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Regional Distribution of Lactate Dehydrogenase Isozymes in Rabbit Brain

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The resolution of lactate dehydrogenase isozymes in tissue samples obtained from various regions of the rabbit brain was carried out by cellulose acetate electrophoresis.

1) Most regions of the brain showed an H-type isozyme pattern.

2) Five clearly differentiated patterns of isozyme activity were found throughout the entire cerebral cortex with no difference between the lobes of the cerebral cortex.

3) All 5 patterns were found in the upper brain, while 4 patterns-LDH-1, 2, 3 and very low activity of LDH-4 were found in the lower brain.

Most tissues of mammals contain five molecular forms of lactate dehydrogenase which can be separated by electrophoresis. The most negatively charged form, isozyme-1 or LDH-1, is a homotetramer of polypeptide units and is designated either "B" or "H". At the other end of the electrophoretic spectrum is isozyme-5 or LDH-5, which is also a homotetramer, but composed of different units ("A" or "M"). Isozymes 2, 3 and 4 are the heterotetramers formed by the association of A and B chains (A_1B_3, A_2B_2 and A_3B_1).

Within a given species the isozymes have similar molecular weight (130,000~135,000; Wieland & Pfleider, 1961; Pesce *et al.*, 1967) and they differ in their net electric charge and in their amino acid composition. LDH-1 has more aspartic acid and glutamic acid, while it has less lysine than LDH-5 (Locke, 1963; Wilkinson, 1970). The fact that the isozymes may be readily separated by electrophoresis and their biochemical activities differ together with the fact that not all isozymes appear in a given tissue at the same time, makes them ideal molecular markers for the study of cellular differentiation and developmental physiology. The pattern of isozyme activity is tissue specific rather than species specific and is related to the physiological activity and microenvironment of the tissue (Cahn *et al.*, 1962).

For example, LDH-1 predominates in heart muscle, whereas LDH-5 predominates in skeletal muscle. This distribution has been correlated with local oxygen tensions, pyruvate inhibition and lactate accumulation (Stambaugh

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and Post, 1966).

The isozymes differ in their catalytic activities, for LDH-1 is inhibited by concentrations of pyruvate or lactate which do not affect the activity of LDH-5 (Plagemann *et al.*, 1960; Cahn *et al.*, 1962).

This finding has been interpreted as an index of physiological significance. LDH-1 would favor an aerobic type of metabolism, since an increase of pyruvate or lactate prevents further accumulation of lactate and would force the oxidation of pyruvate in the Krebs cycle. On the other hand, LDH-5 is able to function at high pyruvate and lactate concentrations, allowing the reoxidation of NADH and the supply of energy under anaerobiosis (Cahn *et al.*, 1962).

That is, under conditions of low oxygen tension LDH-5 would be expected to predominate over LDH-1.

Several studies suggest that individual LDH isozymes have specific cellular and subcellular loci in a given tissue.

Smith and Kissane (1963) have found different forms of LDH in different regions of the rat nephron by subjecting dissected portions of the kidney to chemical analysis.

Therefore it could be assumed that LDH isozyme patterns would be different in various parts of the brain.

This experiment was intended to investigate the regional differences of the LDH isozymes pattern of the rabbit brain.

MATERIALS AND METHOD

Adult rabbits, weighing about 2.5~3.0kg, were used as the experimental animal. The animals were decapitated without anesthesia in order to avoid the side effect of anesthetics on brain enzymes. After decapitation, the skull cap was removed and each part of the brain was dissected immediately, weighed and stored in an ice bath until homogenization.

Each sample was minced and homogenized using a glass tissue grinder prepared in an ice bath in 5 volumes of distilled water and stored in a refrigerator.

Separation of lactate dehydrogenase was carried out by electrophoresis by the method of Kohn (1958).

A horizontal electrophoresis tank for small scale membrane filter electrophoresis, with a gap of 4.5cm was used. Celotate (Millipore) was used as a cellulose acetate membrane strip, size 4.9×2.5cm and it was held by filter paper acting as wicks. Barbitone buffer (pH 8.6, ionic strength 0.75) was used throughout the experiment and 120V was applied for about one hour, providing a pattern of approximately 2cm.

Isozymes were visualized by incubating the acetate strip in the following staining solution at 37°C for 40~60 min. The staining solution was prepared immediately before use with chemicals from the Sigma Chemicals Co. and the prepared amount was three times the amount of these.

1 %	Nicotinamide adenine dinucleotide (NAD)	1 ml
0.1%	Nitro-blue tetrazolium (NBT)	3ml
0.1%	Phenazine methosulphate (PMS)	0.3ml
1.0M	Na DL-lactate, pH 7.0	1ml
0.5M	Tris-HCl buffer, pH 7.1	1ml

The strip was washed in running tap water, blotted and dried.

