Mitochondria e drogas e substancias e hormônios

Effect of estradiol, diethylstilbestrol, and resveratrol on FOF1-ATPase activity from mitochondrial preparations of rat heart, liver, and brain.

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Abstract

The question of whether estrogens or estrogen-like compounds would alter differentially the enzymatic activity of the FOF1-ATPase was addressed. Mitochondrial fractions of the liver, brain, and heart were obtained from adult male rats and solubilized by digitonin. About 85% of the adenosine triphosphate hydrolysis by these three preparations come from the mitochondrial FOF1-ATPase. The enzymatic activity differed in the following order: liver < brain < heart. A concentration of 13 nM estradiol stimulated the FOF1-ATPase activity in heart by 10% (p < 0.01), but not in liver or brain. 17beta-estradiol stopped off the binding of estradiol-17b-carboxymethyl)oxime:125I-labeled bovine serum albumin to mitochondrial preparations of the heart, revealing two binding sites. Resveratrol inhibited the FOF1-ATPase activity in both heart and liver with an IC50 of 13-15 microM, which confirmed our previous report in preparations of brain. Lower doses (picomolar to nanomolar) of resveratrol stimulated the FOF1-ATPase activity in liver by 10% but not in heart. At 6.7 microM, diethylstilbestrol (DES) inhibited the FOF1-ATPase activity in the three preparations by 61-67%. This study demonstrates that estradiol activates rat heart mitochondrial FOF1-ATPase at physiologic concentrations and that the FOF1-ATPase activity is markedly different in rat liver, brain, and heart. In addition, estradiol, DES, and resveratrol alter the FOF1-ATPase activity selectively, probably via different mechanisms.

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Toxicity of amiodarone and amiodarone analogues on isolated rat liver mitochondria.

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Abstract

BACKGROUND: Amiodarone is a well-known mitochondrial toxin consisting of a benzofuran ring (ring A) coupled to a p-OH-benzene structure substituted with 2 iodines and a diethyl-ethanolamine side chain (ring B). AIM: To find out which part of amiodarone is responsible for mitochondrial toxicity. METHODS: Amiodarone, ring A and B without the ethanolamine side-chain and iodines (B0), ring A and B with iodines but no ethanolamine (B2), ring B with 1 iodine and no ethanolamine (C1) and ring B with ethanolamine and 2 iodines (D2) were studied. RESULTS: In freshly isolated rat liver mitochondria, amiodarone inhibited state 3 glutamate and palmitoyl-CoA oxidation and decreased the respiratory control ratios. B0 and B2 were more potent inhibitors than amiodarone and B2 more potent than B0. C1 and D2 showed no significant mitochondrial toxicity. After disruption, mitochondrial oxidases and complexes of the electron transport chain were inhibited by amiodarone, B0 and B2, whereas C1 and D2 revealed no inhibition. Beta-oxidation showed a strong inhibition by amiodarone, B0 and B2 but not by C1 or D2. Ketogenesis was almost unaffected. CONCLUSIONS: Amiodarone, B0 and B2 are uncouplers of oxidative phosphorylation, and inhibit complexes I, II and III, and beta-oxidation. The benzofuran structure is responsible for mitochondrial toxicity of amiodarone and the presence of iodine is not essential.

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Amiodarone induces cytochrome c release and apoptosis through an iodine-independent mechanism.

