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Glycolytic Enzymes in Polyamine-Treated Bovine Retina

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Summary

The retina is characterized by glycolysis under aerobic conditions, mediated by lactate dehydrogenase isoenzyme-5 (LDH-5) as well as by the soluble isoenzyme of malate dehydrogenase. Bovine retina LDH and MDH isoenzymes and their activities were studied after polyamine treatment. Our results showed that LDH-5 isoenzyme presented the highest activity in untreated as well as in putrescine-treated retina. Decreased activity was present when the retina was treated with spermidine or spermine. It was demonstrated that retinic LDH-5 had a high affinity for lactate which enabled the isoenzyme to be more effective than the other LDH isoenzymes in the conversion of NADH to NAD. Therefore, the putrescine enhancing LDH-5 activity appeared to be capable of stimulating NAD-mediated rhodopsin regeneration. Putrescine induced a marked increase of both MDH isoenzymes – soluble (s-MDH) and mitochondrial (m-MDH), while spermine and spermidine mostly affected the soluble form of the enzyme. Putrescine induced a three-fold increase in s-MDH and m-MDH activities, while spermine and spermidine induced a four to five-fold increase in s-MDH. These results document the differential effects of polyamine treatment on LDH and MDH isoenzyme activities.

Key words

LDH isoenzymes • MDH isoenzymes • Polyamines • Retina

Introduction

The retina is characterized by its glycoside metabolism similar to that observed in neoplastic cells, since a high consumption of glucose with a high production of lactate occurs under aerobic conditions (Saavedra and Anderson 1983, Saavedra *et al.* 1985). Aerobic glycolysis in tumor cells is mediated by a lactic acid dehydrogenase (L-lactate: NAD^+ oxidoreductase, E.C.1.1.1.27), isoenzyme 5 (LDH-5) consisting of four M subunits and expressed in muscle cells after birth, as well as by malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.11.37) soluble isoenzyme (s-MDH). Retina cells also show an elevated expression of LDH-5, with respect to the other four LDH isoenzymes, as was previously demonstrated (Hochachka and Somero 1984). The similar LDH isoenzyme pattern of retina and tumor cells accounts for the aerobic requirement of a given tissue (De Almeida-Val and Val 1993), independently of its malignancy. Furthermore, retina cells also express the MDH enzyme that catalyzes the reversible oxidation of malate to oxalacetate. Its isoenzymes are represented by two major forms in vertebrates, namely s-MDH and mitochondrial m-MDH (Banaszak and Bradshaw 1975). On the other hand, it is well known that transformed cells are characterized by an ornithine decarboxylase (ODC) activity increase of 50-100 % relative to control non-malignant cells, as well

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as enhanced polyamine synthesis, which are responsible for the high rate of proliferation. In connection with the above results and the polyamine regulatory role on cellular metabolism (Dawson *et al.* 1964) and enzyme activities (Raina and Janne 1975, Heby 1981), we studied the characteristics of LDH and MDH isoenzymes in bovine retina in relation to polyamine treatment.

Materials and Methods

Source

Bovine eyes, obtained from the local slaughter-house, were immediately enucleated and dissected. Some retinas were homogenized in a Potter-Elvehjem homogenizer using an appropriate medium (tris-HCl buffer 100 mM, pH 7.0) and centrifuged at 15 000 rpm (30 min, 4 °C). The supernatant obtained was submitted to disc-electrophoresis and enzyme activity assay.

Other retinas were placed in a Petri dish (two retinas in each) with putrescine (P), spermine (SP) and spermidine (SPD) solution.

Polyamine treatment

Retina treatment with polyamines was performed separately using putrescine, spermidine or spermine dissolved in saline at 3.3 x 10^{-5} M concentration for 60 min at 37 °C.

At the end of the incubation time, the retinas were removed and then washed several times with sterile saline, placed in 100 mM tris-HCl buffer (pH 7.0) homogenized and then centrifuged at 15 000 rpm (30 min, 4 °C). At the end of this procedure, samples were submitted to enzyme assay and disc-electrophoresis.

