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The cellular and molecular progression of mitochondrial dysfunction induced by 2,4-dinitrophenol in developing zebrafish embryos

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ABSTRACT

The etiology of mitochondrial disease is poorly understood. Furthermore, treatment options are limited, and diagnostic methods often lack the sensitivity to detect disease in its early stages. Disrupted oxidative phosphorylation (OXPHOS) that inhibits ATP production is a common phenotype of mitochondrial disorders that can be induced in zebrafish by exposure to 2,4-dinitrophenol (DNP), a FDA-banned weight-loss agent and EPA-regulated environmental toxicant, traditionally used in research labs as an uncoupler of OXPHOS. Despite the DNP-induced OXPHOS inhibition we observed using in vivo respirometry, the development of the DNP-treated and control zebrafish were largely similar during the first half of embryogenesis. During this period, DNP-treated embryos induced gene expression of mitochondrial and nuclear genes that stimulated the production of new mitochondria and increased glycolysis to yield normal levels of ATP. DNP-treated embryos were incapable of sustaining this mitochondrial biogenic response past mid-embryogenesis, as shown by significantly lowered ATP production and ATP levels, decreased gene expression, and the onset of developmental defects. Examining neural tissues commonly affected by mitochondrial disease, we found that DNP exposure also inhibited motor neuron axon arbor outgrowth and the proper formation of the retina. We observed and quantified the molecular and physiological progression of mitochondrial dysfunction during development with this new model of OXPHOS dysfunction, which has great potential for use in diagnostics and therapies for mitochondrial disease.

1. Introduction

Most eukaryotic cells are dependent upon mitochondrial energy metabolism through oxidative phosphorylation (OXPHOS) for the majority of their ATP synthesis. Single cells may contain hundreds of mitochondria and each organelle is equipped with at least one (but typically more) copies of the mitochondrial genome, which encodes only 13 of the hundreds of genes required for OXPHOS (Chinnery and Hudson, 2013). Furthermore, patients carrying identical mutations may present with varied pathologies affecting different organ systems (Haas et al., 2008). These complex features of mitochondrial biology have complicated the development of reliable diagnostics and treatment strategies for mitochondrial disease, especially pediatric disease (Koenig, 2008). As a result, many questions remain as to how mitochondrial dysfunction progresses during development. Mitochondrial dysfunction often occurs in tissues with high energetic needs, and a large proportion of patients develop nervous system pathologies that result in sensory and motor deficits (DiMauro and Schon, 2008; Koenig, 2008). The sensitivity of the nervous system may be due to the high energy demands required to maintain the ion gradients across neuronal membranes and neurons have a low capacity to store ATP for later use (Erecinska et al., 2004). Post-mitotic neuronal cells also accumulate deleterious mitochondrial DNA (mtDNA) mutations at a high rate, which can affect expression of proteins involved in OXPHOS and exacerbate mitochondrial dysfunction (Bender et al., 2006; Zsurka and Kunz, 2013). In addition to their critical role as ATP generators, mitochondria also control calcium homeostasis, generate reactive oxygen species

Abbreviations: AO, acridine orange; CaP, caudal primary; DNP, 2,4-dinitrophenol; FCCP, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone; GCL, ganglion cell layer; hpf, hours post-fertilization; INL, inner nuclear layer; L, lens; N, neural tube; N, notochord; PL, photoreceptor layer; RGC, retinal ganglion cell; mtDNA, mitochondrial DNA; OCR, oxygen consumption rate; OVL, otic vesicle length; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; YE, yolk extension

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(ROS), and regulate apoptosis and many other cellular signaling cascades. These non-ATP generating processes are thought to play an underappreciated role in the development of tissue-specific pathologies that result from mitochondrial disease (Raimundo, 2014), and may in part explain why metabolically-demanding tissues (i.e., heart and central nervous system) are not equally affected by mitochondrial dysfunction.

It remains unclear how mitochondrial dysfunction produces the severe sensory and motor deficits seen in patients (DiMauro and Schon, 2008). Hindering the development of therapeutics is the dearth of experimental systems that model progressive mitochondrial disease and mitochondrial dysfunction in vivo. Here we have disrupted the mitochondrial proton gradient in developing zebrafish embryos with 2,4-dinitrophenol (DNP), a FDA-banned weight loss agent and EPA-regulated environmental toxicant, which is used experimentally as an uncoupler of OXPHOS (Harris and Corcoran, 1995). DNP transports protons across the mitochondrial inner membrane, altering the proton gradient and inhibiting ATP production via OXPHOS (Lou et al., 2007). ROS are a byproduct of OXPHOS and as DNP decreases OXHPOS via dissipation of the proton gradient, DNP treatment will decrease ROS production. Indeed, work in mice has demonstrated that DNP treatment results in lowered ROS production (Caldeira da Silva et al., 2008), an observation that follows other work postulating that mitochondrial uncoupling is an actively regulated process employed to decrease oxidative damage and stress (Brand, 2000). In addition to the detrimental effects of ROS, recent work has shown that mitochondrially-generated ROS are critical signaling molecules that regulate a growing list of cell functions (Raimundo, 2014), including regulation of the cell cycle (Trachootham et al., 2008).