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Abstract

Amiodarone (AMD) is one of the most effective antiarrhythmic drugs available. However, its use is often limited by side-effects, mainly hypothyroidism. As AMD displays direct toxic effect on different cell types, we investigated the cytotoxic effect of AMD and its main metabolite, desethylamiodarone (DEA), in thyroid (TAD-2) and nonthyroid (HeLa) cell lines. Both AMD and DEA displayed a dose-dependent toxicity in TAD-2 and HeLa cells, although DEA was more effective. Both TAD-2 and HeLa cells underwent apoptosis, as evidenced by plasma membrane phosphatidylserine exposure and DNA fragmentation. Inhibition of protein synthesis with cycloheximide and inhibition of endogenous peroxidase activity with propylthiouracil did not affect this AMD- and DEA-induced apoptosis in TAD-2 cells. Western blot analysis did not display variations in the expression of p53, Bcl-2, Bcl-XL, and Bax proteins during the treatment with AMD and DEA. Generation of reactive oxygen species, investigated by flow cytometry with dichlorofluorescein diacetate, did not show the production of free radicals during drug treatment. Furthermore, Western blot analysis of cytosolic and mitochondrial fractions prepared from AMD-treated cells demonstrated that AMD induces the release of cytochrome c into the cytosol from the mitochondria. These data indicate that AMD induces cytochrome c release from mitochondria, triggering apoptosis through an iodine-independent mechanism, and that this process is not mediated by modulation of p53, Bcl-2, Bcl-XL, or Bax protein expression and does not involve the generation of free radicals.

PMID: 11095475


Hepatocellular toxicity and pharmacological effect of amiodarone and amiodarone derivatives.

Waldhauser KM, Török M, Ha HR, Thomet U, Konrad D, Brecht K, Follath F, Krähenbühl S.
Drug-induced mitochondrial toxicity.

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Abstract
Mitochondria play a critical role in generating most of the cell’s energy as ATP. They are also involved in other metabolic processes such as urea generation, haem synthesis and fatty acid beta-oxidation. Dysfunction of mitochondrial function by drugs can result in cell death by necrosis or can signal cell death by apoptosis (e.g., following cytochrome c release). Drugs that injure mitochondria usually do so by inhibiting respiratory complexes of the electron chain; inhibiting or uncoupling oxidative phosphorylation; inducing mitochondrial oxidative stress; or inhibiting DNA replication, transcription or translation. It is important to test for mitochondrial toxicity early in drug development as impairment of mitochondrial function can induce various pathological conditions that are life threatening or can increase the progression of existing mitochondrial diseases.

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Melatonin protects against common deletion of mitochondrial DNA-augmented mitochondrial oxidative stress and apoptosis.

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Abstract
Defected mitochondrial respiratory chain (RC), in addition to causing a severe ATP deficiency, often augments reactive oxygen species (ROS) generation in mitochondria (mROS) which enhances pathological conditions and diseases. Previously, we demonstrated a potent endogenously RC defect-augmented mROS associated dose-dependently with a commonly seen large-scale deletion of 4977 base pairs of mitochondrial DNA (mtDNA), i.e. the common deletion (CD). As current treatments for CD-associated diseases are rather supplementary and ineffective, we investigated whether melatonin, a potential mitochondrial protector, provides beneficial protection for CD-augmented mitochondrial oxidative stress and apoptosis particularly upon the induction of a secondary oxidative stress. Detailed mechanistic investigations were performed by using laser scanning dual fluorescence imaging microscopy to provide precise spatial and temporal resolution of mitochondrial events at single cell level. We demonstrate, for the first time, that melatonin significantly prevents CD-augmented mROS formation under basal conditions as well as at early time-points upon secondary oxidative stress induced by H2O2 exposure. Thus, melatonin prevents mROS-mediated depolarization of mitochondrial membrane potential (DeltaPsi(m)) and subsequent opening of the mitochondrial permeability transition pore (MPTP) and cytochrome c release. Moreover, melatonin prevents depletion of cardiolipin which appears to be crucial for postponing later MPTP opening, disruption of the mitochondrial membrane and apoptosis. Finally, the protection provided by melatonin is superior to those caused by the suppression of mitochondrial Ca2+ regulators including the mitochondrial Na+/Ca2+ exchanger (MPTP), and the mitochondrial Ca2+ uniporter and by antioxidants including vitamin E and mitochondria-targeted coenzyme Q, MitoQ. As RC defect-augmented endogenous mitochondrial oxidative stress is centrally involved in a variety of pathological conditions and diseases, melatonin thus may serve as a therapeutic drug to benefit many clinical conditions that involve malfunction of the mitochondria.

