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Differences in Rat Tissue Lactate Dehydrogenase Activity Caused by Giberellic Acid and Homobrassinolide

[Giberellik Asit ve Homobrassinolit Uygulaması ile Sıçan Doku Laktat Dehidrogenaz Aktivitesinde Gözlenen Değişiklikler]

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ABSTRACT

Objective: Lactate dehydrogenase enzyme, a tissue marker for cardiac disorders, reversibly forms pyruvate from lactate in all animal tissues. Low-dose effect of dietary plant hormones homobrassinolide and gibberellic acid on this enzyme activity was therefore investigated in normal rat tissues.

Methods: Hormones were administered intradermally to male albino wistar rat groups (100-120 g) at 10, 50 and 250 µg, in independent trials and the lactate dehydrogenase enzyme activity was assayed two hours later.

Results: Homobrassinolide at 10 µg increased kidney and testicular lactate dehydrogenase enzyme specific activity, but at 50 µg increased heart and testicular lactate dehydrogenase enzyme specific activity. The homobrassinolide responses were however below alcohol control level for all other tissues. Homobrassinolide increased hemoglobin and total protein, decreased blood glucose and altered total cholesterol content. Gibberellic acid increase in blood glucose, cholesterol content and changes in white cell count were noted.

Conclusion: Dietary gibberellic acid and homobrassinolide therefore affected cellular energy production, blood glucose and cholesterol content while homobrassinolide affected protein synthesis and hemoglobin content.

Keywords: lactate dehydrogenase, gibberellic acid, homobrassinolide, pyruvate, hemoglobin, glucose

ÖZET

Amaç: Laktat dehidrogenaz tüm hayvan dokularında piruvattan laktat oluşumunu gerçekleştiren enzim olup kalp rahatsızlıkları için belirteç olarak kullanılır. Çalışmada diyetle alınan düşük dozda bitki hormonları homobrassinolit ve giberellik asidin bu enzime etkileri incelenmiştir.

Gereç ve yöntemler: Hormonlar bağımsız çalışmalarda erkek albino wistar sıçan gruplarına 10, 50 ve 250 µg dozlarında deri içine verilmiş ve uygulamadan iki saat sonra laktat dehidrogenaz aktivitesi ölçülmüştür.

Bulgular: 10 µg homobrassinolit böbrek ve testiküler laktat dehidrogenaz enziminin spesifik aktivitesini arttırırken 50 µg homobrassinolit uygulaması kalp ve testiküler laktat dehidrogenaz enziminin spesifik aktivitesinin artmasına neden olmuştur. Homobrassinolit yanıtları diğer tüm dokularda kontrol alkol seviyelerinde daha düşük bulunmuştur. Homobrassinolit hemoglobin ve total protein miktarını arttırıken kan glukoz seviyesi düşmüş ve total kolesterol içeriği değişmiştir. Giberellik asit uygulaması ile kan glukozu, kolesterol içeriği ve beyaz küre sayısında değişiklikler gözlenmiştir.

Sonuçlar: Diyet ile alınan giberellik asit ve homobrassinolit hücrenin enerji üretimini, kan glukoz ve kolesterol içeriğini etkiler iken homobrassilonit protein sentezi ve hemoglobin içeriğini değiştirir.

Anahtar kelimeler: : laktat dehidrogenaz, gibberellik asit, homobrassinolit, piruvat, hemoglobin, glukoz

Introduction

Lactate dehydrogenase (LDH) is a bi-directional cytoplasmic enzyme capable of reversible formation of pyruvate and lactate in all eukaryotic and prokaryotic cells (1). This catalytic activity utilized NAD^+ and NADH coenzyme forms dependent upon the nature of substrate being converted to product (2). LDH catalysis involves the transfer of a hydride ion from the C-4 of NADH to C-2 of pyruvate with concomitant transfer of a proton from the imidazole moiety of His 195 in the enzyme structure (3). 34 isoforms for this tetrameric enzyme (228 kDa) containing an alpha chain (MW of 36 KDa) and a beta chain (MW of 21 kDa) had been reported (4-6). Five different types of LDH with subunit composition comprising the M and H forms in different combinations had also been reported. (3). Allosteric inhibition of LDH forms by pyruvate has been noted based on changes in the K_m and V_{max} values of the enzyme (7, 8). Several inhibitors of LDH activity have been identified

(9) including heavy metals, oxygen, glycerate, oxalate, malate, phenylpyruvate and fatty acids. Group specific reagents such as 2,3–butanedione, diethylpyrocarbonate, tetranitromethane and N-bromosuccinimide also bring about complete loss of LDH activity (10).

In a cell metabolic level of pyruvate is normally utilized through one of three pathways, such as 1) conversion of pyruvate to lactate by LDH, 2) generation of glucose through gluconeogenesis and 3) formation of acetyl-coA and its reutilization in the TCA cycle. In contrast, lactate is released into the blood stream by RBC and skeletal muscle cells, for converting it to glucose (11, 12). Hence, modulations in the cellular status of pyruvate or lactate will affect metabolic pathways involving pyruvate in a coordinated manner. The measurement of serum LDH has therefore been used as a diagnostic tool for the clinical elevation of subjects (13-16).

