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Indirubin-3′-oxime impairs mitochondrial oxidative phosphorylation and prevents mitochondrial permeability transition induction

Ana T. Varela [1], Ana P. Gomes [1], Anabela M. Simões, João S. Teodoro, Filipe V. Duarte, Anabela P. Rolo, Carlos M. Palmeira *

Center for Neurosciences and Cell Biology, Department of Zoology, University of Coimbra, 3004-517 Coimbra, Portugal

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ABSTRACT

Indirubin, a red colored 3,2′-bisindole isomer, is a component of *Indigo naturalis* and is an active ingredient used in traditional Chinese medicine for the treatment of chronic diseases. The family of indirubin derivatives, such as indirubin-3′-oxime, has been suggested for various therapeutic indications. However, potential toxic interactions such as indirubin effects on mitochondrial bioenergetics are still unknown. This study evaluated the action of indirubin-3′-oxime on the function of isolated rat liver mitochondria contributing to a better understanding of the biochemical mechanisms underlying the multiple effects of indirubin. Indirubin-3′-oxime incubated with isolated rat liver mitochondria, at concentrations above 10μM, significantly depresses the phosphorylation efficiency of mitochondria as inferred from the decrease in the respiratory control and ADP/O ratios, the perturbations in mitochondrial membrane potential and in the phosphorylative cycle induced by ADP. Furthermore, indirubin-3′-oxime at up to 25μM stimulates the rate of state 4 respiration and inhibits state 3 respiration. The increased lag phase of repolarization was associated with a direct inhibition of the mitochondrial ATPase. Indirubin-3′-oxime significantly inhibited the activity of complex II and IV thus explaining the decreased FCCP-stimulated mitochondrial respiration. Mitochondria pre-incubated with indirubin-3′-oxime exhibits decreased susceptibility to calcium-induced mitochondrial permeability transition. This work shows for the first time multiple effects of indirubin-3′-oxime on mitochondrial bioenergetics thus indicating a potential mechanism for indirubin-3′-oxime effects on cell function.

Introduction

Indirubin, a red colored 3,2′-bisindole isomer, is a component of *Indigo naturalis* and is an active ingredient used in traditional Chinese medicine for the treatment of chronic diseases such as leukemias (Eisenbrand et al., 2004). Several papers describing the properties of the indirubin family as cyclin-dependent kinases (CDKs) (Hoessel et al., 1999; Leclerc et al., 2001) and glycogen synthase kinase-3β (GSK-3β) (Leclerc et al., 2001; Meijer et al., 2003) inhibitors unravelled the potential therapeutic indication of these compounds. Indirubin and derivatives may have important implications for the development of therapies for many diseases such as ischemia–reperfusion, Alzheimer's disease, cancer and type 2 diabetes (Barillas et al., 2007; Meijer et al., 2003; Jope et al., 2007), as well as in stem cell therapy (Sato et al., 2004).

Numerous indirubin analogs have been synthesized to optimize this promising drug scaffold. Indirubin-3′-oxime is an analogue of indirubin commercially available. Addition of a 3-oxime substitution led to an overall increase in kinase inhibitory effects (Zhang et al., 2006) and increased solubility (Meijer et al., 2003). However, drugs for various therapeutic indications frequently have unexpected effects as a result from unknown interactions between the intended drug and biochemical pathways. Such unexpected activities may lead to adverse effects and toxicity, thus disabling the potential therapeutic action. In the last years, several mitochondrial off targets of drug action have been shown as responsible for adverse effects. Such mitochondrial toxicity leads to metabolic failure since mitochondria constitute the principal energy-producing organelles of the cell through oxidative phosphorylation. Therefore, alterations of mitochondrial bioenergetic features by mitochondrial toxicants perturb energetic charge and balance of cell and may cause drastic consequences on cellular function.