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Parabens in male infertility—Is there a mitochondrial connection?

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Abstract
Parabens are widely used as preservatives in many foods, cosmetics, toiletries, and pharmaceuticals due to their relatively low toxicity profile and to a long history of safe use. Parabens are alkyl esters of p-hydroxybenzoic acid and typically include methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben, isopropylparaben and benzylparaben. These compounds are known to have a null or very weak estrogenic activity in estrogen receptor assays in vitro. In recent years, an increasing concern has emerged...
Mitochondrial medicine: pharmacological targeting of mitochondria in disease.

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Abstract

Mitochondria play a central role in cell life and death and are known to be important in a wide range of diseases including the cancer, diabetes, cardiovascular disease, and the age-related neurodegenerative diseases. The unique structural and functional characteristics of mitochondria enable the selective targeting of drugs designed to modulate the function of this organelle for therapeutic gain. This review discusses evidence that parabens may not be as safe as initially thought, and suggests that the interaction between parabens and mitochondrial function in the testis may be key in explaining the contribution of parabens for a decrease in reproductive potential.

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Valproic Acid II: Effects on Oxidative Stress, Mitochondrial Membrane Potential, and Cytotoxicity in Glutathione-Depleted Rat Hepatocytes

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Oxidative stress has been associated with valproic acid (VPA) treatment, and mitochondrial dysfunction has been implicated in the pathogenesis of VPA-idiosyncratic hepatotoxicity. The present study investigated the effect of VPA and the role of GSH on oxidative stress, mitochondrial membrane potential, and toxicity in freshly isolated rat hepatocytes. Hepatocytes were isolated from Sprague-Dawley rats, and total levels of glutathione (GSH) reduced by pretreatment with a combination of L-buthionine sulfoximine (2 mM) and diethylmaleate (0.5 mM) prior to VPA (0–1000 µg/ml) treatment. Oxidative stress was determined by measuring the levels of 15-F2t-isoprostane (15-F2t-Isop) and 2',7'-dichlorofluorescein (DCF). Mitochondrial membrane potential (ΔΨm) was determined by using the dual-fluorescent dye JC-1, and cell viability was evaluated by the water-soluble tetrazolium salt WST-1 assay. Exposure of rat hepatocytes to VPA (0–1000 µg/ml) resulted in a time- and dose-dependent increase in 15-F2t-isoprostane and DCF fluorescence, and these levels were further elevated in GSH-reduced hepatocytes. In control hepatocytes, VPA had no effect on cell viability; however, significant cytotoxicity was observed in the glutathione-depleted hepatocytes treated with 1000 µg/ml VPA. The ΔΨm was only reduced in glutathione-reduced hepatocytes at 500 and 1000 µg/ml VPA. Our novel findings indicate that acute treatment of freshly isolated rat hepatocytes with VPA resulted in oxidative stress, which occurred in the absence of cytotoxicity, and that glutathione confers protection against hepatocyte injury associated with mitochondrial damage by VPA.

Key Words: Valproic acid; hepatocytes; 15-F2t-isoprostane; mitochondrial membrane potential; glutathione, 2',7'-dichlorofluorescein.


The causes of cancer revisited: "mitochondrial malignancy" and ROS-induced oncogenic transformation - why mitochondria are targets for cancer therapy.

