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Cytoplasmic Enzyme Activities Involved in Energy and Amino Acid Metabolism in Pathological Human Renal Cortex

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Enzyme levels of lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (HBDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in the cytosol of renal cortex samples from either normal and pathologic kidney tissue. The mean enzyme activity values, expressed in Units per gram of cytosolic protein decreased in the following order: normal cortex (LDH = 4,299 ± 654; AST = 522 ± 101; ALT = 197 ± 44). chronic pyelonephritis (LDH = 2,360 ± 876; AST = 297 ± 117; ALT = 90 ± 48), hydronephrosis (LDH = 2,208 ± 1,264; AST = 279 ± 165; ALT = 82 ± 61), pyonephrosis (LDH = 1,410 ± 596; AST = 158 ± 69; ALT = 23.4 ± 16.4) and renal tuberculosis (LDH = 1,149 ± 481; AST = 93 ± 34; ALT = 5.6 ± 2.8). The decrease in the enzyme activities paralleled tissue damage and it was shown to affect cellular functionality in relation with energy and amino acid metabolism.

Key words: Alanine aminotransferase, Aspartate aminotransferase, Lactate dehydrogenase, Renal tissue.

Energy metabolism of normal renal cortex cells is mainly aerobic. The renal cortex exhibits the highest level of lactate dehydrogenase (LDH, EC 1.1.1.27) per gram of wet weight of the organism (9). Furthermore, the renal cortex is a very active site in the synthesis of amino acids with high levels of aminotransferases (4,6, 8). Decreased levels of cytoplasmic enzymes involved in energy metabolism and in amino acid metabolism in hypernephrona have recently been reported by us (3). In order to study the extent of involvement of cytoplasmic enzyme activities in various kidney diseases lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (HBDH), aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2)

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have been measured in renal cortex samples from various kidney diseases.

Materials and Methods

The study was performed on human renal samples obtained from nephrectomy. None of the subjects had received endocrine treatment prior to the extraction of the kidney. The samples were divided into two aliquots, for both histological control and enzyme activity measurements. Although it is well recognized that normal and pathological kidney tissues have heterogeneous cell populations, no attempts were made to isolate them.

For light microscopy, tissue samples were fixed in 10 % formalin and embedded in paraffin. Five-μm-thick sections were stained with hematoxylin-eosin. For enzyme analysis the samples were placed in liquid nitrogen immediately after nephrectomy and then stored at -70° until assayed.

On the basis of the microscopic findings, the samples were classified into the following groups: 1) Normal; 2) Chronic pyelonephritis; 3) Hydronephrosis; 4) Pyonephrosis; and 5) Renal tuberculosis.

For enzyme analysis, a 6/1 (v/w) homogenate of each sample was prepared in ice-cold TEDG buffer (0.01 M Tris-HCl; 0.0015 M EDTA; 0.001 M DL-dithiothreitol and 10 % glycerol, pH 7.4) using a Polytron homogenizer (four 10-second phases with 1 min cooling intervals). The homogenate was centrifuged at 800x*g* for 20 min; the pellet was discarded and the supernatant fluid centrifuged at 100,000x*g* for 60 min to obtain the cytosol. All these operations were carried out at 4°C.

Enzymes were measured by optimized standard methods (1, 2) at 30°C using a Hitachi 705 automatic analyzer. Protein was measured by the method of LOWRY *et al.* (5). Enzyme activities (Mean±SD) were expressed in U/g cytoplasmic protein.

Analysis of variance (ANOVA) and Newman-Keuls test were used to assess significance. All p values were two-tailed.

Results

Enzyme activities and the LDH/HBDH and AST/ALT ratios measured in normal renal cortical tissue and in different pathologic renal tissue samples are shown in Table I. The highest LDH and HBDH values corresponded to normal tissue, and decreased in the following order: chronic pyelonephritis, hydronephrosis, pyonephrosis and renal tuberculosis. Analysis of variance shows statistically significant differences between the groups ($p = 9.0 \times 10^{-9}$ for LDH; $p = 1.7 \times 10^{-9}$ for HBDH and 9.8×10^{-3} for LDH/HBDH). When Newman-Keuls analysis for each pairs of groups was done statistically significant differences were observed for LDH, HBDH and LDH/HBDH ratio between normal renal cortical tissue and all of the other groups ($p < 0.001$), except for LDH/HBDH ratio between normal cortical tissue and the group of renal tuberculosis tissue that was not significant.