Hormones are actively involved in the regulation of growth and development both in plant and animal species. In plants giberellic acid (GBA) comprises a family of greater than 90 molecular species of plant terpenes. GBA is a member of this family. GBA and homobrassinolide (HB) are produced in plants through extension of cholesterol biosynthetic pathway available only there (17-18). HB (over 40 varieties) activates signal transduction pathways in plants, and promotes cell elongation and cell division. They also promote differentiation of xylem and other tissues, and retard leaf abscission (19). Absence of HB results in plant dwarfing. Among the naturally occurring HB, epi- and homo- isoforms are significantly bio-potent molecules (20).

Since animals including man consume GBA and HB in their diet, it was hypothesized that dietary factors influenced metabolic events in animal cells. Hormones in general being potent physiological mediators, the biological significance of consuming dietary phytohormones GBA and HB was studied in relation to their possible effect on a marker enzyme function like that of the cardiac marker LDH. Since GBA and HB were both derived as extended biosynthetic products of cholesterol metabolism in plant cell, it was believed that structural similarities between these compounds perhaps allowed for their recognition by molecular systems that employed the steroid structure for biological activities in the animal cells. This study therefore aimed at elucidating certain biological responses detectable in selected male rat tissues when exposed to low amounts of these plant hormones.

Materials

Male albino Wistar strain (8-10 weeks old) rats weighing 170-190gm obtained from the vivarium of JIPMER, Pondicherry, were used for the study. GBA was a gift from Dr.B.Kannabiran, our department. HB was obtained courtesy of Dr.Vyas, Godrej Agrovet, Mumbai, India. All chemicals and bio chemicals used in the study were of analytical grade. Glass distilled water was used in the preparation of all reagents.

Methods

Investigations were aimed at studying the effect of GBA and HB on the catalytic activity of LDH in the brain, heart, liver, kidney and testis of male rat. Control and test rats were organized into 4 groups. The control rats were administered 10 µl and 50 µl of 95 % ethanol intradermally since ethanol was used as a solvent for solubilization of HB. Test rats were administered 10 µg and 50 µg HB (from a stock of 1mg/ml) in 95 % ethanol. A normal control group which is untreated was used as control for GBA. A fourth group of rats was given 250 µg of GBA intradermally. Dose selection was based on preliminary trials on rat for identifying minimum quantum of this compound required to elicit a reproducible response in blood markers cited. All animals were anaesthetized two hours following HB and GBA dose using anesthetic ether and each tissue was surgically removed. Tissues were washed in ice cold 1.15 % KCl and were cut into small pieces, for homogenization. To obtain a 10 % (w/v) homogenate, the tissue was homogenized by several strokes in a Potter-Elvehjem homogenizer using a teflon pestle in 0.1 M sodium phosphate buffer, pH 7. The homogenate was centrifuged at 10,000 x g for 10 minutes in Sorvall Rc5c Remi high speed refrigerated centrifuge at 4°C. The clear supernatant obtained was used for the measurement of LDH activity (22) and protein content (23). Blood was collected (in 1.15 % EDTA) by cardiac puncture and was used for hematological studies. Serum was obtained from a second sample of rat blood collected without an anticoagulant. LDH enzyme activity was determined and expressed in International units (IU). One IU of enzyme activity converted one micromole of the substrate per minute to product under standard conditions. Blood glucose was estimated by the method of Asatoor and King (24) and total cholesterol by the method of Zak et al (25). Hemoglobin content

was measured by the acid hematin method, erythrocyte, leukocyte and differential counts were obtained by the Leishman staining method (26).

Statistical analysis

All values were expressed as mean ± SEM. Statistical analysis was carried out using the SPSS 11. The statistical significance of differences between the means was assessed by ANOVA. A difference at $P<0.05$ was considered statistically significant

Results

LDH enzyme activity in rat brain, heart, liver, kidney and testis homogenate 10,000 x g supernatant was determined independently three times in control and treated animal tissues following intradermal GBA (250 µg) and HB (10 & 50 µg) administration to male rats. Normal control rats were used for the GBA study and ethanol control rats were used for HB study, since HB was solublized in ethanol. Percentage change in LDH activity between treated and control values were determined. The tissue LDH activity was determined expressed in IU/ml; protein content in mg/ml and, specific activity in IU/mg (Table 1).

HB at 10 µg increased kidney and testicular LDH specific activity, but at 50 µg increased heart and testicular LDH specific activity. The HB responses were however below alcohol control level for all other tissues (Table 2). Percentage changes in mean LDH specific activity were also determined. Results indicated reduction of LDH activity by HB in rat brain, and liver and augmentation of LDH activity in heart, kidney and testis.

