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Functional and metabolic implications of biotin deficiency for the rat heart

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ABSTRACT

The tricarboxylic acid (TCA) cycle is the main ATP provider for the heart. TCA carbons must be replenished by anaplerosis for normal cardiac function. Biotin is cofactor of the anaplerotic enzymes pyruvate and propionyl-CoA carboxylases. Here, we found that in biotin deficient rats, both carboxylases decreased 90% in adipose tissue, jejunum and spleen, but in heart they conserved about 60% residual activity. We then investigated if under biotin deficiency (BtDEF), the heart is able to maintain its function in vivo and in isolated conditions, and during ischemia and reperfusion, where metabolism drastically shifts from oxidative to mainly glycolytic. Neither glucose nor octanoate oxidation were severely affected in BtDEF hearts, as assessed by mechanical performance, oxygen uptake or high-energy metabolite content; however, myocardial hexokinase activity and lactate concentration were reduced in deficient hearts. When challenged by ischemia and reperfusion injury, BtDEF hearts did not suffer more damage than the controls, although they lowered significantly their performance, when changed to ischemic conditions, which may have clinical implications. Post-ischemic increase in ADP/ATP ratio was similar in both groups, but during reperfusion there was higher rhythm perturbation in BtDEF hearts. By being relatively insensitive to biotin deficiency, cardiac tissue seems to be able to replenish TCA cycle intermediates and to maintain ATP synthesis.

Introduction

Fatty acids and ketone bodies are a major energy source for the heart [14], although glucose is dominant in the post-prandial state [13]. Both types of metabolic fuels provide acetyl CoA for the functioning of the tricarboxylic acid (TCA) cycle. Since heart TCA carbons are lost, mainly via citrate release [27], anaplerosis is a critical process for cardiac function. As a matter of fact, when the heart is perfused only with ketone bodies, it stops functioning after a short time unless glucose is added, providing TCA cycle intermediates through PC [30,27,11,19]. The vitamin biotin is very important for anaplerosis because it is the prosthetic group of two major anaplerotic enzymes. Thus, we were interested to investigate if under biotin deficiency, the heart is able to maintain its function, using different substrates as fuel in both, in vitro and in vivo models. In the same way, we tested the response to a metabolic challenge such as ischemia and reperfusion, where metabolism drastically shifts from oxidative to glycolytic energy production and thus, the metabolic role of biotin-dependent anaplerotic processes is evidenced.

Biotin is the prosthetic group of pyruvate carboxylase (PC) (EC 6.4.1.1.), propionyl CoA carboxylase (PCC) (EC 6.3.4.10), 3-methyl crotonyl CoA carboxylase (MCC) (EC 6.4.1.4) and two forms of acetyl CoA carboxylase (ACC) (EC 6.4.1.2), the first three and one ACC located in the mitochondria, and the other ACC in the cytosol [38]. PC is the first enzyme in gluconeogenesis in liver and kidney; it participates in fatty acid synthesis in adipose tissue, and has a prominent TCA cycle anaplerotic role in many organs like brain, heart and skeletal muscle [15]. MCC is an enzyme in the leucine catabolic pathway. PCC catalyses the conversion of propionyl CoA into methylmalonyl CoA (MMA), in the degradation of valine, isoleucine, threonine, odd-chain fatty acids and cholesterol. MMA is converted to succinate to replenish the TCA cycle. ACC is the first enzyme in fatty acid synthesis, besides contributing to TCA cycle anaplerosis. Thus, these enzymes have a prominent role in the metabolism of carbohydrates, lipids and proteins.

Biotin is not synthesized in mammals and is obtained from the diet, although small amount may derive from the intestinal flora, because the combined daily urine and stools output exceeds the

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dietary intake [2]. In its role as the carboxylation cofactor it is covalently bound to a lysine residue of inactive apocarboxylases, which in this way become active holoenzymes, a two-step reaction catalyzed by holocarboxylase synthetase (HCS). When the active holocarboxylases are degraded during their turnover, free biotin is released by the breakdown of biocytin, a compound of linked biotin and lysine, by the action of biotinidase. There is therefore a "biotin cycle" through which biotin can be reutilized, biotinidase performing as a "salvage" enzyme. It is also quite important in meeting this vitamin nutritional requirement since most of the dietary biotin is protein bound.

Besides its catalytic role, biotin has regulatory functions. We found that in intact rats this vitamin participates in the genetic expression of the enzymes to which it is functionally related [28,25]. Biotin has also been reported as regulator of the expression of several proteins not related to carboxylation, including key enzymes of carbohydrate metabolism: liver glucokinase and phosphoenol pyruvate carboxykinase. There have been reported many human recessive mutations leading to Multiple Carboxylase Deficiency (MCD), a syndrome with catastrophic phenotypic effects, involving the genes for HCS and biotinidase [38].

Biotin deficiency in the rat paradoxically diminishes the carboxylases activities in liver, kidney and skeletal muscle as well as HCS and the biotin transporter (SMVT), but not in the brain [26], in an analogous way with most organs sparing glucose in favor of the brain. In the present work, we found a similar preservation effect in the heart. Herein we present these results, as well as the effect of biotin deficiency on heart metabolism and function.

