CAPABILITIES OF APPLICATION OF OXIDOREDUCTASES IN ENZYME THERAPY

F. N. Gylmiarova, V. M. Radomskaya, G. M. Baisheva, and M. S. Kleiman

We clarified organs—target evaluated the metabolic effect of exogenic, isotope-marked lactate dehydrogenase (LDH) in conditions *in vivo* in an organism animal-recipient was evaluated. Isotope-marked enzymes received by a method of hyperthermal solid-phase of a catalytic isotopic exchange with gaseous tritium. The conducted researches have shown, that the exogenic dehydrogenases call the metabolic answer, being stored predominantly in blood and tissues on stretch of two day. The changes in proteinaceous—lipide and carbohydrate metabolism emerge at miscellaneous levels of structural organization of a liver, cardiac and sceletal muscles, a brain and have a specific character for LDH. The availability of the metabolic answer, specifity of exchange shifts and distributions, gated in testifies to a perspective of analysis of possibility of using of oxidoreductases like a means in enzyme therapy.

The role of dehydrogenases in the cell-oxidizing metabolism was enlarged by the facts proving its participation in the molecular processes, formed not only by the redox-pairs and reoxidized nicotinamidum coenzymes, but also due to the interaction of the fermental proteins with other bio-molecules.

The aim of this research is to clear out the distribution of the exogenous lactate dehydrogenase, i.ė., the topography of the enzyme input. We also want to measure the total activity of lactate dehydrogenase in the target-organs having indirectly proved the viability of the exogenous lactate dehydrogenase in the dynamics, to trace the metabolic response to the entrance of dehydrogenases in the respective tissues and organs. This aim is achieved by visualization of the lactate dehydrogenase in the endogenous environment, i. e., in the organism of the recipient animal. Radioactive marking is used for this purpose. In spite of the fact that "the marked atoms method" is one of the well-known methods in the protein chemistry, the main difficulty was caused by the necessity of preserving the enzyme's native features and activity during the process of radioactive marking.

Methods

Lactate dehydrogenase was obtained out of the mink's skeleton-muscles. The method of R.K. Scopes and A. Stoter was taken as the foundation of the enzyme isolation method. The muscle tissue was washed out of blood, released of connective tissue and grease and reduced to homogeneous mess. The extraction of soluble proteins was carried out using the cooled 0.03 N solution KOH (1 : 1), containing 1 mM EDTA 0.02 mM DTT. We centrifuged at 1500–2000 g for 40 minutes. Stepped saturation of the extract by the ammonium sulphate and heat processing at 58 °C contributed to the precipitation of the ballast proteins. The lactate dehydrogenase rectification was carried out according to the gel-filtration method using Toyopearl HW-40. Spectrophotometrically defined the protein concentration and the lactate dehydrogenase specific activity. The determination of the specific activity of malate dehydrogenase and activity and α-glycerophosphate dehydrogenases was carried out spectrophotometrically [1].

Isotope-marked enzyme was obtained by the method of hyperthermal solid - phase of catalytic exchange with gasiform tritium [2]. The activity of the samples obtained was controlled spectrophotometrically and by the liquid styntilator. Gaseous tritium is sorbed on the catalytic metal of the platinum group and its excess replaces hydrogen in the enzyme, which in such conditions is a organic component. During this all a radioactive-marked protein is formed. The tritium-marked enzyme was put into the rat's tail vein (dose 7.5 μCi per 1 kg). The biological effect was measured in the head, brain, heart, skeleton-muscles, liver, kidney, and spleen in 1 h, 24 h, and 48 h after the enzyme input. The change of the enzyme's quantity was counted proportionally to the change of the rate of radioactivity in comparison with the its starting level. The resulting activity was recounted per 1 g of tissue. Current and concluding control showed that the procedure of the enzyme marking input is entailed with the reduce of activity (4–8% in comparison with the starting) data.

The metabolic concentration was studied spectrophotometrically using the fermentative method in the deproteinized tissue extracts [3].

Results and Discussion

As is well-known lactate dehydrogenase is the final ferment of the process. By providing reversible oxidizing of the lactic, and piryvic's reduction in presence of NAD, the enzyme fulfills an important function of reoxidizing of cytoplaszmatic NADH, making its contribution into maintenance of optimum balance in the system of oxidized and reduced forms of the NAD coenzymes, which is the key regulator of the cell metabolic processes.

Samara State Medical University, Department of biological and clinical chemistry, fax 846-2) 332-976, e-mail: kleiman@smr.ru.

The input of the isotope-marked lactate dehydrogenase showed that only about 95% of the enzyme input is traced in the animal organism (Fig. 1). The marking can be found in all the tissues and organs including the brain. The tritium marked lactate dehydrogenase can be traced in the organism within 2 days.

