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TOTAL ACTIVITY AND ISOENZYMIC PATTERNS OF LACTATE — DEHYDROGENASE (LDH) IN VARIOUS RAT TISSUES

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Lactate-dehydrogenase (LDH) isoenzyme patterns were measured by microdisc gel electrophoresis in various tissues (cardiac muscle, kidneys, adrenals, thyroid, liver) and different parts of the brain (hypothalamus, hippocampus, Nucleus caudatus, cortex cerebri, cerebellum) of albino male Wistar rats. Total LHD activity had the highest value in cardiac muscle and liver, and the smallest value in kidneys. In cardiac muscle the greatest activity was shown by isoenzyme LDH_2 (LDH_2>LDH_3>LDH_1>LDH_4>LDH_5), while the greatest activity in liver was shown by the electrophoretically "slowest" component, LDH_5. In the kidneys isoenzyme LDH_2 (LDH_2> LDH_1>LDH_4>LDH_3>LDH_5) had the greatest activity. In half of the examined animals a discrete subfraction of LDH_4 was found in the kidneys. The adrenals and the thyroid showed great similarity in LDH isoenzyme patterns, with the highest value for the electrophoretically "slow" components LDH_5 and LDH_4 (LDH_5>LDH_4> LDH_3>LDH_2>LDH_1). The brain structures had lower total activity when compared with the other tissues and organs. The smallest total LDH activity was shown by the cerebellum. However, all examined brain structures showed marked similarity in LDH isoenzyme patterns: the highest activity for LDH_4 and LDH_3 and the smallest activity in LDH_1. Our results agree with earlier findings that different tissues and organs of both animals and humans have characteristic LDH isoenzyme patterns.

Key words: Lactate-dehydrogenase (LDH), rat, cardiac muscle, liver, kidneys, adrenals, thyroid, brain.

INTRODUCTION

Several isoenzymes of lactate-dehydrogenase (LDH) separable by electrophoresis have been identified and characterized in animal tissues (Wroblewski and Gregory, 1961). It has been established that these isoenzymes are tetramers of all possible combinations of M and H subunits (Markert, 1963;

Dimitrijević, 1981). These isoenzymes were found to differ from each other in several properties in a regular manner that could be correlated with their electrophoretic mobilities in starch gel (Plagemann et al., 1960). Isoenzymic patterns of various tissues were studied and tissue specific patterns obtained. It has been suggested that patterns found in certain organs may be associated with particular modes of metabolism (Dawson et al., 1964).

The present investigation was performed in order to examine LDH isoenzyme distribution in various rat tissues.

MATERIAL AND METHODS

The experiments were performed on 20 albino male Wistar rats of mean body weight 255±20 g. The rats were killed by decapitation. Immediately after wards the tissue fragments were removed and cooled to +4°C. The total activity of LDH was estimated by the method of Wroblewski-La Due (1955). The isoenzymic patterns of LDH were determined by the modified method of micro-disc gel electrophoresis according to Ornstein-Davis (1964), Vlajnić et al. (1983) and Dimitrijević (1981). Electrophoregrams were stained by the method of Van der Helm (1962). Relative isoenzymic activity was measured by direct densinometry of the enzymograms. The total activity of enzymes was expressed in Units/g of wet tissue (U/g w.t.) and the activity of individual enzymes as a percentage of total activity.

RESULTS AND DISCUSION

Our results show that lactate dehydrogenase (LDH) a "biomarker" cytoplasmic enzyme catalyzing the reversible reaction of lactate — piruvate, was represent by five electrophoreticaly distinct isoenzymes in all examined tissues except liver. The amounts of each isoenzyme were tissue specific.

Yasuda el al. (1990) used histoelectrophoresis for the direct analysis of LDH and found that LDH isoenzymes of rat tissues could be separated into four fractions.

Table 1. shows the total activity (Unitis/g of wet tissue) and distribution of LDH isoenzymes in the different rat tissues examined here.

The LDH isoenzyme electrophoregrams for various tissues in normal Wistar male rats (1-cardiac muscle, 2-kidneys, 3-liver, 4-thyroid, 5-adrenals) are given in Figure 1.