Spectrophotometric assay

LDH activity was spectrophotometrically assayed by measuring the changes in NADH adsorbancy at 340 nm/min, using a Perkin-Elmer DU70 spectrometer. The LDH assay was carried out using 0.1 M lactic acid and 7.0 mM NAD. All reactions were performed in triplicate and they were always started with the addition of small amounts of extracts to the reaction mixture. LDH values were expressed as IU/g wet weight of tissue.

MDH assay was carried out in 0.1 M potassium phosphate buffer (pH 7.0) containing 70 mM malic acid and 3.0 mM NAD.

Disc-electrophoresis

LDH was analyzed by 7 % polyacrylamide disc gel electrophoresis (PAGE). As soon as the electro-phoresis was accomplished, the gel was removed, stained and incubated at 37 °C in the dark according to the method of Ferris and Whitt (1979). The LDH staining solution contained 0.125 M lactic acid, 0.125 nM NAD, 0.075 mM nitroblue tetrazolium (NBT) and 0.05 mM phenazime-metha-sulphate (PMS) in 0.1 M phosphate buffer (pH 7.0). The reaction was stopped by washing in distilled water and the gel was fixed in 7 % acetic acid.

The MDH staining solution contained 25 mM malic acid, 0.25 mM NAD, 0.075 mM NBT and 0.05 mM PMS in 100 mM phosphate buffer (pH 7.0). The reaction was stopped by washing in distilled water and the gel was fixed in 7 % acetic acid.

Table 1. Protein content and LDH total activity variations in polyamine-treated bovine retina.

	Proteins mg/g w.w.	**LDH IU/g w.w.**	**% LDH increase**	**Specific activity**
R	65.12±3.22	8.53±0.51	–	0.13
Rput	75.11±4.11	12.56±0.92	+42.6*	0.16
Rsp	75.79±3.95	11.46±0.70	+34.3**	0.15
Rspd	76.80±4.41	10.51±0.63	+23.2**	0.13

*Data are mean values ± S.D. of eight separate samples. R – control retina; Rput – retina treated with putrescine; Rsp – retina treated with spermine; Rspd – retina treated with spermidine. Significantly different from control retina: * $P<0.001$, ** $P<0.01$.*

Protein determination

The protein content was determined with Bio-Rad protein Kit by the method of Bradford (1976) using purified bovine serum albumin as standard. Results were expressed as mg/g wet weight of tissue.

Statistical analysis

The statistical significance of these differences, which was assessed by Student's t-test, was considered to be significant at $p<0.05$.

Results

The protein content and LDH total activity in polyamine-treated and untreated retina are shown in Table 1. Both proteins and LDH activity were increased by the incubation with putrescine, spermine and spermidine. Putrescine affected LDH activity (+42.6 %) more than the protein content when compared to the other polyamines, whereas spermidine was more effective in increasing the protein content.

The effects of polyamine treatment on LDH isoenzymes are summarized in Table 2. LDH-5 isoenzyme presented the highest activity in the untreated and putrescine-treated retina, while its activity decreased after spermidine or spermine treatment. LDH-4 and LDH-3 activity was not significantly altered when the retina was incubated with any of the three polyamines. LDH-2 activity was enhanced when the retina was treated with spermine and spermidine. On the other hand, LDH-1 activity was increased by all the polyamines studied, particularly by spermine. Table 2 also shows the percentage variations of LDH isoenzyme activities after incubation with putrescine, spermine and spermidine, relative to their activity in untreated retinas. It is evident that the highest variations occurred in LDH-1 isoenzyme activity, especially after spermine and spermidine treatment, and in the LDH-5 isoenzyme. The latter was differentially modulated by the three polyamines studied. In fact, putrescine enhanced its activity, while the other two polyamines decreased it with a variable intensity. The other isoenzymes showed a slight increase in their activity, except LDH-2, whose increment with spermine was more marked.

Table 2. LDH isoenzyme activities and their percent variations in bovine retina treated with polyamines.

