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Relative sensitivity of soluble guanylate cyclase and mitochondrial respiration to endogenous nitric oxide at physiological oxygen concentration

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Nitric oxide (NO) is a widespread biological messenger that has many physiological and pathophysiological roles. Most of the physiological actions of NO are mediated through the activation of soluble guanylate cyclase and the subsequent production of cGMP. NO also binds to the binuclear center of cytochrome c oxidase and inhibits mitochondrial respiration in competition with oxygen and in a reversible manner. Although soluble guanylate cyclase is more sensitive to endogenous NO than cytochrome c oxidase at atmospheric oxygen tension, the more relevant question is which enzyme is more sensitive at physiological oxygen concentration. Using a system in which NO is generated inside the cells in a finely controlled manner, we determined cGMP accumulation by immunoassay and mitochondrial oxygen consumption by high-resolution respirometry (Oroboros Oxygraph-2k) at 30 μM oxygen. We report here that the NO concentration that caused half-maximal activation (EC_{50}) of soluble guanylate cyclase was around 2.9 nM whereas that required to achieve half-maximal inhibition of respiration (IC_{50}) was 141 nM (the basal oxygen consumption in the absence of NO was 14 ± 0.8 pmol $O_2.s^{-1}.10^{-6}$ cells). **In accordance with this, the NO-cGMP signalling transduction pathway was activated at lower NO concentrations than the AMP-activated protein kinase (AMPK) pathway**. We conclude that guanylate cyclase is around 50-fold more sensitive than cellular respiration to endogenous NO under our experimental conditions. The implications of these results for cell physiology are discussed.

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