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Review

The importance of controlling *in vitro* oxygen tension to accurately model *in vivo* neurophysiology

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ABSTRACT

The majority of *in vitro* studies modeling *in vivo* conditions are performed on the lab bench in atmospheric air. However, the oxygen tension (pO_2) present in atmospheric air (160 mm Hg, ~21% O_2) is in great excess to the pO_2 that permeates tissues within the brain (5–45 mm Hg, ~1–6% O_2). This review will discuss the differentiation between pO_2 in the *in vivo* environment and the pO_2 commonly used during *in vitro* experiments, and how this could affect assay outcomes. Also highlighted are studies linking changes in pO_2 to changes in cellular function, particularly the role of pO_2 in mitochondrial function, reactive oxygen species production, and cellular growth and differentiation. The role of hypoxia inducible factor 1 and oxygen sensing is also presented. Finally, emerging literature exploring sex differences in tissue oxygenation is discussed.

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1. Introduction

Although its concentration has varied over the course of time, oxygen now composes ~21% of the earth's atmosphere (Canfield, 2014). While increases in pO_2 allowed for larger organisms to exist, these organisms needed to develop mechanisms to ensure that oxygen was effectively delivered to all of their cells (Halliwell and Gutteridge, 2015). As O_2 has a limited solubility in water, the majority of O_2 in mammalian blood is carried by hemoglobin (Revsbech and Fago, 2017). Oxygen reversibly binds to heme at higher O_2 concentrations in the lungs, and dissociates at lower concentrations of O_2 in tissue (Halliwell and Gutteridge, 2015). Due to its reactive nature, O_2 is utilized during mitochondrial energy

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metabolism as an electron acceptor, but can also act as a toxin (Ndubuizu and LaManna, 2007). The possible toxicity of O_2 requires that a range of oxygen tensions must exist that are advantageous for cellular function, and, as such, mechanisms must exist to properly regulate oxygen concentrations in various tissues (Ndubuizu and LaManna, 2007). Atmospheric pO_2 (160 mm Hg, ~21% O_2) is in great excess compared to the pO_2 that reaches tissues within the brain (5–45 mm Hg, ~1–6% O_2) (Erecińska and Silver, 2001; Feng et al., 1988; Grote et al., 1996). The vast majority of *in vitro* research has been performed at 21% O_2 out of ease and affordability. While there is growing recognition that studies performed *in vitro* are not a perfect model for the *in vivo* environment (Hellwig et al., 2013; Neiva et al., 2014; Ransohoff, 2016), surprisingly few have attempted to bridge the gap in order to more accurately model *in vivo* physiological functions in an *in vitro* setting.

The considerable amount of literature exploring hypoxia and hyperoxia *in vivo* have revealed that oxygen tension changes can drastically affect a wide variety of cellular functions and will be briefly reviewed. However, many *in vitro* studies attempting to model hypoxia or hyperoxia simply use the same oxygen tensions to which an entire organism would be exposed, *i.e.* culturing 'normoxic' cells at ~21% O_2, culturing 'hypoxic' cells at ~1% O_2, and culturing 'hyperoxic' cells at ~100% O_2. While those may be the oxygen tensions that whole organisms are exposed to, particular tissues within the organism will experience a far lower pO_2.

Here, we review evidence linking changes in pO_2 to changes in cellular function. We also discuss the possible role of pO_2 in mitochondrial function, as well as in reactive oxygen species production. Additionally, we review advances in monitoring tissue oxygenation *in vivo*, and discuss how these techniques can be paired with other assays to more accurately determine the influences of oxygen tension on physiological functions.

2. Methods to measure cellular/tissue oxygenation

Initial experiments measuring brain tissue oxygenation were performed using surface microelectrodes or needle electrodes (Grote et al., 1996, 1981). While these methods revealed that the oxygen tensions reaching brain tissues *in vivo* were far lower than the 21% O_2 present in the atmosphere, their resolution was limited (Xu et al., 2017). Excitingly, a new technique which utilizes two-photon phosphorescence lifetime microscopy provides detailed values of the absolute pO_2 in different tissues (Sakadžić et al., 2010; Xu et al., 2017). This method allows for pO_2 measurements with three-dimensional spatial resolution, a measurement depth of up to 250 μm, and sub-second temporal resolution (Xu et al., 2017). High-resolution images of blood oxygen concentration gradients can then be obtained, allowing for detailed descriptions of pO_2 in different brain regions (Xu et al., 2017). These techniques have revealed the great heterogeneity in brain tissue oxygenation by region and allowed researchers to visualize the dramatic differences between pO_2 in the brain compared to pO_2 in the atmosphere (Fig. 1, modified from (Xu et al., 2017)).

