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Differences in the Distribution of Energy-Metabolizing Enzymes in Rat Brain Regions

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The activities of several enzymes of glucose metabolism (glycolytic and tricarboxylic acid pathways) in four different regions of rat brain (cerebellum, medulla oblongata and pons, cerebral cortex and diencephalon) have been studied. Statistical differences were found in the activities of all the enzymes analyzed in the four regions, except in the case of the soluble hexokinase and pyruvate kinase. The changes observed in citrate synthase activity may account for physiological differences in those areas related to myelin formation and energy metabolism. Cerebral cortex and diencephalon showed enzyme activities which were generally greater than those of he cerebellum and medulla oblongata and pons. The results obtained lend support to the concept of a differential energy metabolism in brain regions.

Key words: Energy-metabolizing enzymes, Brain enzymes, Regional distribution of enzymes.

Glucose serves as the main fuel for energy metabolism in the adult mammalian brain, being metabolised via the glycolytic and citric cycle pathways (15). Furthermore, it has been suggested that different regions of the brain may differ in their energy metabolism (8, 16). Although developmental profiles of enzyme activities in similar brain regions (11, 12, 19) have been reported and histochemical studies have demostrated the distribution of various enzymes of energy metabolism (1, 6), there are very few reported values of activities of enzymes of glycolytic and Krebs cycle pathways in the normal adult rat brain (9, 13).

Based on this information, the purpose of the present study was to examine the activities of several key enzymes of glucose metabolism in brain regions reported previously. The knowledge of these regional differences was useful for the study of energy metabolism changes in rat brain after acute and chronic dextroamphetamine administration (18).

Materials and Methods

Male and female Wistar albino rats weighing 180 to 200 g at the time of

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sacrifice were used. Rats were housed three per cage under optimal environmental conditions (22°C, 12 h day cycle) and food and water were available *ad libitum*. Six groups of five animals were employed and one series per week was analyzed. The animals were sacrificed by decapitation, their brains were quickly removed from the skull, placed in liquid nitrogen at —196°C, and dissected in four different regions (7): cerebellum, medulla oblongata and pons, cerebral cortex and diencephalon.

Fresh tissues were homogenized according to BAQUER *et al.* (2) with the addition of 1 mM EDTA (17), with ten strokes of an all-glass Potter-Elvehjem homogenizer. The homogenate obtained was then centrifuged for 10 min at 2,060 × *g* to remove contaminating materials. A crude mitochondrial fraction was obtained after centrifugation at 12,900 × *g* (10 min) and mitochondrial pellets were resuspended in 0.7 ml of the extraction buffer and Triton X-100 at 0.5% (w/v) final concentration, immediately before use. This mitochondrial residue was used for the analysis of the particulate enzymes: hexokinase (EC 2.7.1.1), citrate synthase (EC 4.1.3.7.) and malate dehydrogenase (EC 1.1.1.37), and the supernatant was used for the determination of the soluble enzymes: hexokinase (EC 2.7.1.1.), phosphofructokinase (EC 2.7.1.11), pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27). For the assay of NAD+ -dependent isocitrate dehydrogenase (EC 1.1.1.41) and NADP+ -dependent isocitrate dehydrogenase (EC 1.1.1.42) brain tissues were homogenized according to SUGDEN and NEWSHOLME (17). The homogenates were processed in the same manner as described above. The remaining pellet was solubilized with 1 ml of the extraction medium and then sonicated (19). A W-375 Sonicator cell disruptor (Heat system, Ultrasonic, INC) with refrigerating compartment was used. The enzymatic assays were carried out as soon as possible after sonication. The methods used for the measurement of enzyme activities were as reported earlier (3 - 5, 9, 17). Protein content was evaluated (14) in all samples.

All enzyme assays were carried out at 25°C and measures were made with a Beckman 25 Spectrophotometer recorder by following the rate of change of extinction at 340 nm except in the case of the citrate synthase that was assayed at 412 nm. Results were expressed as specific activities (nmol · min^{-1} · mg $protein^{-1}$) and statistically analyzed with a three way nested analysis of variance (A: series, B: rats and C: regions).

Results

Figure 1 shows the activities of the key enzymes of the glycolytic pathway

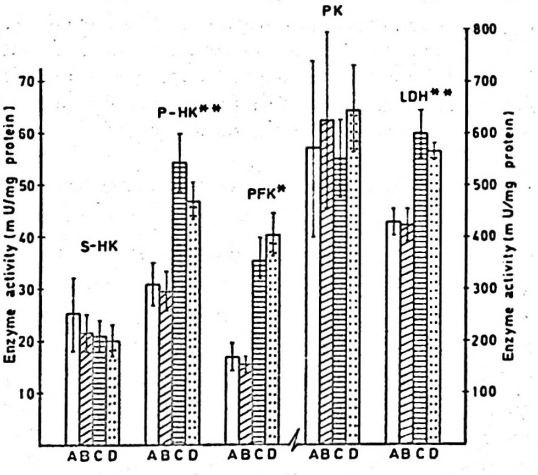

Fig. 1. *Enzyme activity in (A) cerebellum, (B) medulla oblongata and pons, (C) cerebral cortex and (D) diencephalon.*
S-HK = Soluble Hexokinase, P-HK = Particulate Hexokinase, PFK = Phosphofructokinase, PK = Pyruvate kinase and LDH = Lactate dehydrogenase. Each value represents the mean ± S.E.M. of six series with five animals each. *($p < 0.01$) and ** ($p < 0.001$).