In our experimental conditions, total LDH activity had the highest value in cardiac muscle and liver, and the smallest value in kidneys. In cardiac muscle the greatest activity was shown by LDH_2, LDH_3 and LDH_1, which are proportionately the richest in LDH-H subunits.

The greatest activity in liver was shown by the electrophoretically "slowest" component LDH_5 (81%). The LDH1 isoenzyme was not detected in any rat and the discrete fraction of LDH_2 was found only in three animals.

Table 1. Total LDH activity and LDH isoenzyme activity in aqueous extracts of various rat tissues

TISSUE	L D H activity					
	Total U/g w.t.	LDH isoenzyme activity – % of total				
		LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
CARDIAC MUSCLE	632±73 (n=20)	22±3 (n=19)	32±4	28±3	14±4	4±2
KIDNEY	193±20 (n=19)	23±6 (n=19)	29±5	19±4	22±9	7±3
ADRENALS	248±96 (n=19)	2±2 (n=12)	10±3	23±10	29±7	35±16
THYROID	346±20 (n=19)	3±2 (n=12)	8±3	17±8	32±7	40±12
LIVER	500±69 (n=15)	— (n=15)	—	5±2	14±5	81±6

Kassner et al. (1988) found that LDH isoenzyme patterns of isolated fat-storing cells from rat liver, was different from that of other non-parenchymal liver cells. Fat-storing cells contain LDH_4 as the main isoenzyme and do not exibit LDH_1, whereas the other non-parenchymal cells had all five LDH isoenzymes, among which LDH_5 dominates.

In the kidneys the greatest activity was shown by LDH_2 isoenezyme. The discrete subfraction of LDH_4 in half of the examined animals was found. The adrenals and the thyroid showed great similarity in LDH isoenzyme patterns, with the highest value in the electrophoretically "slow" components LDH_5 and LDH_4, (Figures 1 and 2).

Table 2. shows the total LDH activity (Units/g wet tissue) and distirbution of LDH isoenzymes in diferent parts of the rat brain. The brain LDH isoenzyme electophorgrams for normal Wistar male rats are given in Figure 3. (M_1-hypothalamus, M_2-cortex cerebri, M_3-Nucleus caudatus, M_4-hippocampus, M_5-cerebellum), (Figure 3 and 4).

Table 2. Total LDH activity and LDH isoenzyme activity in aqueous extracts of diffferent parts of the rat brain

BRAIN	L D H activity					
	Total U/g w.t.	LDH isoenzyme activity – % of total				
		LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
HYPOTHALAMUS	159±10 (n=9)	20±2 (n=10)	20±2	24±5	24±4	11±4
HIPPOCAMPUS	154±7 (n=8)	19±5 (n=12)	21±4	25±6	27±6	8±3
NUCLEUS CAUDATUS	156±1 (n=9)	16±3 (n=10)	21±4	26±4	27±4	9±4
CORTEX CEREBRI	161±13 (n=8)	18±3 (n=11)	22±3	26±5	25±5	9±4
CEREBELLUM	117±9 (n=6)	23±6 (n=5)	24±3	25±5	21±3	7±3

