INTRODUCTION

High concentrations of phytanic acid (Phyt) are found in tissues and biological fluids of patients affected by Refsum disease (RD). Cardiomyopathy and heart failure are common features manifested by the affected patients that may lead them to sudden death.

OBJECTIVES

Considering that the pathogenesis of cardiac dysfunction in RD is poorly understood, the present work evaluated the in vitro effects of Phyt (50-200 µM) on mitochondrial homeostasis in rat heart mitochondrial preparations supported by glutamate plus malate as substrates.

MATERIAL AND METHODS
RESULTS AND DISCUSSION

Phyt markedly increased state 4 (Figure 1A) and decreased state 3 respiration (Figure 1B) and the RCR (Figure 1D). Phyt also reduced the Ca²⁺ retention capacity (Figure 2), NAD(P)H content (Figure 3A) and Δψm (Figure 4A) in the presence of exogenous Ca²⁺. More importantly, some of these deleterious effects were prevented by cyclosporin A and ADP, implying the involvement of mitochondrial permeability transition (MPT) pore opening (Figures 3B and 4B).

Figure 1. Effects of phytanic acid (Phyt) on respiratory parameters measured by oxygen consumption in heart mitochondria. (A) State 4 (oligomycin-stimulated), (B) state 3 (ADP-stimulated), (C) uncoupled (CCCP-stimulated) respiration and (D) respiratory control ratio (RCR). Glutamate plus malate (2.5mM each) were used as respiratory substrates. Mitochondrial preparations (0.1 mg protein.ml⁻¹) and Phyt (50-200µM) were added to the incubation medium at the beginning of the assays. Controls (Ctrl) were performed in the absence of Phyt. Values are means ± standard deviation calculated as pmol O₂.s⁻¹.mg of protein⁻¹. ** P<0.01, ***P< 0.001 compared to controls (Tukey multiple range test).

Figure 2. Effects of phytanic acid (Phyt) on mitochondrial Ca²⁺ retention capacity in heart mitochondria. All experiments were performed in a reaction medium containing mitochondrial preparations (0.35mg protein.ml⁻¹) supported by glutamate plus malate (2.5mM). Phyt (50-200µg) was added at the beginning of the assay. All panels refer to mitochondrial preparations in the presence of 30µM Ca²⁺, as indicated. Controls were performed in the absence of Phyt. CCCP was added in the end of the assays. Traces are representative of four independent experiments (N) and are expressed as fluorescence arbitrary units (FAU).
RESULTS AND DISCUSSION

Figure 3. Effects of phytanic acid (Phyt) on mitochondrial NAD(P)H content in heart mitochondria. All experiments were performed in a reaction medium containing mitochondrial preparations (0.35mg protein.ml⁻¹) supported by glutamate plus malate (2.5mM each). (A) Phyt (50-200µM) was added 50 s after the beginning of the assay. (B) CsA (1µM) plus ADP (300µM) were added at the beginning of the assays. All panels refer to mitochondrial preparations in the presence of 30µM Ca²⁺, as indicated. Controls were performed in the absence of Phyt. CCCP was added in the end of the assays. Traces are representative of four independent experiments (N) and are expressed as fluorescence arbitrary units (FAU).

Figure 4. Effects of phytanic acid (Phyt) on mitochondrial membrane potential in heart mitochondria. All experiments were performed in a reaction medium containing mitochondrial preparations (0.35mg protein.ml⁻¹) supported by glutamate plus malate (2.5mM each). (A) Phyt (50-200 µM) was added 50 s after the beginning of the assay. (B) CsA (1µM) plus ADP (300µM) were added at the beginning of the assays. All panels refer to mitochondrial preparations in the presence of 30µM Ca²⁺, as indicated. Controls were performed in the absence of Phyt. CCCP was added in the end of the assays. Traces are representative of four independent experiments (N) and are expressed as fluorescence arbitrary units (FAU).

CONCLUSIONS

Taken together, the data indicate that Phyt disrupts mitochondrial energy and Ca²⁺ homeostasis, acting as a strong uncoupler, a metabolic inhibitor and a MPT inducer. It is presumed that these pathomechanisms may contribute to the cardiomyopathy of patients with RD.

REFERENCES


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