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Original Contribution

Mitochondrial hydrogen peroxide production alters oxygen consumption in an oxygen-concentration-dependent manner

Shane E. Munns, James K.C. Lui, Peter G. Arthur*

School of Biomedical & Chemical Sciences, M310, University of Western Australia, Crawley, WA 6009, Australia

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Abstract

Metabolic responses of mammalian cells toward declining oxygen concentration are generally thought to occur when oxygen limits mitochondrial ATP production. However, at oxygen concentrations markedly above those limiting to mitochondria, several mammalian cell types display reduced rates of oxygen consumption without energy stress or compensatory increases in glycolytic ATP production. We used mammalian Jurkat T cells as a model system to identify mechanisms responsible for these changes in metabolic rate. Oxygen consumption was 31% greater at high oxygen (150–200 μM) compared to low oxygen (5–10 μM). Hydrogen peroxide was implicated in the response as catalase prevented the increase in oxygen consumption normally associated with high oxygen. Cell-derived hydrogen peroxide, predominately from the mitochondria, was elevated with high oxygen. Oxygen consumption related to intracellular calcium turnover was shown, through EDTA chelation and dantrolene antagonism of the ryanodine receptor, to account for 70% of the response. Oligomycin inhibition of oxygen consumption indicated that mitochondrial proton leak was also sensitive to changes in oxygen concentration. Our results point toward a mechanism in which changes in oxygen concentration influence the rate of hydrogen peroxide production by mitochondria, which, in turn, alters cellular ATP use associated with intracellular calcium turnover and energy wastage through mitochondrial proton leak.

Keywords: Mitochondria; Hydrogen peroxide; Hypoxia; Metabolic rate; Proton leak; Oxygen sensing; Free radicals

ATP production from oxidative phosphorylation is critically dependent on an adequate oxygen supply. An inadequate oxygen supply results in a decline in pericellular oxygen concentration and elicits a multitude of responses in mammals aimed at maintaining ATP production. Systemic responses include increased cardiac output, red blood cell production, and the redistribution of blood flow (systemic arterial vasodilation and pulmonary arterial vasoconstriction) [1]. At the molecular level a declining oxygen concentration activates transcription of proteins such as erythropoietin, nitric oxide synthase, and vascular endothelial growth factor [2]. These responses are designed to increase oxygen delivery to cells, minimizing the effects of the reduced oxygen supply.

Metabolic responses of mammalian cells toward declining oxygen concentration are generally thought to occur when the concentration of oxygen limits cytochrome oxidase activity. It is not certain when oxygen becomes limiting, but it has been suggested to be less than 3 μM (0.2 kPa) for isolated mitochondria [3]. A lack of oxygen at the mitochondria typically causes a decline in oxygen consumption, cellular energy stress (reduced ATP synthesis leading to a decrease in ATP concentration), and a partial compensatory increase in the rate of glycolytic ATP production [4]. However, a novel metabolic response has been identified in which changes in the oxygen consumption of mammalian cells and tissue have been found to occur at oxygen concentrations markedly above (up to 90 μM)

Abbreviations: FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; HBS, Hepes-buffered saline; CM-H_2DCFDA, 5-(and 6-)chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide; EFA, ethoxyformic anhydride diethyl pyrocarbonate.

* Corresponding author. Fax: (08) 6488 1148.
E-mail address: parthur@cyllene.uwa.edu.au (P.G. Arthur).

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those limiting to the mitochondria. Characteristically, a decline in oxygen concentration causes a decrease in oxygen consumption, but without the expected cellular energy stress (e.g., decline in ATP or phosphocreatine if present) or compensatory increase in glycolytic ATP production [5–7]. This response has been identified in a variety of mammalian models, including isolated primary hepatocytes [5], isolated primary cardiomyocytes [6], the C_2C_{12} muscle cell line [7], whole tissue muscle hind-limb preparations [8,9], and in vivo hypoxic studies on rats [10]. There has also been a suggestion that low oxygen concentration affects cardiac basal metabolism [11]. The biological significance of this response toward low oxygen concentration is not known, but it may be related to prolonging function and/or viability of cells during times of compromised oxygen supply [12,13].

