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Isocitrate Oxidation in Dog Heart Mitochondria Under Anoxic Conditions

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The roles of NAD- Specific Isocitrate Dehydrogenase (NAD- IDH) (EC 1.1.1.4), NADP- Specific Isocitrate Dehydrogenase (NADP- IDH) (EC 1.1.1.42) and Piridin Nucleotide Transhydrogenase (Transhydrogenase) (EC 1.6.1.1) in the mitochondrial oxidation of isocitrate through the respiratory chain in conditions of normal and increased energy requirements have been studied in submitochondrial particles isolated from healthy and ischemic dog hearts. The activities of both, NAD- IDH and NADP- IDH were increased in conditions of anoxia, while Transhydrogenase remained unchanged. The results obtained showed that the mitochondrial oxidation of isocitrate in dog myocardium occurs mainly through the NAD- IDH pathway in normoxic and anoxic conditions.

Isocitrate oxidation in the mitochondria, can occur either through the NAD- Specific Isocitrate Dehydrogenase (NAD- IDH) (EC 1.1.1.4) pathway or through the pathway involving NADP- Specific Isocitrate Dehydrogenase (NADP- IDH) (EC 1.1.1.42) and Piridin Nucleotide Transhydrogenase (transhydrogenase) (EC 1.6.1.1). Changes in redox and phosphorylation ratios, as indicated by NADH / NAD^+ and ATP / ADP quotients, are of importance in the control of intramitochondrial oxidation of isocitrate (6). In rat liver coupled mitochondria, NICHOLLS and GARLAND (4) observed that isocitrate oxidation occurs mainly through the NAD- IDH pathway because of the relatively low activity of transhydrogenase. However, in uncoupled mitochondria, the Transhydrogenase system would be probably stimulated. LEE and ERNSTER (3) suggested that the NADP- IDH and transhydrogenase would increase its contribution during periods of enhanced energy requirements. In this paper has been studied the role of these enzymes in the isocitrate oxidation under normal and anoxic conditions, in submitochondrial particles from both normal and ischemic myocardial tissue,

in which the energy requirements are increased, the oxidative phosphorylation uncoupled and ATP production reduced (1, 5, 8).

Materials and Methods

Mongrel dogs weighting 14 to 22 kg fed *ad libitum* were anesthetized with sodium thiopental (25 mg/kg i.v.) and intubated with an endotracheal cannula attached to an Palmer- type respiratory pump providing atmospheric air. A thorachotomy and pericardiotomy were carried out and ischemia was induced by hand traction of the left coronary artery near its branched derivation followed by ligation (10). The hearts were then removed and pieces of affected and unaffected myocardium were excised and used for enzyme assay. During the experiments electrocardiographic controls were performed with epicardial monopolar leads. Heart rate and blood pressure were monitorized. Morphological controls of healthy and ischemic myocardium by electron microscopy have shown alterations similar to those described by JENNINGS and GANOTE (2) after ischemic injury.

Mitochondria from myocardium were obtained by differential centrifugation, following the method of TYLER and GONZE (9). Submitochondrial particles were obtained after low frequency ultrasonic treatment.

The enzymatic determinations were carried out under optimal conditions by spectrophotometric methods. NAD— IDH and NADP— IDH activities were recorded by the method of PLAUT ad AOGAICHI (6). Transhydrogenase was assayed as described by RYDSTROM *et al.* (7). Protein was measured by the deoxycholate biuret method (11).

Results and Discussion

NAD-IDH and NADP- IDH activities are higher than transhydrogenase activity in normal conditions (Table I). In ischemic myocardium they are increased by fifty per cent, while the transhydrogenase activity remains unchanged.

Table I. *Enzymatic activities of NAD- IDH, NADP- IDH and Transhydrogenase of healthy and ischemic myocardium.*
The enzymatic activities are expressed in nmoles of substrate × min^{-1} × mg of $protein^{-1}$. Results are mean ± SD for 20 experiments.

	NAD- IDH	NADP- IDH	Transhydrogenase
Healthy myocardium	64 ± 6	146 ± 19	25 ± 2
Ischemic myocardium	94 ± 17	209 ± 40	25 ± 3

These results are consistent those obtained by NICHOLLS and GARLAND (4) showing that in rat liver coupled mitochondria, isocitrate is mainly oxidized by NAD- IDH, since the low transhydrogenase activity would prevent the rapid oxidation of NADPH generated by the NADPH- IDH. Indeed, the results shown that NAD- IDH activity is three times higher than that of transhydrogenase, therefore, the flux through the NADP-IDH/transhydrogenase would be lower, despite of the higher activity of the NADP— IDH. These results are different of those obtained by NICHOLLS and GARLAND (4) in rat liver chemically uncoupled mitochondria, since in dog ischemic myocardium transhydrogenase is not stimulated, while NAD— IDH increases by fifty per cent, that is, four times higher than transhydrogenase.

The data presented here suggest that the mitochondrial oxidation of isocitrate in myocardium, through the respiratory chain, under conditions of anoxia would proceed mainly through the NAD- IDH pathway and not through the NADP-IDH/transhydrogenase pathway, as proposed by NICHOLLS and GARLAND for rat liver uncoupled mitochondria (4).

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Resumen

Se estudia la actividad de las enzimas isocitrato deshidrogenasa NAD- específica (EC 1.1.1.4), isocitrato deshidrogenasa NADP- específica (EC 1.1.1.42) y piridín nucleótido transhidrogenasa (1.6.1.1) en partículas submitocondriales de corazón sano e isquémico de perro y su papel metabólico en la oxidación intramitocondrial del isocitrato, vía cadena respiratoria, en circunstancias de demanda energética aumentada (isquemia miocárdica). Las actividades de la isocitrato deshidrogenasa NAD-específica y de la isocitrato deshidrogenasa NADP-específica aumentan en condiciones de anoxia, mientras que la actividad de la transhidrogenasa permanece invariable. Los resultados indican que la oxidación del isocitrato en las condiciones señaladas ocurre preferentemente a través de la vía que requiere la isocitrato deshidrogenasa NAD- específica.

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