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Abstract

The role of oncogenes and tumor suppressor proteins in promoting the malignant transformation of mammalian cells by affecting properties such as proliferative signalling, cell cycle regulation and altered adhesion is well established. Chemicals, viruses and radiation are also generally accepted as agents that commonly induce mutations in the genes encoding these cancer-causing proteins, thereby giving rise to cancer. However, more recent evidence indicates the importance of two additional key factors imposed on proliferating cells that are involved in transformation to malignancy and these are hypoxia and/or stressful conditions of nutrient deprivation (e.g. lack of glucose). These two additional triggers can initiate and promote the process of malignant transformation when a low percentage of cells escape cellular senescence. It is becoming apparent that hypoxia causes the progressive elevation in mitochondrial ROS production (chronic ROS) which over time leads to stabilization of cells via increased HIF-2alpha expression, enabling cells to survive with sustained levels of elevated ROS. In cells under hypoxia and/or low glucose, DNA mismatch repair processes are repressed by HIF-2alpha and they continually accumulate mitochondrial ROS-induced oxidative DNA damage and increasing numbers of
mutations driving the malignant transformation process. Recent evidence also indicates that the resulting mutated cancer-causing proteins feedback to amplify the process by directly affecting mitochondrial function in combinatorial ways that intersect to play a major role in promoting a vicious spiral of malignant cell transformation. Consequently, many malignant processes involve periods of increased mitochondrial ROS production when a few cells survive the more common process of oxidative damage induced cell senescence and death. The few cells escaping elimination emerge with oncogenic mutations and survive to become immortalized tumors. This review focuses on evidence highlighting the role of mitochondria as drivers of elevated ROS production during malignant transformation and hence, their potential as targets for cancer therapy. The review is organized into five main sections concerning different aspects of "mitochondrial malignancy". The first concerns the functions of mitochondrial ROS and its importance as a pacemaker for cellular growth versus senescence and death. The second considers the available evidence that cellular stress in the form of hypoxic and/or hypoglycaemic conditions represent two of the major triggering events for cancer and how oncoproteins reinforce this process by altering gene expression to bring about a common set of changes in mitochondrial function and activity in cancer cells. The third section presents evidence that oncoproteins and tumor suppressor proteins physically localize to the mitochondria in cancer cells where they directly regulate malignant mitochondrial programs, including apoptosis. The fourth section covers common mutational changes in the mitochondrial genome as they relate to malignancy and the relationship to the other three areas. The last section concerns the relevance of these findings, their importance and significance for novel targeted approaches to anti-cancer therapy and selective triggering in cancer cells of the mitochondrial apoptotic pathway. Crown Copyright 2010. Published by Elsevier Ltd. All rights reserved.

Mitochondrial bioenergetic adaptations of breast cancer cells to aglycemia and hypoxia.

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Abstract

Breast cancer cells can survive and proliferate under harsh conditions of nutrient deprivation, including limited oxygen and glucose availability. We hypothesized that such environments trigger metabolic adaptations of mitochondria, which promote tumor progression. Here, we mimicked aglycemia and hypoxia in vitro and compared the mitochondrial and cellular bioenergetic adaptations of human breast cancer (HTB-126) and non-cancer (HTB-125) cells that originate from breast tissue. Using high-resolution respirometry and western blot analyses, we demonstrated that 4 days of glucose deprivation elevated oxidative phosphorylation five-fold, increased the spread of the mitochondrial network without changing its shape, and decreased the apparent affinity of oxygen in cancer cells (increase in C_50), whereas it remained unchanged in control cells. The substrate control ratios also remained constant following adaptation. We also observed the Crabtree effect, specifically in HTB-126 cells. Likewise, sustained hypoxia (1% oxygen during 6 days) improved cell respiration in non-cancer cells grown in glucose or glucose-deprived medium (+32% and +38%, respectively). Conversely, under these conditions of limited oxygen or a combination of oxygen and glucose deprivation for 6 days, routine respiration was strongly reduced in cancer cells (-36% in glucose medium, -24% in glucose-deprived medium). The data demonstrate that cancer cells behave differently than normal cells when adapting their bioenergetics to microenvironmental conditions. The differences in hypoxia and aglycemia tolerance between breast cancer cells and non-cancer cells may be important when optimizing strategies for the treatment of breast cancer.

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