Isoenzymes	R IU/g w.w.	R*put* IU/g w.w.	% isoenzyme variations	R*sp* IU/g w.w.	% isoenzyme variations	R*spd* IU/g w.w.	% isoenzyme variations
LDH-5	3.05±0.13	5.21±0.26	+70.8*	2.76±0.07	–9.5	1.90±0.06	–37.7**
LDH-4	1.46±0.06	1.67±0.05	+14.3	1.48±0.04	---	1.83±0.05	+25.3**
LDH-3	2.15±0.08	2.57±0.09	+19.5	2.44±0.09	+13.5	2.72+0.09	+26.5**
LDH-2	1.25±0.05	1.59±0.04	+27.2**	2.16±0.08	+72.8*	1.91+0.06	+52.8*
LDH-1	0.62±0.02	1.11±0.03	+79.0*	2.57±0.06	+314.3*	2.14+0.08	+245.2*

*R – control retina; Rput – retina treated with putrescine; Rsp – retina treated with spermine; Rspd – retina treated with spermidine. LDH isoenzyme activities are expressed in percent variations of the correspondent isoenzyme activities in control retina. Significantly different from control retina: * $P<0.001$, ** $P<0.01$.*

The LDH isoenzyme patterns obtained by electrophoretic analysis are given in Table 3. It has been shown that the retina treatment with various polyamines affected the activities of all LDH isoenzymes. Table 4 presents the variations of two MDH isoenzyme in bovine retina following putrescine, spermine and spermidine incubation. All the polyamines studied significantly increased the activity of both isoenzymes, although spermine and spermidine mostly affected the soluble form of the enzyme. The percentage variations of MDH isoenzyme activities in polyamine-treated retinas indicated that putrescine induced a three-fold increase in s-MDH and m-MDH activities, and that spermine and spermidine induced a four- to five-fold increase in

s-MDH and almost one and a half-fold increase in m-MDH. It is clear that both s-MDH and m-MDH were enhanced by putrescine. A greater activity of the soluble isoenzyme was evident when the retina was incubated with spermine and spermidine.

Table 3. LDH isoenzyme pattern in untreated bovine retina and after polyamine treatment.

Isoenzymes	R	R*put*	R*sp*	R*spd*
LDH-5	7.32±0.15	9.14±0.23	22.43±1.24	20.37±1.78
LDH-4	17.07±0.86	13.71±0.82	12.90±0.77	17.43±0.97
LDH-3	25.20±1.13	21.14±0.95	21.66±1.58	25.87±1.87
LDH-2	14.63±0.64	13.14±0.72	18.89±0.97	18.23±0.84
LDH-1	35.77±2.17	42.86±2.23	24.12±1.73	18.10±0.75

R – control retina; Rput – retina treated with putrescine; Rsp – retina treated with spermine; Rspd – retina treated with spermidine. Values are expressed as percentage of total LDH activity.

Table 4. MDH isoenzyme activities and their percent variations in bovine retina and after polyamine treatment.

	m-MDH IU/g w.w.	% MDH variations	s-MDH IU/g w.w.	% MDH variations
R	1.30±0.07	–	2.46±0.13	–
Rput	3.93±0.15	+302.3*	7.38±0.42	+300.0*
Rsp	1.95±0.05	+150.0*	9.84±0.55	+400.0*
Rspd	2.16±0.08	+166.1*	11.07±0.73	+450.0*

*R – control retina; Rput – retina treated with putrescine; Rsp – retina treated with spermine; Rspd – retina treated with spermidine. MDH isoenzyme activities are expressed in percent variations of the correspondent isoenzyme activities in non-treated retina. Values reported are related to the total MDH activity. Significantly different from control retina: * $P<0.001$.*

Discussion

It is difficult to propose an unequivocal interpretation of the mechanism by which polyamines influence cellular metabolism. However, it is possible that they act by modifying the functional properties of the cell membrane and/or controlling gene activities (Canellakis *et al.* 1989). When exogenous polyamines are added to the cell culture, their growth promoting ability might really be considered an important step in controlling cellular homeostasis and the genetic activity affecting important gene derepression mechanisms (Russell *et al.* 1976). Our report shows that all polyamines influence either total LDH and MDH enzyme activities, the values of which in non-incubated retina were 8.53±0.51 and 3.78±0.42 IU/g w.w., respectively, or the isoenzymes with different potencies. These effects are only partially related to the their well-known ability to activate DNA and RNA synthesis, which in turn leads to enhanced protein synthesis (Canellakis *et al.* 1989, Bachrach 1973, Russell and Durie 1978, Raina and Cohen 1966). In fact, the LDH and MDH increase is mainly due to a specific action of polyamines on these enzymes, since they exerted a preferential influence on some LDH and MDH isoenzymes. In particular, the present results indicate that all the polyamines increase