Similarly to LDH and HBDH, the highest aminotransferase values were obtained in normal cortical tissue, and decreased in the following order: chronic pyelonephritis, hydronephrosis, pyonephrosis and renal tuberculosis. On the other hand, the lowest AST/ALT ratios were obtained in the normal tissue. Analysis of variance shows statistically significant differences between the groups ($p = 3.4 \times 10^{-8}$ for AST; $p = 1.2 \times 10^{-9}$ for ALT and $p = 1.5 \times 10^{-9}$ for AST/ALT). Newman-Keuls comparison for each pair of groups gave significant differences for AST and ALT between normal renal cortical tissue and all of the other groups ($p < 0.001$). Also, ALT shows significant differences between the pyelonephritis and the pyonephrosis ($p < 0.05$) and renal tu-

Table I. *Cytoplasmic enzyme activities (U/g protein) and LDH/HBDH and AST/ALT ratios in renal cortex tissues with various diseases.*

Group	n	LDH	HBDH	LDH/HBDH	AST	ALT	AST/ALT
1	14	4.299±654	2.568±425	1.70±0.10	522±101	197±44	2.73±0.62
		(5.752—3.312)	(3.325—1.818)	(1.82—1.51)	(689—359)	(278—139)	(3.78—1.52)
2	6	2.360±876	1.282±587	1.92±0.30	297±117	90±48	3.72±1.31
		(3.488—1.101)	(1.986—549)	(2.48—1.67)	(466—169)	(163—31)	(5.40—2.22)
3	5	2.208±1.264	1.242±694	1.78±0.24	279±165	82±61	4.12±1.31
		(3.723—746)	(2.075—495)	(2.10—1.51)	(439—93)	(148—17.5)	(6.11—2.98)
4	7	1.410±596	702±295	2.03±0.33	158±69	23.4±16.4	8.68±4.67
		(2.211—492)	(989—266)	(2.50—1.61)	(240—63)	(46.5—5.2)	(17.31—5.12)
5	3	1.149±481	547±239	2.11±0.15	93±34	5.6±2.8	18.33±4.69
		(1.452—549)	(720—275)	2.24—1.94)	124—57)	(7.8—2.4)	23.7—15.0)

Values are mean ± SD. 1) Normal, 2) Chronic pyelonephritis, 3) Hydronephrosis, 4) Pyonephrosis, and 5) Renal tuberculosis. n = number of samples.

berculosis ($p<0.05$) groups, and between the hydronephrosis and the pyonephrosis ($p<0.05$) and renal tuberculosis ($p<0.05$) groups. The ratio AST/ALT shows statistical differences ($p<0.001$) between normal renal cortical tissue and pyonephrosis and tuberculosis, between pyelonephritis and pyonephrosis and renal tuberculosis, between hydronephrosis and pyonephrosis and tuberculosis, and between pyonephrosis and tuberculosis.

Discussion

The results obtained show that the pathologic human kidney tissues analyzed exhibited decreased LDH, HBDH, AST and ALT levels when compared with normal renal cortical tissue. The fall of enzyme activities in the cytosol paralleled the extent of tissue damage. It is noteworthy that while in normal kidney the activities measured are very constant in diseased tissues they are subjected to extensive scatter. This difference may be due to different stages in the pathological process.

The decrease in LDH and HBDH activities is accompained by an increase in the LDH/HBDH ratio, which reflects the aerobic capacity of a tissue (7). Tissues with a predominantly aerobic metabolism have low LDH/HBDH ratios, while tissues with predominantly anaerobic metabolism have high LDH/HBDH ones. The renal cortex is an active site of aerobic metabolism, demonstrated by very high levels of lactate dehydrogenase and low LDH/HBDH ratio (3, 6, 8). In renal diseases the decrease in LDH activity produced as tissue deteriorates is accompanied by an increase in the LDH/HBDH ratio, that is, an increase in anaerobic metabolism.

In regard to aminotransferases, they are key enzymes in amino acid metabolism. The decrease in aminotransferase activities observed in the pathologies studied is accompanied by an increase in the AST/ALT ratio (greater decrease of ALT than AST). These facts point to a decreased synthesis of nonessential amino acids and an apparent lower availability of pyruvate in diseased tissue, utilized by lactate dehydrogenase in the anaerobic direction. Pyruvate could act as an inducer of aspartate aminotransferase synthesis.

In summary, these results show an increase in the anaerobic metabolism and a decrease in the synthesis of nonessential amino acids in the cytosol of diseased re-

nal cortex. The extent of enzyme activities disorders runs in parallel to tissue deterioration.

Resumen

Se han medido los niveles de las enzimas láctato deshidrogenasa (LDH), α-hidroxibutirato deshidrogenasa (HBDH), aspartato aminotransferasa (AST) y alanina aminotransferasa (ALT) en el citosol de cortex renal procedente de riñones normales y con diferentes patologías. Las actividades medias expresadas en U/g de proteína citosólica descienden en el orden: cortex normal (LDH = 4.299 ± 654; AST = 522 ± 101; ALT = 197 ± 44), pielonefritis crónica (LDH = 2.360 ± 876; AST = 297 ± 117; ALT = 90 ± 48), hidronefrosis (LDH = 2.208 ± 1.264; AST = 279 ± 165; ALT = 23,4 ± 16,4) y tuberculosis renal (LDH = 1.149 ± 481; AST = 93 ± 34; ALT = 5,6 ± 2,8). El descenso de las actividades enzimáticas es paralelo al daño tisular y afecta la funcionalidad celular en relación con el metabolismo energético y de los aminoácidos.

Palabras clave: Alanina aminotransferasa, Aspartato aminotransferasa, Lactato deshidrogenasa, Tejido renal.

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