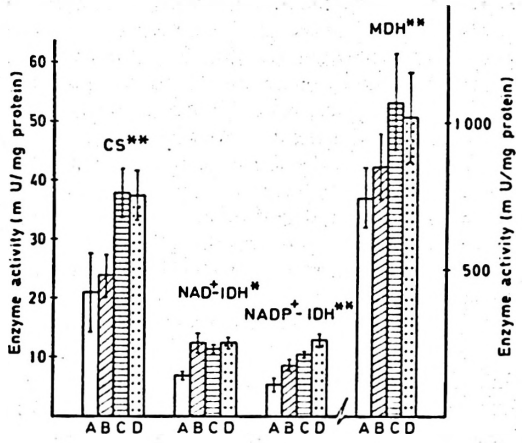

Fig. 2. *Enzyme activity in (A) cerebellum, (B) medulla oblongata and pons, (C) cerebral cortex and (D) diencephalon.*
CS = Citrate synthase, NAD+-IDH = NAD+-isocitrate dehydrogenase, NADP+-IDH = NADP+-isocitrate dehydrogenase and MDH = Malate dehydrogenase. Each value represents the mean ± S.E.M. of six series with five animals each. *($p < 0.025$) **($p < 0.001$).

and the lactate dehydrogenase in four rat brain regions (cerebellum, medulla oblongata and pons, cerebral cortex and diencephalon). The enzyme activities of the particulate hexokinase, phosphofructokinase and lactate dehydrogenase showed statistical differences in the areas studied. No significant differences were found, however, for soluble hexokinase and pyruvate kinase activities.

Figure 2 shows the activities of some enzymes of the tricarboxylic acid pathway and one of the NADPH-generating enzymes (NADP+ -isocitrate dehydrogenase) in the same brain regions. Statistical differences were found in the activities of citrate synthase, NADP+ -dependent isocitrate dehydrogenase, malate dehydrogenase and NAD+ -dependent isocitrate dehydrogenase in those four regions.

Citrate synthase catalyzes a flux-generating step of the tricarboxylic acid cycle and plays a role in lipid metabolism. In fact, the changes observed in citrate synthase activity may account for physiological differences in the four areas studied.

Discussion

The relationship between the maximal activity and metabolic flux is complex, but several investigators have considered the measurement of enzyme activities as a useful approach to relating metabolic potential to tissue function.

Statistical differences were found in the distribution of glycolytic pathway enzyme activities in the four rat brain regions studied, except in the case of soluble hexokinase and pyruvate kinase activities (fig. 1). Cerebral cortex and diencephalon have enzyme activities that are generally greater than those of the cerebellum and medulla oblongata and pons. The first two areas showed similar activities in contrast to the cerebellum which generally exhibited the lowest of all the areas studied. Other authors (13) found the highest activities in cerebral cortex and cerebellum, in contrast to the results obtained in this study.

Hexokinase partitioning between the soluble and particulate fractions of brain has been observed to change according to the energy status of the brain (9). The extent of binding on mitochondria is inversely related to the ATP content. Therefore, conditions in which energy utilization exceeds supply shift the solubilization equilibrium to the bound form, which is more active (10, 20). Statistical differences were found in the hexokinase particulate activities in the regions with higher values in cerebral cortex and diencephalon. This fact would be consistent with major energy requirements in those regions.

The ratios between the two forms of hexokinase in cerebellum (47% soluble

hexokinase and 53% particulate hexokinase) and brain (31% and 69%) previously reported (9) are quite comparable with the results presented here (45%-55% and 30%-70%, respectively).

As for the activities of the Krebs cycle enzymes assayed, statistical differences were also found in the distribution in the four rat brain regions studied (fig. 2). The regional differences in citrate synthase activities are particularly relevant due to the participation of this enzyme in some metabolic reactions occurring in the nervous tissue like energy generation and myelin formation. Again the cerebral cortex and diencephalon showed the highest activities. The data reported by other authors (13) are not in agreement with these results except in the case of cerebral cortex.

From the data presented here the various regions of the brain appear to vary in their potential enzymic capabilities to utilize glucose. Therefore, the differences found in the areas studied could be related to the importance of glycolytic and tricarboxylic acid pathways in each area.

Although the conclusions that can be drawn from the analysis of the gross regions examined are limited, the highest activities found in cerebral cortex and diencephalon could suggest a higher metabolic activity in these areas related to the different physiological functions of the brain regions.

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Resumen

Se estudia la actividad de varias enzimas del metabolismo de la glucosa (glucólisis y ciclo de los ácidos tricarboxílicos), en cuatro áreas cerebrales diferentes del cerebro de rata: cerebro, médula oblongata y puente, corteza cerebral y diencéfalo. Se encontraron diferencias estadísticamente significativas en las actividades de todas las enzimas analizadas, entre las cuatro áreas cerebrales, excepto en el caso de la hexoquinasa soluble y la piruvatoquinasa. Los cambios observados en la actividad de la citrato sintasa podrían estar relacionados con diferencias fisiológicas entre las zonas en procesos como la obtención de energía y la formación de mielina. La corteza cerebral y el diencéfalo presentaron actividades enzimáticas que fueron generalmente superiores a las presentadas por el cerebro y la médula oblongata y el puente. Estos resultados refuerzan el concepto de la existencia de un metabolismo energético diferente en las distintas regiones cerebrales.

Palabras clave: Metabolismo energético cerebral; Enzimas cerebrales; Distribución regional de las enzimas.

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