These *in vivo* analyses of brain pO_2 have led to an increasing number of studies utilizing lower oxygen tensions *in vitro* in an attempt to more closely model physiology. However, it is important to remember that simply placing cells at the 'correct' oxygen tension does not necessarily result in those cells experiencing the same pO_2 as their environmental surroundings. Notably, studies measuring the oxygen concentration at the cell surface of cultured cells demonstrate that the pO_2 at the cell surface is lower than the pO_2 in the surrounding gaseous environment, likely due to high levels of oxygen consumption by some *in vitro* cell types (Bambrick et al., 2011; Lewis et al., 2017, 2016). It is therefore not only important for future studies hoping to model *in vitro* cultures in a more physiologically-relevant manner to regulate environmental chambers to 'correct' oxygen

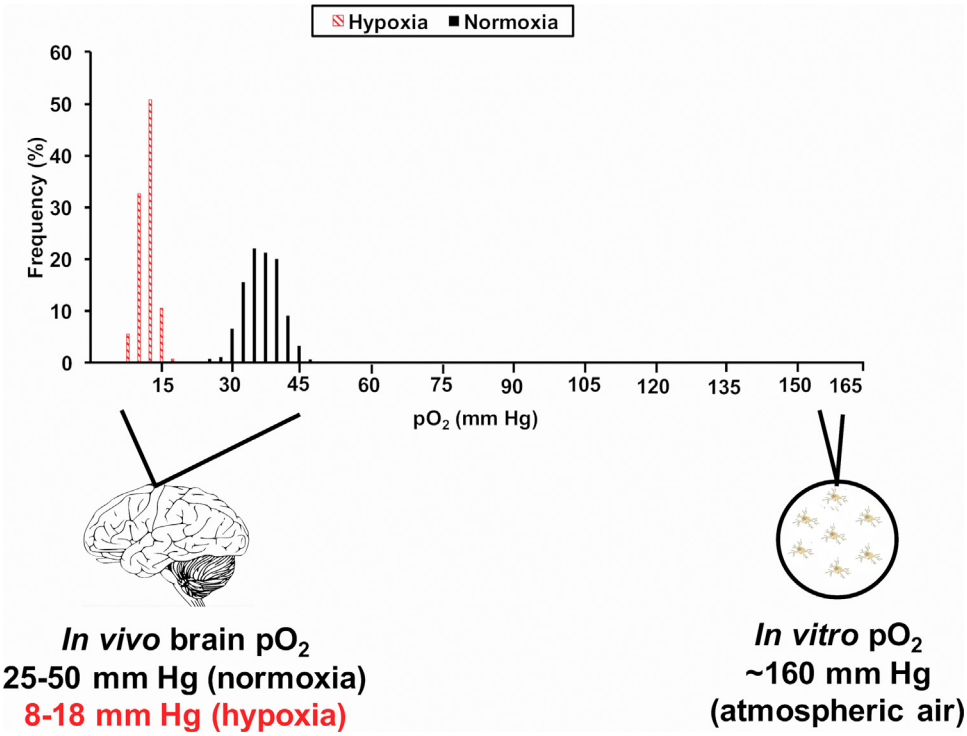

Fig. 1. Visualization of *in vitro* and *in vivo* oxygen tension differences. Frequency graphs of pO_2 distributions in mouse cortex under normoxic (21% O_2) or hypoxic (10% O_2) conditions, modified from (Sakadžić et al., 2010). Overlayed is the pO_2 (~21% O_2, ~160 mm Hg) at which the majority of *in vitro* research is performed. Modified with permission from Xu et al. (2017) *Adv. Exp. Med. Biol.* 977: 149–153.

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