Rat blood analysis showed changes in RBC and WBC content. Increase in hemoglobin, decrease in blood glucose and differences in total cholesterol were due to HB treatment of the animal. GBA influenced changes in the RBC and WBC content, and increased hemoglobin, blood glucose and total cholesterol (Table 3).

Table: 1 Mean LDH specific activity in normal control and GBA (250 µg/250 µl) treated rat tissue homogenate 10,000 x g supernatant.

Specimen	Specific activity IU/mg of protein x10^{-2}		
	Normal control	Treated	% change
Brain	49.5±5.1	60±5.8	21.3↑
Heart	89.6±4.8	85.6±0.6a	4.4↓
Liver	22±2.5	18.4±0.8a	16.1↓
Kidney	34.4±4.8	41.3±1.9a	19.9↑
Testis	84.9±0.5	42.9±7.1	45.2↓

[Normal control refers to a set of 3 rats which is untreated (group 1); Treated refers to a set of 3 rats which is GBA treated (group 2)]
Arrows indicate change; ↓ - decrease; ↑ - increase)
$p<0.01$; [a] $p<0.05$, compared to normal control

Table: 2 Mean LDH specific activity in ethanol control (10 & 50 µl) and HB (10 & 50 µg) treated rat tissue homogenate 10,000 x g supernatant.

Specimen	Specific activity IU/mg of protein x10^{-2}					
	Ethanol Control(10µl)	Ethanol Control(50µl)	Treated(10µg)	Treated(50µg)	% change	% change
Brain	48.9±14.6[a]	40.7±3.2[a]	35±14.6[a]	8±1	22↓	82↓
Heart	0.7±0.1[a]	0.6±0.1[a]	0.4±0.0	18.9±3	55↓	2011↑
Liver	33.8±3.1[a]	34.8±3.3[a]	12±2.5	0.4±0.1	68↓	99↓
Kidney	8.3±0.1[a]	8.6±0.5	16.3±3	0.9±0.2	15900↑	900↓
Testis	52.5±11.1[a]	33.2±1[a]	54.2±1.7	44.3±4.3[a]	69↑	37↑

[Ethanol control refers to two sets of 3 rats each treated with 10 µl & 50 µl ethanol separately (group 3); Treated refers to two sets of 3 rats each treated with 10 µg & 50 µg HB separately (group 4)]
(Arrows indicate change; ↓ - decrease; ↑ - increase)
$p<0.01$; [a] $p<0.05$, compared to normal control

Table.3 Cell counts in normal control and treated rat blood.

Specimen	RBC x 10^6 µl	WBC/ mm^3	Neutro-phils (%)	Lympho-cytes (%)	Eosino-phils (%)	Hb gm/dl	Blood glucose (mg/dl)	Total cholesterol (mg/dl)
Normal control	3.5	9400	62	36	02	9.5	84	42.50
10 µg of HB	4.9	9,900	59	41	05	13.3	72	32.75
50 µg of HB	5.3	10,100	64	38	04	14.4	68	48.38
250 µg of GBA	3.9	12,600	28	72	06	10.6	109	63.0

Discussion

Lactate dehydrogenase enzyme activity of animal cells can be modulated by the plant derived growth regulators such as GBA and HB. GBA remained as an uncompetitive inhibitor of LDH activity in all the rat tissues studied. GBA increased the protein content in rat testicular cells, whereas it reduced protein content in all other tissues. Protein content of rat kidneys and brain decreased 40- 44 % respectively. The protein content of rat brain and rat testis however showed an inverse relationship.

At GBA induced LDH I_{50} activity, greater than 50 % pyruvate (contrary to the normal 8 %) shall remain directed to enter TCA cycle for ATP synthesis. The cell's ability to recover NAD^+ from NADH through anaerobic lactate production by LDH activity utilizing the residual pyruvate (available 50 %) is however expected to continue.

The use of ethanol was required for solublizing of HB. HB in ethanol decreased LDH activity in the brain and liver, and increased the enzyme activity in kidneys and testis. Heart LDH activity exhibited a flip-flop behavior associated with the level of HB administered. Specific activity changes were similar to the activity changes noted for LDH in response to either ethanol, or HB. HB treatment altered the RBC and WBC content, increased hemoglobin content, decreased blood glucose and altered the total cholesterol level. The increase in serum cholesterol level by HB and GBA was perhaps related to the mobilization of this compound from membrane stores or due to the activation of HMG –CoA reductase for increased synthesis of cholesterol. This proposition was however not experimentally determined and much work has to be done in this regard. Serum cholesterol level along with blood glucose and hemoglobin content was measured due to their critical importance in blood markers that are susceptible to changes in response to modulatory factors affecting an animal, and in this case, the use of the novel inductory GBA and HB.

The metabolic consequence of LDH inhibition will result in utilization of pyruvate for the TCA cycle and alanine production by transamination. Elevated levels of alanine will aid glutamate production and energy yield through pyruvate in the TCA cycle. Inhibition of LDH prevented lactic acid accumulation in tissues, whereas increased lactic acid production in testicular cells aided spermatogenesis.

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