Marked bioenergetics deregulation provoked by D-2-hydroxyglutaric acid in the heart may contribute to the cardiomyopathy of D-2-hydroxyglutaric aciduria

Ribeiro R T 1, Roginski AC 1, Marschner R 2, Wajner SM 2, Leipnitz G 1, Amaral A U 1,3, Wajner M 1,4

1PPG Bioquímica, Depto Bioq, ICBS, UFRGS, Porto Alegre, Brasil; 2Seção de Tireoide, Divisão Endócrina, HCPA, UFRGS, Porto Alegre, Brasil 3Depto Ciencias Biologicas, URI, Erechim, Brasil 4Servicio de Genética Medica, HCPA, Porto Alegre, Brasil

BACKGROUND

D-2-hydroxyglutaric acid (D-2-HG) concentrations are highly elevated in tissues and biological fluids of patients affected by D-2-hydroxyglutaric aciduria type II (D2HGA2), an inherited metabolic disease characterized by severe cardiomyopathy, apart from neurological abnormalities. Considering that the mechanisms of cardiac injury in D-2-HGA are poorly understood, this study evaluated whether D-2-HG could be toxic to the heart by investigating its effects on a large spectrum of bioenergetics parameters.

METHODS

The present study investigate the role of D-2-HG on important parameters of mitochondrial bioenergetics using isolated mitochondria and crude homogenates from heart of developing rats, as well as cultivated H9c2 cardiac myoblasts. The parameters of bioenergy were evaluated in the presence of D-2-HG, namely state 3 (ADP-stimulated), state 4 (non-phosphorylating), uncoupled (CCCP-stimulated) respiration and RCR, the activities of the respiratory chain complexes I to IV and of the citric acid cycle enzymes and ATP production.

RESULTS

The present study investigate the role of D-2-HG on important parameters of mitochondrial bioenergetics using isolated mitochondria and crude homogenates from heart of developing rats, as well as cultivated H9c2 cardiac myoblasts. The parameters of bioenergy were evaluated in the presence of D-2-HG, namely state 3 (ADP-stimulated), state 4 (non-phosphorylating), uncoupled (CCCP-stimulated) respiration and RCR, the activities of the respiratory chain complexes I to IV and of the citric acid cycle enzymes and ATP production.
RESULTS

Heart mitochondria (glutamate)

Fig. 1. Effects of D-2-HG on respiratory parameters measured by oxygen consumption in glutamate supported heart mitochondria. State 3 (ADP-stimulated) (A), state 4 (resting) (B), uncoupled (CCCP-stimulated) (C) respiration and respiratory control ratio (RCR) (D). Glutamate (5 mM) was used as substrate. Mitochondrial preparations (0.1 mg protein. mL$^{-1}$) and D-2-HG (0.5 – 5.0 mM) were added to the incubation medium in the beginning of the assays. Values are means ± standard deviation of four independent experiments (N) and were expressed as pmol O$_2$. s$^{-1}$. mg of protein$^{-1}$. *P < 0.05, **P < 0.01, ***P < 0.001, compared to control (Tukey’s multiple range test).

Heart homogenates (SUIT protocol)

Fig. 2 Fig. 3. Effects of D-2-HG on respiratory parameters in heart homogenates using the substrate-uncoupler inhibitor titration (SUIT) protocol. State 3 (ADP-stimulated) (A and B), state 4 (resting) (C) and uncoupled (CCCP-stimulated) (D and E) respiration. Heart homogenates (1 mg tissue. mL$^{-1}$) and D-2-HG (5 mM) were added to the incubation medium in the beginning of the assays. Pyruvate (5 mM), malate (0.5 mM) plus glutamate (10 mM) (A and D) and succinate (10 mM) (B and E) were used as substrates. Values are means ± standard deviation of four independent experiments (animals) and were expressed as pmol O$_2$. s$^{-1}$. mg of protein$^{-1}$. *P < 0.05, **P < 0.01, compared to control (Student’s t test for unpaired samples).
RESULTS

Fig. 3 Effects of D-2-HG on respiratory parameters in permeabilized cardiac cells (H9c2) using the substrate-uncoupler inhibitor titration (SUIT) protocol. Digitonin (8 μM) was used to permeabilize cells. State 3 (ADP-stimulated) (A and B), state 4 (resting) (C) and uncoupled (CCCP-stimulated) (D and E) respiration. H9c2 cells (1.5 million cells/mL) and D-2-HG (5 mM) were added to the incubation medium in the beginning of the assays. Pyruvate (5 mM), malate (0.5 mM) plus glutamate (10 mM) (A and D) and succinate (10 mM) (B and E) were used as substrates. Values are means ± standard deviation of four independent experiments (N) and were expressed as pmol O₂·s⁻¹·million cells⁻¹.

*P < 0.05, **P < 0.01, compared to control (Student’s t test for unpaired samples).

Fig. 4 Effects of D-2-HG on ATP production in heart mitochondria. Experiments were performed in an incubation medium containing heart mitochondrial preparations (0.1 mg protein. mL⁻¹) supported by glutamate (5mM). D-2-HG (0.5 − 5.0 mM) was added to the incubation medium in the beginning of the assays. Oligomycin A (Oligo, 1 μg. mL⁻¹) was used as a positive control. Values are means ± standard deviation of five independent experiments (animals) and were expressed as nmol ATP·min⁻¹·mg⁻¹.

**P < 0.01, ***P < 0.001, compared to control (Tukey’s multiple range test).
RESULTS

**Fig. 5** Effects of D-2-HG on the activities of the mitochondrial respiratory chain complexes and creatine kinase in H9c2 cells. Complex I (A), complex II (B), SDH (C), complex II-III (D), complex IV (E) and creatine kinase (F) activities. Mitochondrial preparations or H9c2 cells were pre-incubated for 30 min with D-2-HG (5 mM). Values are means ± standard deviation of four to six independent experiments (N) and were expressed as nmol.min⁻¹.mg protein⁻¹ or μmol.min⁻¹.mg protein⁻¹. ***P < 0.001, compared to control (Student’s t test for unpaired samples).

**CONCLUSIONS**

Taken together, our data indicate that mitochondrial bioenergetics is markedly compromised by D-2HG in the heart. It is therefore presumed that D-2HG-induced disruption of bioenergetics may possibly contribute to the cardiomyopathy commonly observed in D2HGA2 patients.