatant. Of the activity present in the pre-gradient pellet, approximately 50–70%, depending upon the

TABLE I

SPECIFIC ACTIVITIES OF ACID HYDROLASES

Values expressed as nmoles product formed per mg protein per h. All enzymes were assayed in the cell preparation from eight brains. The activities were determined in three separate cell preparations. The values represent the separate preparations with the mean shown in parentheses.

| *Enzyme* | *Neurons* | *Glia* | *Pre-gradient* | *Neurons/ pre-gradient* | *Glia/ pre-gradient* |
|---|---|---|---|---|---|
| β-Galactosidase | 55.6, 110.3, 123.5 (96.5) | 106.3, 55.6, 169.1 (110.3) | 36.1, 49.4, 65.2 (50.2) | 1.54, 2.23, 1.89 (1.9) | 2.94, 1.12, 2.59 (2.2) |
| β-Glucosidase | 15.6, 15.3, 20.7 (17.2) | 18, 8.6, 19.2 (15.3) | 9.0, 8.1, 12.3 (9.8) | 1.73, 1.89, 1.68 (1.78) | 2.0, 1.06, 1.56 (1.54) |
| α-Galactosidase | 15.1, 16.7, 29.4 (20.4) | 23.2, 15.0, 26.7 (21.6) | 12.0, 11.1, 17.2 (13.4) | 1.26, 1.50, 1.71 (1.5) | 1.93, 1.35, 1.55 (1.6) |
| α-Mannosidase | 28.2, 31.4, 48.4 (36.0) | 45.2, 30.6, 51.6 (42.5) | 9.9, 9.1, 14.2 (11.1) | 2.85, 3.45, 3.41 (3.2) | 4.56, 3.36, 3.63 (3.8) |
| *N*-Acetyl-β-glucosaminidase | 2635, 2547, 4821 (3334) | 4242, 2558, 4620 (3807) | 650, 656, 889 (732) | 4.05, 3.88, 5.42 (4.45) | 6.5, 3.90, 5.2 (5.2) |
| *N*-Acetyl-β-galactosaminidase | 146, 134, 274 (185) | 176, 146, 218 (180) | 40, 44, 57 (47) | 3.65, 3.04, 4.81 (3.83) | 4.4, 3.32, 3.82 (3.85) |
| Acid phosphatase | 1894, 2611, 4217 (2907) | 2735, 1972, 5003 (3237) | 478, 486, 1050 (671) | 3.96, 5.37, 4.02 (4.45) | 5.72, 4.06, 4.76 (4.8) |
| β-Glucuronidase | 5.2, 2.7, 8.2 (5.4) | 2.3, 3.3, 5.0 (3.5) | 10, 8.1, 11.5 (9.9) | 0.52, 0.33, 0.71 (0.52) | 0.23, 0.41, 0.43 (0.36) |
| Arylsulfatase | 3.4, 4.6, 4.3 (4.1) | 4.4, 3.6, 4.3 (4.1) | 2.2, 2.8, 3.3 (2.8) | 1.54, 1.64, 1.30 (1.5) | 2.0, 1.28, 1.30 (1.53) |
| Glucocerebrosidase | 15.0, 29.3, 43.7 (29.3) | 19.2, 20.6, 20.8 (20.2) | 10.1, 15.6, 12.3 (12.7) | 1.48, 1.88, 3.55 (2.30) | 1.9, 1.32, 1.69 (1.64) |
| Galactocerebrosidase | 0.06, 0.1, 0.07 (0.08) | 0.04, 0.11, 0.06 (0.07) | 0.01, 0.04, 0.05 (0.03) | 6.0, 2.5, 1.4 (3.3) | 4.0, 2.75, 1.2 (2.65) |

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specific enzyme, were recovered in neuronal and glial enriched fractions. The solutions used in the washings of neurons and glia contained only about 20% of the activities recovered in the cells. It seems likely that the major cell damage occurs during the initial preparation of the cell suspension.

While the present investigation was in progress, Idoyaga-Vargas and Sellinger[25], and Sellinger *et al.*[26] reported that β-*N*-acetylglucosaminidase, β-*N*-acetylgalactosaminidase and α-mannosidase activities are similarly distributed in the neurons and glia isolated from rat brain according to their technique. This observation agrees with the data reported here. In contrast, Sinha and Rose[27] noticed that in the cells prepared according to their procedure, the lysosomal enzymes are concentrated more in the neuronal than in the neuropil fraction. They suggested that β-galactosidase may indeed be a neuronal marker. The results reported here do not support this observation. The apparent discrepancy may lie in the differences in technique adopted for the isolation of cells. In a recent communication, Sinha and Rose[28] have compared their technique with that of Norton and Poduslo[21] and that of Sellinger *et al.*[29], and suggested that substantial cell damage may occur in the latter two procedures. It is difficult to ascertain how the effects of such cellular damage, if they occur during the isolation procedure would alter the distribution of lysosomal enzymes between the neurons and glia. At present, it is hard to judge which method of preparation yields the least altered cells. This decision can only be arrived at after a significant number of reports have appeared.

Earlier observations from this laboratory[20] indicated that glia contain more lipid than neurons. There is very little difference in the fatty acid composition of cerebrosides present in the two cell types although it differs from that present in myelin.

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