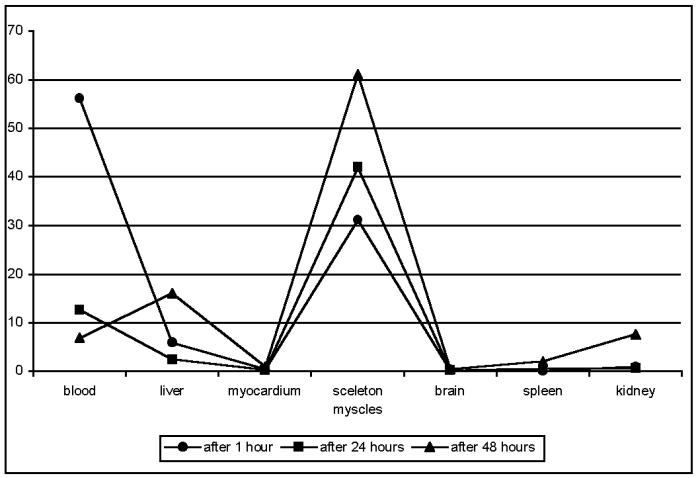

Fig. 1. Changing of the distribution of the tritium-marked lactatdehydrogenase in the organs and tissues after the intravenously injection.

The isotope marking is to great extend concentrated and kept in the circulating blood. The research showed that within 1 h after the marked ferment input its share in the blood reaches 56.2%. The isotope marking penetration rate into the skeleton muscles is 31.2% per hour. About 5.9% of the marking can be found in the liver; 1%—in the kidney; 0.4—myocardium; 0.2—brain; 0.1—spleen. In a day the quantity of the isotope marking is 7.2% less then in an hour after the input. The isotope marking quantity in blood decreases to 12.6% of the total quantity of lactate dehydrogenase in the rat's organism. The increased concentration of the ferment is found in the skeleton muscle tissues and in the spleen, where we find 42% (+34.6%) and 0.5 (5 times). In the liver and kidney the concentration reduces to 2.4 and 0.6%. Practically no changes occur in the concentration in the brain and myocardium. In 2 days the tritium marked lactate dehydrogenase concentration in the rat's organism is reduced by 20.6%. Now its concentration in the blood is 6.9% but the concentration in all other tissues and organs increases: 61.1%—skeleton muscles, 16%—liver, 7.6%—kidney, 2%—spleen, 3 times increased concentration in the myocardium and 2 times in the brain.

The tritium marked lactate dehydrogenase input causes the changing in the activity of endogenous malate dehydrogenase (Table 1). The malate dehydrogenase activity in the blood reduces from 0.023 E/mg to 0.014 E/mg in an hour. In 24 h practically reaches the starting level—0.022 and in 48 h remains practically unchanged.

The activation of the fermentative process is traced in the liver. The process is catalyzed by malate dehydrogenase: in an hour for 80.3% ($p < 0.001$), in 24 hours—for 82.6% ($p < 0.001$) which is in average 2.859 ± 0.117 and 2.896 ± 0.102 E/mg accordingly. In 48 h the activity of the given enzyme returns to the starting level. The same changes can be traced in the brain tissue. The malate dehydrogenase activity increases for 52.6% in an hour, for 104.7% in 24 h, and in 48 h the activity reduces to the starting level. In the myocardium the activation of malate dehydrogenase occurs in an hour—for 74.1% ($p < 0.01$) which further on is substituted by the lasting reduce of the activity in the next 2 days for 12 and 11.7% accordingly. The reduce of malate dehydrogenase activity is traced in the skeleton muscles as well. Starting with the rate of this fermentative process at about 3.091 ± 0.173 E/mg, in an hour we go to -27.5 and in 24 h to -27.9% in comparison

Table 1

Changing of the activity of malatdehydrogenase in various rat's organs and tissues after the intravenously injection of the tritium-marked lactatdehydrogenase

Organs and tissues	Start	After 1 h	After 24 h	After 48 h
Blood ($M \pm m$)	0.023 ± 0.001	0.014 ± 0.001	0.022 ± 0.001	0.021 ± 0.001
Liver ($M \pm m$)	1.568 ± 0.081	2.859 ± 0.117	2.896 ± 0.102	1.568 ± 0.094
Brain ($M \pm m$)	0.447 ± 0.014	0.682 ± 0.019	0.915 ± 0.023	0.484 ± 0.016
Myocardium ($M \pm m$)	2.771 ± 0.176	4.825 ± 0.325	2.438 ± 0.236	2.448 ± 0.210
Skeleton muscles ($M \pm m$)	3.091 ± 0.173	2.241 ± 0.154	2.229 ± 0.144	3.004 ± 0.195
Kidney ($M \pm m$)	0.954 ± 0.029	0.692 ± 0.027	0.998 ± 0.034	1.006 ± 0.039
Spleen ($M \pm m$)	0.381 ± 0.023	0.854 ± 0.012	0.410 ± 0.018	0.327 ± 0.018