LDH-1 activity to different extent, while there are differential effects on LDH-5 activity. This isoenzyme activity is decreased by spermine and spermidine and is enhanced by putrescine. The specific isoenzyme activation by the polyamines supports a significant change in the functional role of LDH which is of particular interest. Several years ago, Whitt and Booth (1970) suggested that LDH-5 played an important role in the regeneration of rhodopsin in photoreceptor cells. As is well known, the oxidation of vitamin A to retinene catalyzed by retinol dehydrogenase (Kohen and Shaw 1976) is a limiting process in rhodopsin generation, and is dependent upon coenzymes produced by retinal respiration and glycolysis. NAD acts at the level of the retinol dehydrogenase reaction to provide rhodopsin synthesis. Whitt and Booth (1970) demonstrated that retinic LDH-5 has a high affinity for lactate which would enable this isoenzyme to be more effective than the other LDH isoenzymes in the conversion of NADH to NAD. Thus, LDH-5 is a unique isoenzyme differentially modulated by polyamines, i.e. enhanced by putrescine and inhibited by spermine and spermidine. It is therefore conceivable that polyamines act in a diversified manner on rhodopsin regeneration. Moreover, the striking spermine- and spermidine-induced activation of LDH-1 isoenzyme, may indicate that these polyamines play a modulating role in lactate dehydrogenase activity. Interestingly, LDH-5 and LDH-1, that correspond to the "pure" M form and to the "pure" H form, respectively, are encoded by separate loci.

On the other hand, the existence of several forms of MDH isoenzymes constitutes a qualitative strategy of biochemical adaptation which permits to adapt the metabolism to variations in extra- and intracellular environments. Retina is more sensitive to this phenomenon and exhibits more rapid glucose oxidation, compared to other tissues (including the brain tissue).

Our results have demonstrated that in adult bovine retina all the polyamines induce a remarkable increase of the MDH cytoplasmic form (s-MDH), while the mitochondrial isoenzyme showed a greater response to putrescine as compared to the effects induced by spermine and spermidine.

It can therefore be postulated that, besides the enhancing effect of all the three polyamines on both MDH isoenzymes, m-MDH is more affected than s-MDH, since there is a considerable difference between the effect of putrescine (+302 %) and of the other two polyamines (+156 % and +166 %) on this isoenzyme. These data indicate a more significant and specific role of putrescine on oxalacetate synthesis and Krebs' cycle functioning, because m-MDH is specifically oriented to oxalacetate production, a metabolite whose concentration is a critical factor in Krebs' cycle regulation (Friedrich *et al.* 1988). Our data are in agreement with those of Toninello *et al.* (1985, 1988) showing that spermine is able to increase m-MDH activity significantly. The energizing effect of spermine, which is able to enter the mitochondrial matrix space simultaneously with inorganic phosphate (Toninello *et al.* 1985, 1988), may be related to the effect of this polyamine on m-MDH activity as demonstrated in the present paper. The almost uniform increase of s-MDH activity in response to the three polyamines treatment accounts, on the contrary, for a generic enhancing role of polyamines in pyruvate metabolism, since s-MDH affects glycogenesis and lipogenesis during aerobic glycolysis (Coppes *et al.* 1987). Moreover, s-MDH has a low substrate specificity, being able to reduce both aromatic and aliphatic alpha-ketoacids, as well as oxaloacetate, phenylpyruvate and pyruvate (Dordal *et al.* 1990). The demonstrated equal enhancing effect of putrescine on m-MDH and s-MDH as well as on LDH-5 and LDH-1 isoenzymes, indicates that this polyamine activates both aerobic and anaerobic metabolism in the retina. On the contrary, spermidine seems to exert a specific effect on anaerobic metabolism, because of the significant increase of LDH-1 and s-MDH isoenzyme activities. Further studies are in progress in our laboratories to clarify the interrelationships that link the glycidic retina metabolism to exogenous polyamine treatment.

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