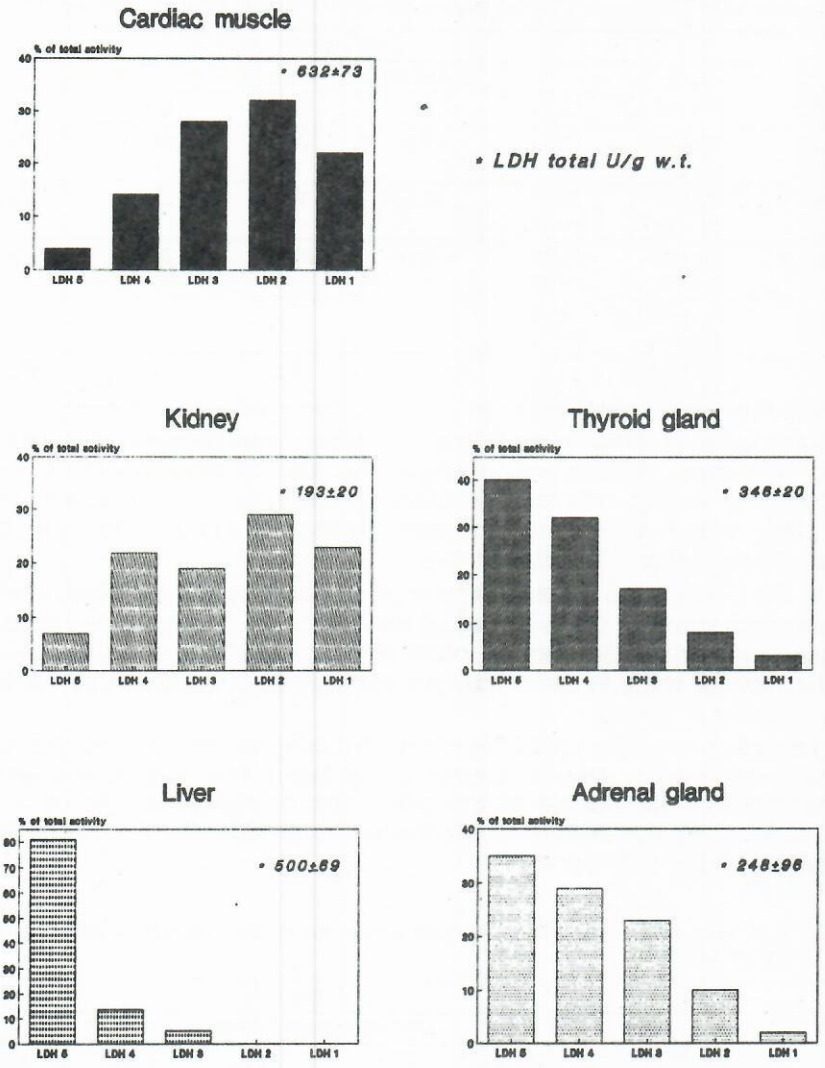

Figure 1. LDH isoenzyme composition in aqueos extracts of various rat tissues

In our investigations the brain structures had lower total LDH activity when compared with the other tissues and organs. The smallest total LDH activity was found in the cerebellum. However, all examined brain structures showed a marked similarity in LDH isoenzyme patterns: the highest activity of LDH_4 and LDH_3 and smallest activity in LDH_5. This could be the consequence of regional capillary density and glucose metabolism.

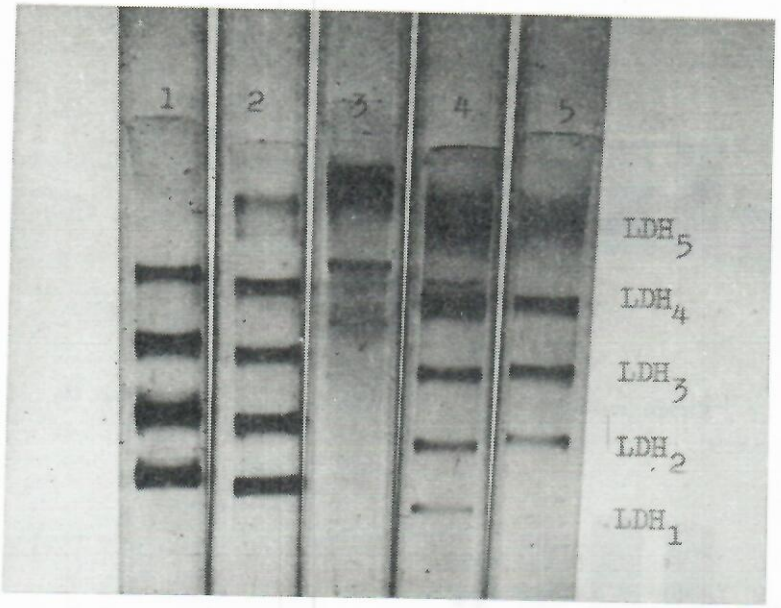

Figure 2. LDH isoenzyme patterns in the cardiac muscle (1), kidneys (2), liver (3), thyroid(4) and adrenals (5) of rats