Table 2

Changing of the activity of α-glycerophosphatedehydrogenases in various rat's organs and tissues after the intravenously injection of the tritium-marked lactatdehydrogenase

Organs and tissues	Start	After 1 hour	After 24 hours	After 48 hours
Blood ($M \pm m$)	0.018 ± 0.002	0.026 ± 0.002	0.025 ± 0.002	0.021 ± 0.002
Liver ($M \pm m$)	0.301 ± 0.065	0.332 ± 0.020	0.300 ± 0.009	0.317 ± 0.015
Brain ($M \pm m$)	0.279 ± 0.010	0.171 ± 0.012	0.280 ± 0.014	0.286 ± 0.011
Myocardium ($M \pm m$)	0.190 ± 0.011	0.454 ± 0.017	0.405 ± 0.021	0.345 ± 0.017
Skeleton muscles ($M \pm m$)	0.334 ± 0.022	0.543 ± 0.026	0.419 ± 0.025	0.309 ± 0.015
Kidney ($M \pm m$)	0.301 ± 0.015	0.142 ± 0.007	0.165 ± 0.009	0.294 ± 0.010
Spleen ($M \pm m$)	0.363 ± 0.014	0.526 ± 0.019	0.396 ± 0.013	0.315 ± 0.016

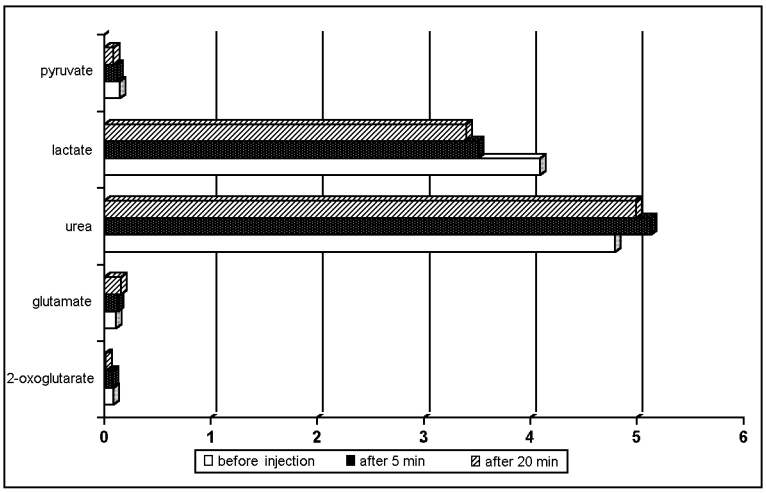

Fig. 2. The dynamics of the concentration of the lactate, pyruvate, urea, glutamate, and 2-oxoglutarate in the animals blood after the intravenously injection of the tritium-marked lactatdehydrogenase.

with the starting data. In 48 hours the ferment activity is practically at the starting level 3.004 ± 0.195 E/mg. The marked lactate dehydrogenase input causes short-time decrease of the malate dehydrogenase activity in the bud tissue (in an hour for 27.5%, $p < 0.01$) and then—its increase in 24 and 48 h to 0.998 ± 0.34 and 1.006 ± 0.039 E/mg. Drastic increase in the malate dehydrogenase activity in 1 h after the input was traced in the spleen (+137.3%). But to the end of the first day it decreases practically down to the starting level 0.410 ± 0.018 E/mg (+76%) and in 2 days this index is 14.2% ($p < 0.05$) lower then the starting level (0.327 ± 0.018 E/mg and 0.381 ± 0.023 E/mg accordingly).

The study of the impact of the isotope marked lactate dehydrogenase on the α-GPD in various tissues showed that its activity in the blood increases for 44.4% in an hour ($p < 0.001$) and in 24 h for 39.9% ($p < 0.001$) and in 48 h for 16.7 (Table 2).

Before the ferment input the activity of α-glycerophosphate dehydrogenases equals 0.018 ± 0.002 E/mg. The changes in the liver are not sufficient and are of the short-time nature. The short time reduce of the α-glycerophosphate dehydrogenases activity was traced in the brain tissue: from 0.279 ± 0.011 to 0.171 ± 0.012 E/mg (−38.7%). In the next measurements in 24 and 48 h no declinations in the brain tissue have been traced. Steady increase of the activity is traced in the myocardium where it increases in an hour from 0.190 ± 0.011 to 0.454 ± 0.117 E/mg; in 24 h—0.405 ± 0.021 E/mg ($p < 0.01$) and even in 48 h for 81.6% ($p < 0.001$), resulting in 0.345 ± 0.017 E/mg.