The analysis of the resultant stained bands was accomplished by use of a "Quick scan" (Helena Laboratories, Texas) densitometer.

RESULTS

Electrophoresis was carried out on various parts of the adult rabbit brain and they exhibited mostly five LDH isozymes. For purposes of description the fractions were numbered 1~5 according to the system of Apella and Markert(1961).

The regions studied are the frontal, parietal, temporal and occipital lobes of the cerebral cortex, rhinencephalon, thalamus, cerebellum,

Table 1. Percentage distribution of the isozymes

Regions of the brain	LDH-1 (B4)	LDH-2 (B3A1)	LDH-3 (B2A2)	LDH-4 (B1A3)	LDH-5 (A4)
Frontal lobe	30.1±0.23	26.3±0.08	23.8±0.71	16.2±1.17	3.6±0.35
Parietal lobe	30.7±1.58	27.0±0.40	24.7±0.36	13.5±0.86	3.8±1.30
Temporal lobe	31.1±2.79	27.6±3.31	23.8±2.42	16.6±0.8	2.1±0.25
Occipital lobe	34.6±3.83	28.9±0.75	24.8±1.58	9.5±2.36	2.3±0.21
Rhinencephalon	34.6±5.28	20.6±2.79	23.1±0.40	17.3±0.57	2.3±0.51
Thalamus	33.8±4.13	21.0±3.37	22.6±0.49	16.1±1.77	6.6±0.49
Cerebellar hemisphere	43.2±3.11	33.2±1.42	19.3±2.93	4.1±0.53	—
Cerebellar vermis	47.4±3.35	33.6±2.09	18.0±1.33	—	—
Superior colliculus	60.0±8.38	25.4±3.87	10.5±2.12	4.0±0.31	—
Inferior colliculus	53.4±5.69	32.4±1.57	11.6±3.38	3.9±0.36	—
Pons	29.7±2.16	22.9±0.43	23.8±0.53	17.7±0.87	5.9±1.25
Medulla oblongata	49.6±3.11	32.8±0.86	14.2±1.94	3.7±0.85	—
Spinal cord	67.4±2.05	21.6±2.71	11.0±0.72	—	—

Values are the mean of three to six separate determinations±S.E.

Table 2. Sequence of isozymes by their activity strength

Regions of Brain	Isozymes					
Frontal lobe	LDH	1	2	3	4	5
Parietal lobe		1	2	3	4	5
Temporal lobe		1	2	3	4	5
Occipital lobe		1	2	3	4	5
Rhinencephalon		1	3	2	4	5
Thalamus		1	3	2	4	5
Cerebellar hemisphere		1	2	3	4	
Cerebellar vermis		1	2	3		
Superior colliculus		1	2	3	4	
Inferior colliculus		1	2	3	4	
Pons		1	3	2	4	5
Medulla oblongata		1	2	3	4	
Spinal cord		1	2	3		

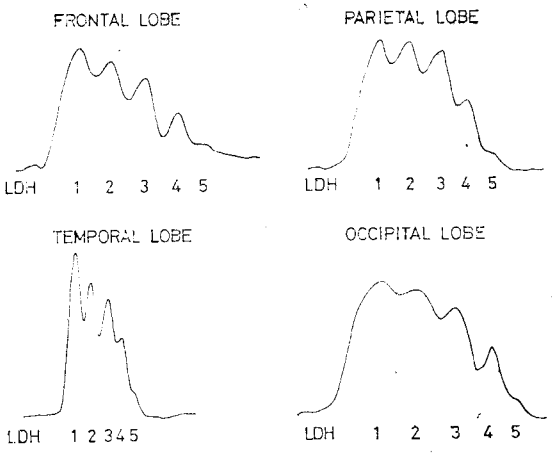

Fig. 1. Densitometric patterns of LDH isozymes in cerebral cortex of the adult rabbit brain.

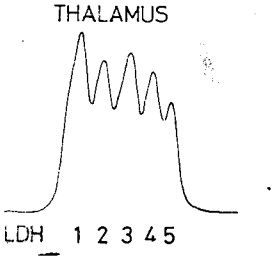

Fig. 2. Densitometric patterns of LDH isozymes in thalamus of the adult rabbit brain.

superior and inferior colliculus, pons, medulla and spinal cord.

The results are represented in Table 1 &2, Fig. 1, 2, 3 and 4.

The percentage mean values of the isozymes of each part of the brain is shown in Table 1 and the isozymes are arranged in the sequence of the strongest activity in various parts of the brain (Table 2).

CEREBRAL CORTEX: The LDH isozymes of each lobe of the cerebral cortex, that is, the frontal lobe, parietal lobe, temporal lobe and occipital lobe did not differ much from each other.

Generally, they showed a fairly uniform distribution with the highest activity in the first three isozymes, LDH-1, LDH-2 and LDH-3.