In healthy cells, mitochondria are plastic and cells respond to changing energy demands by regulating ATP synthesis, as well as altering the number of mitochondria (Liesa and Shirihai, 2013). We were interested in quantifying the effects of DNP-induced mitochondrial dysfunction on the molecular and cellular development of embryos, in order to determine the extent to which embryos are capable of mounting molecular defenses to compensate for chronic mitochondrial dysfunction. Within hours of

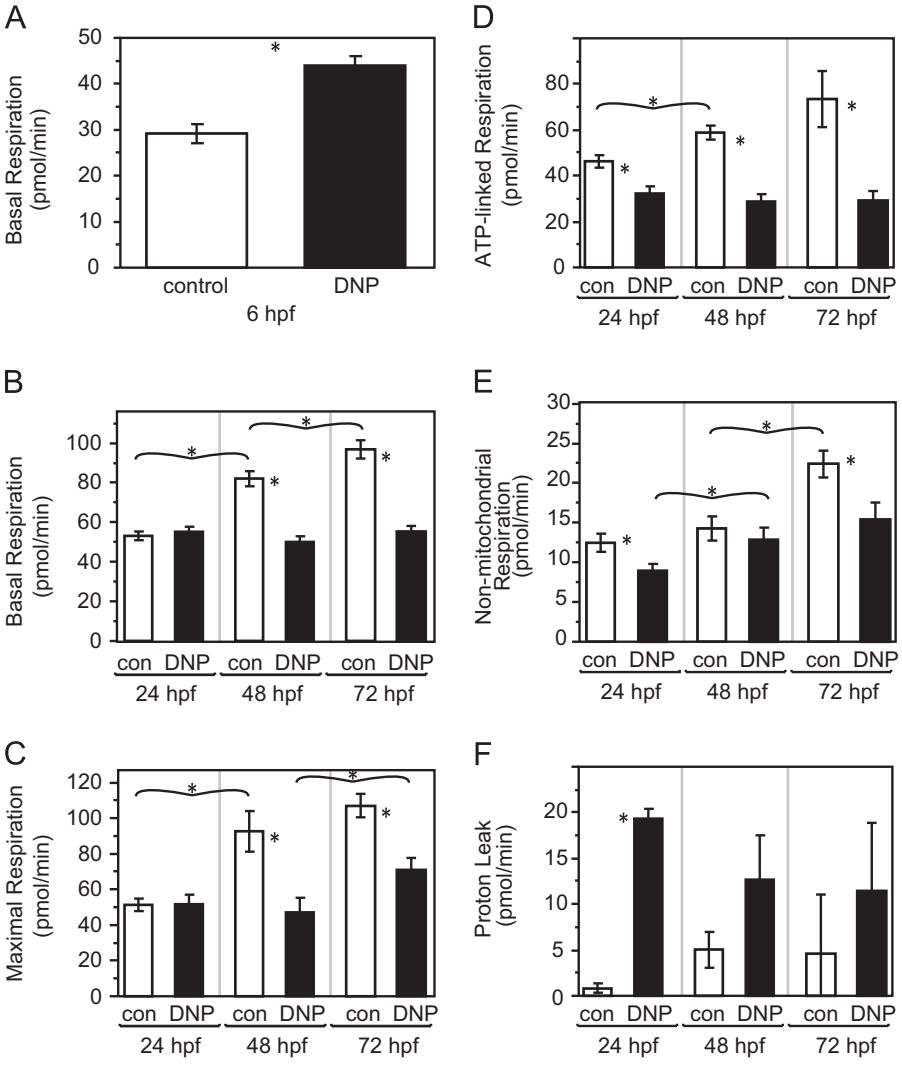

Fig. 1. DNP induces mitochondrial dysfunction, inhibits mitochondrial ATP production and prevents normal developmental increases in mitochondrial output. In vivo respiration (oxygen consumption) rates were measured from intact zebrafish embryos. (A) At 6 hpf, basal respiration was significantly increased in DNP-treated embryos compared to control fish indicating DNP uncoupled the mitochondria and compromised OXPHOS. $n=9$ and 8. (B) At 24 hpf, basal respiration of DNP treated embryos is not significantly different from control levels. The normal developmental increases basal respiration seen in the control animals are inhibited by DNP exposure. (C) Maximal respiration is decreased in DNP-exposed embryos at the later 48 and 72 hpf time points, but is not changed at 24 hpf. (D) Compared to controls, the ATP linked (oligomycin-sensitive) respiration of DNP-treated embryos is significantly decreased at all time points. (E) Non-mitochondrial respiration (azide-insensitive) respiration is decreased in DNP-exposed embryos at 24 and 72 hpf. (F) Proton leak in DNP-treated embryos is significantly higher than controls only at 24 hpf, $n=27–38$ embryos per time point. * Indicates significant ($p<0.05$) difference from control values or significant difference between time points.