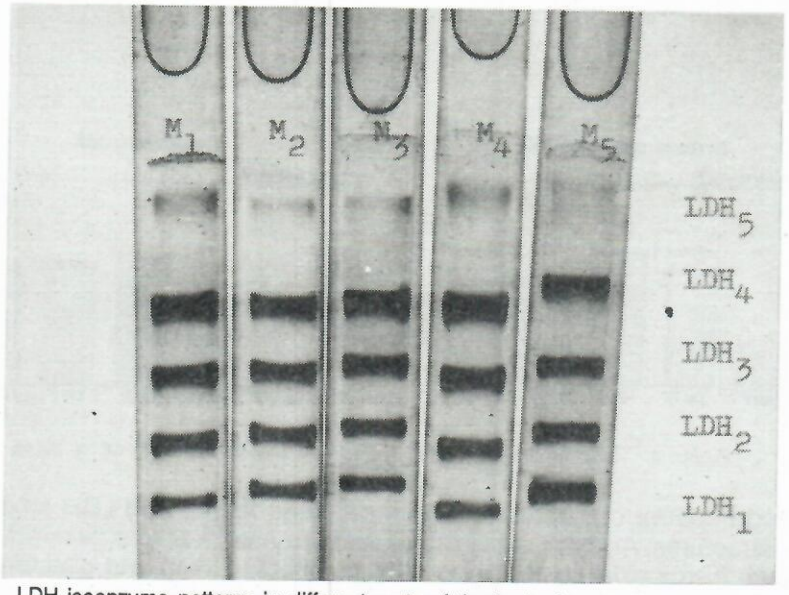

Figure 3. LDH isoenzyme patterns in different parts of the brain: hypothalmus (M_1), cortex cerebri (M_2), Nucleus caudatus(M_3), hipocampus (M_4) and cerebellum (M_5) of rats

Borowsky and Collins (1989) analysed 18 grey and 5 white matter regions and found a positive correlation between capillary density and glucose utilization