The distribution of the LDH-4 and LDH-5 were lower than the first three isozymes. As seen from Fig.1, the activity of LDH isozymes in the frontal lobe was most active in LDH-1 and LDH 2 & 3 declined gradually. The LDH-5 declined suddenly and its mean value of the percentage distribution was 3.6 ± 0.35 to compare with 16.2 ± 1.17 of the LDH-4.

In parietal lobe, LDH-1 was most active and LDH-2 & 3 declined slightly compared with frontal lobe. LDH-4 and LDH-5 declined suddenly and especially the activity of LDH-5 was very low.

In temporal lobe LDH-1 was most active and LDH-2 & 3 declined exponencially. The percentage distribution of LDH-4 was obviously lower than the LDH-3 and the lowest activity was shown by LDH-5.

In occipital lobe, LDH-1 showed the highest activity among the lobes of the cerebral cortex. The distribution of LDH-2 & 3 was appeared similar to the distribution pattern of the other lobes. The percentage of LDH-4 was 9.5 ± 2.36 and it is the lowest in the lobes of the cerebral cortex (Fig. 1).

RHINENCEPHALON: In rhinencephalon, there was no prominent characteristics compared to the cerebral cortex. It showed five well-defined bands. Rhinencephalon is regarded as the part of the cerebral cortex, so it showed similar distribution pattern to the cerebral cortex. The activity of LDH-1 was the highest but the activity of the percentage distribution were 20.6 ± 2.79 (LDH-2) and 23.1 ± 0.4 (LDH-3).

DIENCEPHALON: The thalamus showed five well-defined bands. LDH-1 showed the highest activity, its mean value of the percentage distribution was 33.8 ± 4.13 & LDH-3 was higher than LDH-2. Their mean value were 21.0 ± 3.37 (LDH-2) and 26.6 ± 0.49

3) respectively. LDH-5 was high compared to the other regions and its mean value was 6.6±0.49. This mean value of the LDH-5 was the highest throughout this experiment (Fig. 2).

CEREBELLUM: Cerebellum showed four bands in total and the action of the LDH-1 was prominent in this region. In cerebellar vermis, the mean value of the percentage distribution of LDH-1 was 47.4±3.35 and this is higher value compared to the upper brain region, that is, cerebrum and diencephalon. LDH-2 and LDH-3 of the cerebellar vermis declined exponencially and LDH-4 was not appeared here. In cerebellar hemishere, general figure of distribution of LDH-1, LDH-2 and LDH-3 was similar to cerebellar vermis but the differences of the mean value between LDH-1 and LDH-2, LDH-2 and LDH-3 were more prominent. Very low activity of LDH-4 was shown in this hemisphere (Fig. 3).

BRAIN STEM; Superior and inferior colliculus exhibited four bands, LDH-1, 2, 3 and 4 and LDH-1 showed highest activities, which were 60.0±8.38 in superior colliculus and 53.4±5.69 in inferior colliculus. There was no significant difference beween these two portions. The pons showed five well-defined bands and their distribution pattern was similar to the diencephalon. That is, LDH-2 is lower than LDH-3 and LDH-5 was rather high, 5.9±1.25. The medulla had four isozymes and LDH-1 was high as 49.6±3.11. LDH-3 declined suddenly compared to LDH-2 and LDH-4 showed very low activity, 3.7±0.85. LDH-5 was not observed.

The spinal cord showed only three bands and the activity of LDH-1 was the highest of all the regions of the brain area. The decreasing rate of the LDH-2 & 3 was very big, about one third in case of LDH-2 and about one half in case of LDH-3. LDH-4 and LDH-5 were not observed (Fig. 4).

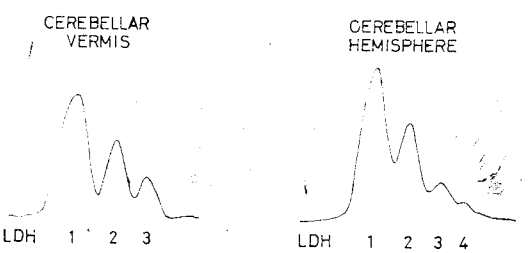

Fig. 3. Densitometric patterns of LDH isozymes in the cerebellum of the adult rabbit brain.