Methods

Animals and biotin-deficient diet

At the start of each experiment 5 Wistar rats, aged 21 days (weight: 51.3 ± 2.7 g), were made biotin-deficient and another 5 rats, of similar gender, age and weight, served as controls. Biotin deficiency was induced by feeding a commercial biotin-deficient diet containing 30% of white egg as source of avidin (TD.81079 ICN Nutritional Biochemicals, Cleveland, OH). The control animals were fed a similar diet that lacked avidin (TD.97126 ICN Nutritional Biochemicals, Cleveland, OH). Each rat was housed individually in an air-filtered cage on a 12 h. light/dark cycle and was allowed free access to water. Control and deficient rats were studied at week 8; at this time, all of the biotin-deficient animals had developed the typical phenotypic features of the deficiency [38]; furthermore, they were excreting large amounts of 3-hydroxy isovaleric acid, a well known marker of biotin deficiency [22]. All the experiments in this article were repeated on at least three different occasions; the results presented are from a representative experiment. The experiments were carried out according to the current national legislation (NOM-062-ZOO-1999 and NOM-087-3COL-SSA1-2002) and were revised and approved by the Instituto de Investigaciones Biomédicas and the Instituto Nacional de Pediatría Ethics Committees.

Tissue homogenates

Rats were anaesthetized with sodium pentobarbital (50 mg/kg of body weight, i.p.) so that under deep anaesthesia, periepididymal adipose tissue, heart, jejunum and spleen could be quickly removed and washed with ice-cold phosphate buffer saline (PBS) pH 7.0. All subsequent steps were carried out at 4 °C. One gram of each organ and tissue was homogenized in 5 volumes of buffer (0.5% Triton X-100, 0.15 M NaCl, 2 mM EDTA, 10 mM Tris–HCl, pH 4.5, containing a proteases inhibitor ("Complete", Roche Diagnostics, Mannheim, Germany)), by three passes with a polytron (Kinmatica, Krienslu). The homogenates were then sonicated with a Branson Cell Disruptor 200 (Danbury, CT) with three pulses of 15 s each. Aliquots of the homogenate were centrifuged at 10,300*g* for 30 min. The fat layer was discarded and 1 ml of the supernatant was ultracentrifuged at 103,000*g* and stored at −70 °C for analyses.

Enzyme assays

PCC and PC were determined by a modified assay of Suormala et al. [33], measuring the enzyme-dependent incorporation of ^{14}C-bicarbonate into acid non-volatile products. The assay mixture for PC contained 50 mM Tris–HCl, pH 8.0, 0.5% Triton X-100, 10 mM MgCl2, 5 mM dithiothreitol (DTT), 0.6 mM ATP, 4 mM sodium pyruvate, 2.9 mM acetyl-CoA and 10 mM $NaH^{14}CO_3$ (specific activity 1 mCi/mmol). The assay mixture for PCC contained 50 mM Tris–HCl, pH 8.0, 1% Triton X-100, 10 mM $MgCl_2$, 94 mM KCl, 5 mM DTT, 0.8 mM ATP, 2.9 mM propionyl-CoA and 10 mM $NaH^{14}CO_3$ (specific activity 1 mCi/mmol). Blanks were prepared by omitting sodium pyruvate and acetyl-CoA in the PC assay, and propionyl-CoA in the PCC one. All assays were performed in triplicate at 30 °C for 30 min in a final volume of 125 μl containing 100 μg of total protein. These determinations were made under initial rate conditions and they were linear with respect to protein concentration. Specific activities were expressed as nmol $^{14}CO_2$ fixed per min per mg protein. The protein concentration of tissue homogenates was measured by the Lowry method. The assay for hexokinase was carried out at 37 °C in 1 ml of buffer containing 100 mM KCl, 50 mM Pops, 0.5 mM EGTA, pH 7.0 plus 2 U G_6PDH, 1 mM NADP+, 5 mM $MgCl_2$ and 10 mM glucose. The reaction was started by addition of 5 mM ATP after 2 min incubation, as described elsewhere [20].

Determination of metabolites

At the end of the in vitro experiments, the isolated beating heart was rapidly removed, frozen in liquid N2 and powdered in a mortar under liquid N_2. A portion of 0.3 g of the frozen tissue was mixed with five volumes of 3% (v/v) perchloric acid/20 mM EDTA. The last suspension was neutralized with KOH/Tris; after centrifugation, the supernatant was stored at −72 °C until HPLC assays. Lactate was measured in aliquots of 250 μl from the neutralized perchloric extracts by standard enzymatic methods [1].

Physiological measurements

Hearts were excised from male Wistar rats of 300–350 g weight and mounted by the Langendorff technique as previously described [4]. Briefly, the animals were anaesthetized with sodium pentobarbital (50 mg/kg of body weight, i.p.). The chest was opened and the heart quickly removed and placed briefly in ice-cold perfusion buffer to arrest it and prevent ischemic preconditioning. The heart was then suspended from a cannula and perfused retrogradely through the ascending aorta at a constant perfusion pressure of 60 mm Hg and at a constant flow rate of 10 ml/min by means of a peristaltic pump. The composition of the perfusion buffer was (in mM): 120 NaCl, 23.4 $NaHCO_3$, 4.8 KCl, 1.2 KH_2PO_4, 0.86 $MgSO_4$, 2.5 $CaCl_2$ and 10 glucose or 1 octanoate as oxidizable substrate 10 glucose, at pH 7.4; the temperature was maintained at 37 °C. The perfusion medium was continuously bubbled with a gas mixture of 95% O_2–5% CO_2. After the heart began contracting spontaneously, a latex balloon, connected to a pressure transducer, was inserted into the left ventricle. Once inserted, the balloon was filled with perfusion buffer at a steady diastolic pressure of 10 mm Hg. Two silver electrodes were attached; one at the apex and the other at one atrium, for electrocardiogram monitoring. The pulmonary artery was cannulated and connected to a closed chamber to measure the O_2