A reduced rate of oxygen consumption without a compensatory increase in glycolytic ATP production implies that the rate of ATP synthesis is decreased at low oxygen. A feature of this response, however, is the maintenance of intracellular ATP concentration at low oxygen. This implies that ATP-using processes decrease in conjunction with the rate of oxygen consumption, permitting the cell to maintain the concentration of intracellular ATP.

The adjustment of cellular ATP use in response to declining oxygen concentrations suggests an intricate pathway of metabolic regulation via oxygen sensing. We have utilized mammalian Jurkat T cells as a model system, because they can be cultured and used in suspension, to identify the mechanisms underlying the oxygen-concentration-sensitive changes in cellular oxygen consumption. The main objectives of our study were to: (1) elucidate the mechanism through which oxygen consumption (cellular ATP/energy use) was altered by oxygen concentration and (2) identify ATP/energy-using processes sensitive to oxygen concentration. Our results point toward a mechanism in which changes in oxygen concentration influence the rate of hydrogen peroxide production by mitochondria, which, in turn, alters cellular ATP use associated with intracellular calcium turnover and energy wastage through mitochondrial proton leak.

Materials and methods

Jurkat cell culture

Jurkat cells were cultured in a spinner flask with continuous agitation (75 rpm) at 37°C in a humidified incubator with 95% air and 5% CO_2. Culture medium (RPMI 1640) was supplemented with 10% FCS, 2 mM glutamine, 50 IU/ml penicillin, and 50 μg/ml streptomycin. All experiments and cell preparations were carried out in darkened work areas under red light to limit the production of reactive oxygen species from the exposure of culture/experimental medium to light [14].

Jurkat cell preparation for experiments

The experimental medium was a Hepes-buffered saline (HBS) containing (in mM) Hepes, 20; $Ca(NO_3)_2$, 0.42; KCl, 5.33; $MgSO_4$, 0.41; NaCl, 139; Na_2HPO_4, 5.63; glucose, 5; glutamine, 2, at pH 7.4, supplemented with FCS (10%). Unless otherwise stated, Jurkat cells were prepared for experiments by centrifugation (3 min × 500*g*) and then resuspended at a cell concentration of 1×10^6 cells/ml in 37°C HBS pregassed with a humidified low-oxygen (10 μM) gas mix. Aliquots of Jurkat cells were transferred to the experimental chamber and incubated for 30 min at low (10 μM) or high oxygen (150–200 μM) before experimental use.

Oxygen consumption

A Clark-type electrode was used to measure rates of oxygen consumption [15]. Briefly, Jurkat cells were suspended in HBS and stirred in a closed glass chamber maintained at 37°C. Within the sealed closed chamber the oxygen electrode measured decreases in oxygen concentration as a function of cellular oxygen consumption. Oxygen consumption measurements taken between 5 and 10 μM were defined as low oxygen and between 150 and 200 μM as high oxygen.

Fluorescence measurements

Fluorescence measurements were carried out in a Shimadzu RF5000 spectrofluorophotometer modified to contain a glass closed-chamber system and oxygen electrode. Fluorescence measurements from cell suspensions were taken through the walls of the glass closed chamber, allowing the monitoring of oxygen concentration while taking simultaneous measurement of fluorescence and cellular oxygen consumption.

Intracellular hydrogen peroxide

The rate of intracellular hydrogen peroxide production in Jurkat cells was monitored by 5-(and 6-)chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA), a derivative of 2′,7′-dichlorodihydrofluorescein diacetate more efficiently retained by cells [16]. CM-H_2DCFDA (1 μM) was added to cells in the spectrofluorophotometer closed chamber and incubated for 10 min, then fluorescence was measured at the excitation/emission wavelengths of 488 and 526 nm, respectively.

Extracellular hydrogen peroxide

Hydrogen peroxide in the extracellular medium was measured by the horseradish peroxidase substrate Amplex red (*N*-acetyl-3,7-dihydroxyphenoxazine) [17]. Amplex red (10 μM) and horseradish peroxidase (1 unit/ml) were added to HBS in the spectrofluorophotometer closed chamber and