DISCUSSION

Two pure lactic dehydrogenases occur in mammals. One of them is found principally in the skeletal muscle(M subunit) and the other in the heart(H subunit). These two enzymes are separate entities as judged by physical, enzymatic and immunochemical criteria(Kaplan & Ciotti, 1961). Several other enzymes have also been shown to exist in

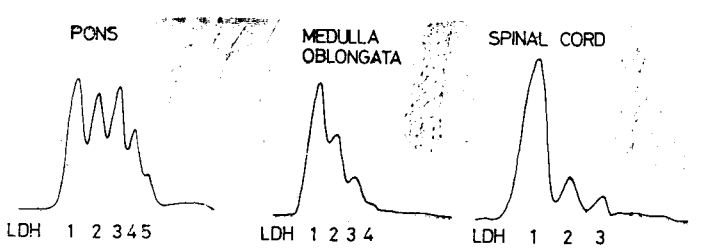

Fig. 4. Densitometric patterns of LDH isozymes in lower brain stem and spinal cord of the adult rabbit brain.

multiple forms, not only within a single organism but even within a single tissue (Markert and Mϕller, 1959). And it has been suggested that these distinguishable molecular types of enzymes be called isozymes. Apella and Markert(1961) followed by Cahn(1962) were able to account for five LDH isozymes, while Nebel *et al.*, (1964) reported six LDH isozymes in the chick embryo. Buta *et al.* (1966) reported a maximum of nine fractions in rat organs but he divided these fractions into five major groups. And also they reported the major differences between rat LDH isozymes and those of other species are the total number of LDH isozymes and five major groups. In the present experiment most regions of the rabbit brain showed the five isozymes with the highest activities in the first three isozymes.

Among these five isozymes, the most anodal migrating fraction is designated as LDH-1, while the isozyme which either exhibits the least mobility or which is most cathodal migrating is termed LDH-5. Brody and Engel (1964) found that the slow moving LDH isozymes are predominant in organs with a relatively large capacity for anaerobic glycolysis, such as liver, whereas organs less capable of anaerobic metabolism, such as heart muscle and brain, which have a preponderance of the faster moving isozymes. The presence of relatively large quantities of all five isozymes was attributed to the influence of transitory high and low levels of oxygen and the consequent necessity to function under both aerobic and anaerobic conditions. Thus their results further indicate that the supply of oxygen plays a large role in determining the relative activities of the A and B genes of LDH and so influences the pattern of isozymes of LDH present within a particular tissue. According to Nebel and Conklin(1964) brain exhibited only slight changes in isozyme content during maturation, however Hazama Uchimura(1974)reported a signifi and cant decrease in LDH-4 and 5 due to maturation in the rat brain.

Swaiman and Wolfe(1970)noted the LDH-1 isozyme activity increased markedly with maturation and there was a decrease in LDH -4 isozyme activity and LDH-5 isozyme activity with age and these findings accord with the present experiment result, the pattern of isozymes in adult rabbit brain.

These findings also parallel the works of Hazama and Uchimura. Most regions of the central nervous system of the rabbit show a fairly uniform distribution with the highest activities in the first three isozymes. This finding is quite different from the works of Bonavita *et al.* (1962) and they reported the percentage mean value of LDH isozymes of the whole brain of the adult rat.

Their results showed even activities of LDH -2, 3 and 4 of around 20% each and 37.6% for LDH-5. This is a tremendously different view in the field of isozyme percentages of the brain of mammals and it is controversial at this time.

Gerhardt and Petri(1965) worked on isozymes of the human brain and in their results, the activity of LDH-5 was insignificant in almost every region of the brain.

The results of the present study indicate that the general distribution of the LDH isozymes in the cerebral cortex, the frontal, parietal, temporal and occipital lobes was equal in LDH-1, 2 and 3 at about 25~30%, LDH-4 about 15% and LDH-5 very low., In the human brain (Gerhardt and Petri, 1965)their results accord with the results of the present study.

Also in the rat(Hazama and Uchimura,1974), parietal cortex was studied and there LDH-1 was rather higher than the others. LDH-5 was not observed in the parietal cortex of the rat brain but in the white matter its activity was around 2%.

In the present experiment the cerebellum exhibited very low activity of isozymes 4 and 5. This finding accords with that of the human brain. They reported the dentate nucleus of the cerebellum to be different from the other regions, due to very low activities of isozyme -4 and isozyme-5, indicating a relatively high rate of aerobiosis.

But in this area the human brain showed an even distribution of LDH-1, 2 & 3, while in the rat LDH-1 is as high as 50%. From this point of view, the rabbit shows similar findings to the rat.

In the lower brain, the pons showed a rather intermediate form, that is, the percentage of LDH-1, 2 & 3 was even and LDH-4 & 5 were observed. However, going down, medulla exhibited stronger activity in LDH-1 than LDH-2 and 3. LDH-5 was not observed here.

This phenomenon is more pronounced in the spinal cord. Therefore, one can assume that the activity of the LDH-1 becomes stronger and that of LDH-4 & 5 becomes weaker going caudally.

In spite of reports by others that there are no regional differences in the brain, this author can conclude from these results that there is a difference between the upper brain and the lower brain.

It can be called an upper brain form which represents five distinct bands of isozymes in the zymogram and a lower brain form which represents LDH-1, 2 & 3 and the highest activity of LDH-1.

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