Previous studies suggest an activity of indirubin and its derivates on the mitochondria (Lee et al., 2005 and MacDonald et al., 2006), however the effect of indirubin in mitochondrial bioenergetics remains unknown. So, this study examines the effects of indirubin-3′-oxime in bioenergetic functions of isolated rat liver mitochondria contributing to a better comprehension of biochemical mechanisms underlying the effects of indirubin. By using a cell-free model such as isolated mitochondria as the experimental model it is possibly to clearly identify if indirubin-3′-

* Corresponding author. Fax: +351 239 855789.
E-mail address: palmeira@ci.uc.pt (C.M. Palmeira).
[1] Both authors contributed equally to the work.

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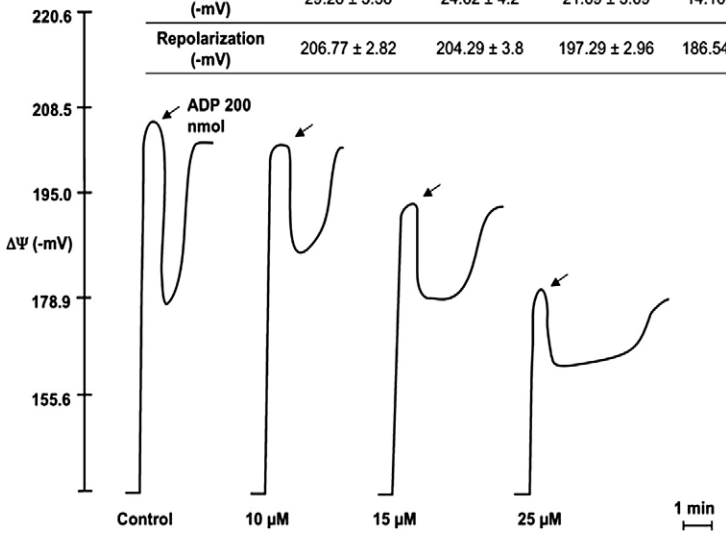
	Control	10μM	15μM	25μM
Initial ΔΨ (-mV)	208.55 ± 2.75	206.23 ± 3.39	199.94 ± 3.96	182.79 ± 4.43*
Depolarization (-mV)	29.28 ± 3.38	24.62 ± 4.2	21.69 ± 3.69	14.16 ± 2.42*
Repolarization (-mV)	206.77 ± 2.82	204.29 ± 3.8	197.29 ± 2.96	186.54 ± 3.44*



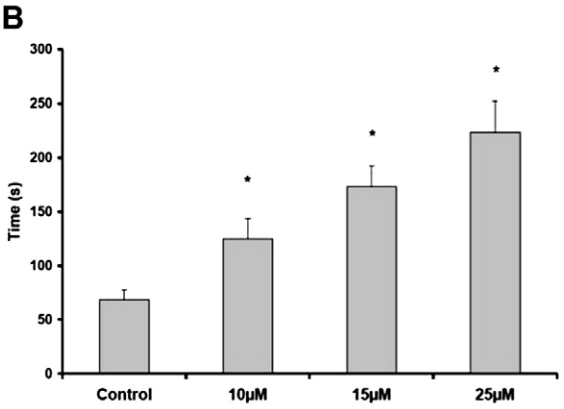


Fig. 1. (A) Mitochondrial transmembrane potential (ΔΨ); (B) Lag phase in succinate-energized liver mitochondria isolated, upon incubation with indirubin-3′-oxime. ΔΨ was measured with a TPP^+-selective electrode. Reactions were carried out in 1 ml of reaction medium, supplemented with 2 μM rotenone and 1 mg of freshly isolated mitochondria, as described in Materials and methods. Energization was achieved with 5 mM succinate and phosphorylation induced by 200 nmol ADP. The traces represent typical direct recordings and data are means±S.E.M of experiments performed with four different mitochondrial preparations. * indicates statistically significant difference versus control ($P<0.05$).