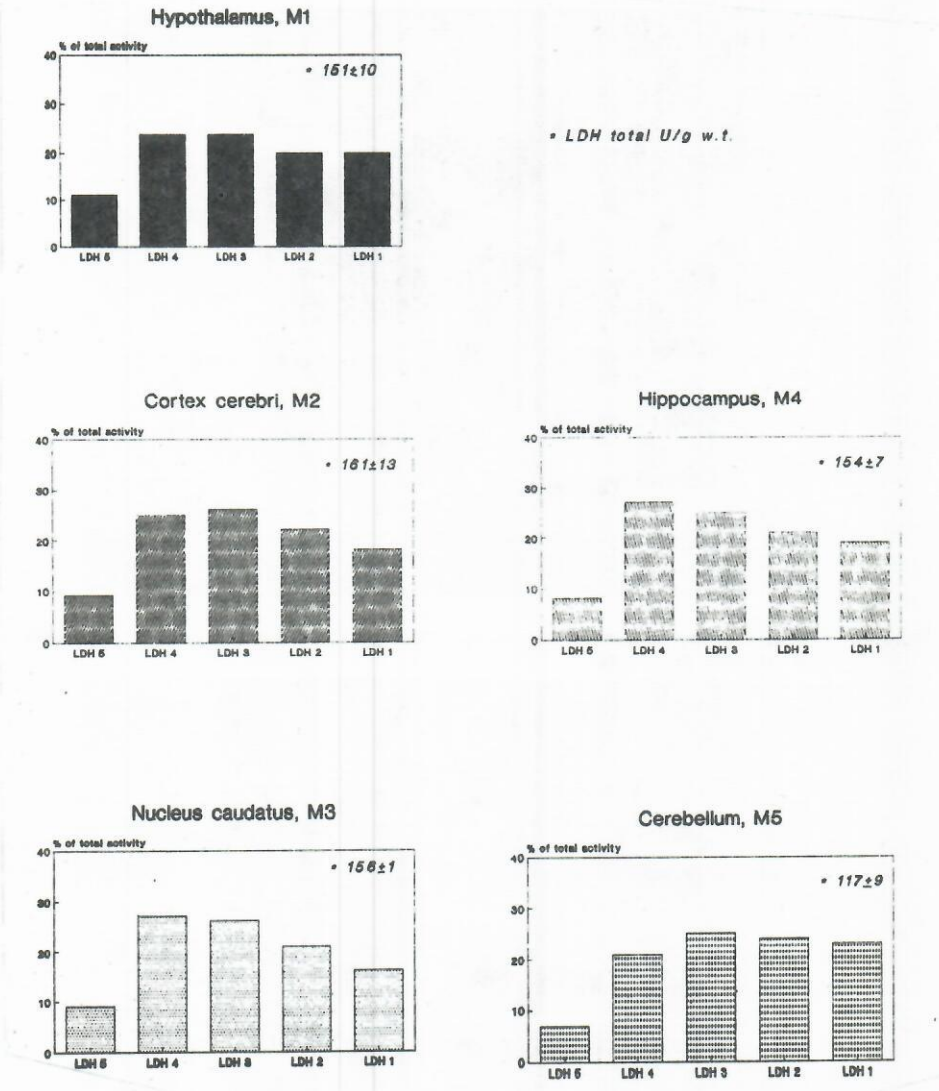

Figure 4. LDH isoenzyme composition in aqueous exstracts of rat brain

rate, and negative correlation between capillary density and LDH among grey matter structures, Analysis of capillaries and enzymes was also performed within laminated histological fields: hippocampus, olfactory bulb and olfactory cortex. In general, this revealed reciprocal patterns of staining for LDH and cytochrome oxidase. Their findings demonstrate distinct distributions of glycolytic and oxidative enzymes within the brain which are at least partly associated with pathway specificity.

Hrachovina and Mourek (1990) investigated the activity of LDH in the neuronal and glial fraction of the cerebral cortex in Wistar strain rats of both sexes aged 10 days and in adult rats (aged 90 days). They found that during maturation the LDH activity declined in the neuronal fraction in a significant way. The LDH activity in the neuronal fraction was always significantly higher than in the glial fractin. The G/N index declines slightly during development (from 0,3 to 0,23).

Assays of the isoenzyme composition of homogenates of normal rat tissues indicated that each tissue (except liver) contained from one to five isoenzymes in characteristic proportions. No two rat tissues examined appeared to have exactly the same LDH isoenzyme patterns.

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Nevena Dimitrijević et. al.: Total activity and isoenzymic patterns of Lactate — dehydrogenase (LDH) in various rat tissues

UKUPNA AKTIVNOST I IZOENZIMSKI PROFIL LAKTAT— DEHIDROGENAZE (LDH) U RAZLIČITIM TKIVIMA PACOVA

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SADRŽAJ

Korišćenjem mikro-disk gel elektroforeze određivan je enzimski profil Laktat-dehidrogeneze (LDH) u raznim tkivima (srčani mišić, bubrezi, nadbubrezi, tireoidea, jetra) i različitim delovima mozga (hypotalmus, hippocampus, Nucleus caudatus, cortex cerebri, cerebellum) u albino pacova, mužjaka, Wistar soja. Ukupna aktivnost LDH najveća je u srčanom mišiću i jetri, a najmanja u bubrezima. U srčanom mišiću najveću aktivnost ima izoenzim LDH_2 (LDH_2>LDH_3>LDH_1>LDH_4>LDH_5). U jetri najveću aktivnost pokazuje elektroforetski "najsporija" frakcija, LDH5. U bubrezima najveću aktivnost ima izoenzim LDH_2 (LDH_2>LDH_1>LDH_4>LDH_3>LDH_5). Kod polovine ispitivanih životinja nađena je diskretna subfrakcija LDH_4 u bubrezima. Nadbubrezi i tireoidea pokazuju veliku sličnost u izoenzimskom profilu LDH, sa najvećom aktivnošću elektroforetski "sporih" izoenzima LDH_5 i LDH_4 (LDH_5>LDH_4>LDH_3>LDH_2>LDH_1). Moždane strukture imaju znatno nižu ukupnu aktivnost LDH odnosu na druga tkiva i organe. Najmanju ukupnu aktivnost ima cerebellum. Međutim, sve ispitivane moždane strukture pokazuju veliku sličnost u izoenzimskom profilu LDH: najveću aktivnost izoenzima LDH_4 i LDH_3, a najmanju aktivnost izoenzima LDH_5. Naši rezultati se slažu sa ranije opisanom činjenicom da različlta tkiva i organi i životinja i ljudi imaju karakteristične izoenzimske profile LDH.