In the skeleton muscle the speed of the glycerol-3-phosphate formation increases in the first hour of the experiment for 62.6% ($p < 0.001$), in 24 h for 24.9%. At the end of the second day some decrease of the α-glycerophosphate dehydrogenases for 7.5% is marked in comparison with the starting data. In kidney the speed of the fermentative reaction, catalyzed by α-glycerophosphate dehydrogenases decreases after the input of the lactate dehydrogenase during the whole time of the experiment: −52.8% in an hour, −45.2% in 24 h, −2.3% in 48 h. In the spleen the activity of α-glycerophosphate dehydrogenases is increased during the 1st day (+44.9%, $p < 0.001$—1 h; +9.1%, $p < 0.01$—in 24 h). In 48 h the decrease of the speed of the formation of α-glycerophosphate dehydrogenases for 13.2% is traced.

The ferments studied here are the ferments of the multifunctional importance that deal with providing the energy exchange in every cell. Intermediates of these dehydrogenases are the products of the catabolic and anabolic transmutations of the proteins, greases and carbohydrates. They by this define the vital activity of the cells, organs and the whole organism.

As many illnesses are the result of the disturbance of some exchange processes the necessity of the search of the means of correction of the disturbed metabolism looks to be pretty vital [4, 5].

Dhydrogenases provide the transformation of the hydrogen — the main source of ATP in the living systems. As known the tissue hypoxia takes place in the pathophysiological scheme of many illnesses [6]. This is entailed by the collection of the metabolites during the overall energetic deficit. This opens the perspectives of the dehydrogenases usage in the pathogenetic therapy of a great variety of illnesses, characterized by the hypoxia syndrome.

The input of lactate dehydrogenase is entailed by the changing in the concentration of lactic and pyruvate which is quite understandable taking into consideration the replenishment of the endogenous reserves of lactate dehydrogenase. However, changes are traced in other reactions not connected with lactate dehydrogenase and in the non connected systems (Fig. 2). Thus glutamate in 20 min after the lactate dehydrogenase input increases and considerablt exceeds the norm for 49%. The rate of 2-oxoglutarate is also above the control indexes. The orientation of the process is such that the correlation between the quantity of the 2-oxoglutarate and the glutamate tends to decrease within 24 h. In a day the quantity of the urea increases which proves the intensity of the nitrogen exchange. In the first day the quantity of the urea remains within the normal rates. The transamination process is intensified which results in the increase of the activity of as ALAT so as of ASAT. These changes are clearly traced during the first 20 min of the observation and by the end of the day practically return to the normal indexes.

The results of the observations where dehydrogenases

were used show that the exogenous ferments penetrate into the tissues, concentrate within them in the regime which is specific for every ferment and provide active impact on the metabolism, including not only the changes in the redox–system formed by its own substrate specific but also a wide range of the carbohydrate–protein exchange.

Conclusions

1. Clear peculiarities of the dynamics of distribution of lactate dehydrogenase in the tissues and organs of the recipient organism have been discovered. It has been determined that the tritium marked lactate dehydrogenase firs concentrated in the blood in 48 h rapidly decreases and in 2 days after the input concentrates mostly in the liver, kidney, spleen, myocardium, skeleton muscles and in the brain.

2. Changes in the carbohydrate-protein exchange are discovered at different levels of the structural organization of the liver, heart and skeleton muscles and brain. Their nature is rather specific for lactate dehydrogenase

3. The fact of the overcome of hematoencephalic barrier has been traced. This results in the increase of the concentration of the marked lactate dehydrogenase of this tissue.

4. The study of the impact of dehydrogenases allows to regard them as a new generation of the biogenetic means of correction of the metabolism and shows the perspectivity of the studying of the possibility of using the oxidoreductases in enzyme-therapy means.

References

1. Ochoa. S (1955) *Meth. Enzymol.*, **1**, 735–739.
2. Zolotarev Yu.A., Zaitsev D.A., and Tatur V.Yu. (1991) *Patent USA* No. 5026909.
3. Gylmiarova F.N. and Radomskaya V.M. (1997) *Comprehension of the essence. An ecology, ecopathology, Natyrsilum.*, 401.
4. Shuster J.J. and McMilliams N.B. (1994) *Am. J. Clin. Oncol.*, **15**, 295–303.
5. Moss D.W. (1994) *Clin. Chem. Acta.*, **255**, 5–11.
6. Singh S.K. and Hasan S.A. (1993), *Indian. J. Physiol. Pharmacol.*, **37**, 93–94.