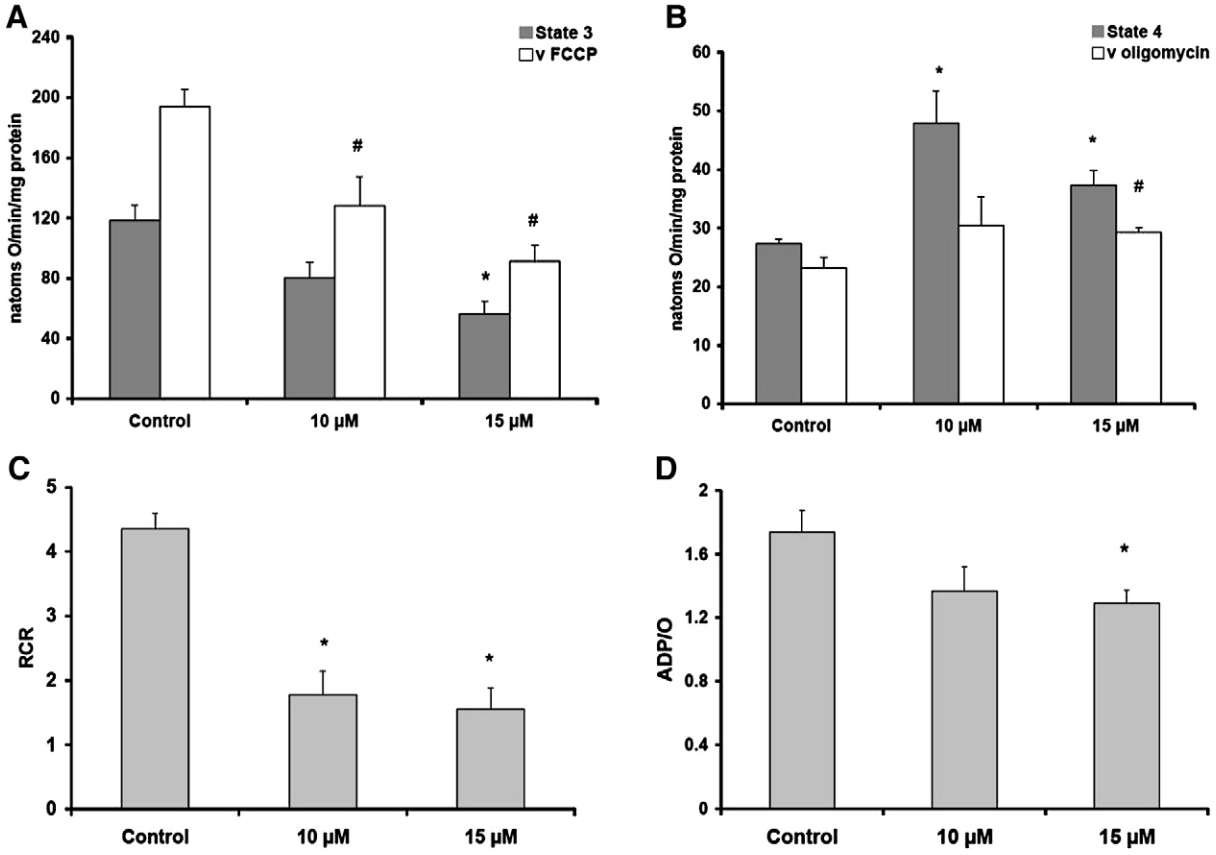


Fig. 2. (A) State 3 respiration and FCCP-stimulated oxygen consumption (V FCCP); (B) State 4 respiration and oligomycin-inhibited oxygen consumption; (C) Respiratory control ratio (RCR); (D) ADP/O in liver mitochondria, upon incubation with indirubin-3′-oxime. Reactions were carried out in 1.4 ml of reaction medium, supplemented with 2 μM rotenone and 1 mg of freshly isolated mitochondria, as described in Materials and methods. Energization was achieved with 5 mM succinate and phosphorylation induced by 200 nmol ADP. Data are means±S.E.M of three different mitochondria preparations. For panel A, * indicates statistically significant difference in state 3 respiration versus control ($P<0.05$), # indicates statistically significant difference in V FCCP versus control ($P<0.05$). For panel B, * indicates statistically significant difference in state 4 respiration versus control ($P<0.05$), # indicates statistically significant difference in V Oligomycin versus control ($P<0.05$). For panels C and D, * indicates statistically significant difference versus control ($P<0.05$).