Biochem. J. (2003) **376** (e5–e6) (Printed in Great Britain)

COMMENTARY

Does interplay between nitric oxide and mitochondria affect hypoxia-inducible transcription factor-1 activity?

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This Commentary discusses recent results from the laboratory of Salvador Moncada (Mateo et al., in this issue of the *Biochemical Journal*) that shed light on the interaction of nitric oxide (NO) and the transcription factor hypoxia-inducible factor 1 (HIF-1). Using cells stably transfected with inducible NO synthase (iNOS) under the control of a tetracycline-inducible promoter, they generated a range of NO concentrations and determined how these affected HIF-1α stability. HIF-1α, a component of the heterodimer HIF-1, is rapidly degraded at high oxygen concentrations, and thus HIF-1 is only active as a transcription factor under hypoxic conditions. The authors found a biphasic effect of NO concentration on HIF-1α stability. Close to hypoxia, low NO concentrations destabilized HIF-1α by inhibiting mitochondrial respiration, thereby increasing the local oxygen concentration. In contrast, high NO concentrations stabilized HIF-1α at both high and low oxygen concentrations by a non-mitochondrial pathway. These data resolve reported discrepancies on the effect of NO on HIF-1α stability at low oxygen concentrations and suggest that inhibition of mitochondrial respiration by NO may affect oxygen sensing by HIF-1 *in vivo*.

Key words: hypoxia, hypoxia-inducible factor-1 (HIF-1), hypoxia-inducible factor-1 subunit α (HIF-1α), mitochondria, nitric oxide (NO).

The physiological roles of nitric oxide (NO) that operate through guanylate cyclase are reasonably well understood. However, there are indications in the literature that NO can also influence physiology and pathology through other pathways, but the mechanisms and significance of these are unclear. One of the most intriguing of these alternative interactions of NO is with mitochondrial respiration, where NO inhibits respiratory Complexes I and IV [1]. Inhibition of Complex IV by NO occurs rapidly through binding to the oxygen reduction site within the enzyme [2,3]. Intriguingly, this inhibition occurs at physiological NO and oxygen concentrations and, because NO binding competes with that of oxygen, NO becomes a more effective inhibitor as the oxygen concentration decreases [4]. The interaction of NO with Complex I also disrupts respiration, but, in contrast with inhibition through Complex IV, this takes time to develop, and the interaction of NO with Complex I is less well understood, although there is evidence for *S*-nitrosothiol formation [5]. That these reactions indicate a physiological role for NO in mitochondria has been suspected for some time, and this is strengthened by reports of a mitochondrial isoform of NO synthase (NOS) [6,7].

A number of physiological roles have been suggested for NO within mitochondria, but the supporting evidence to date is not strong. The interplay between NO and oxygen concentration at the level of the mitochondrion suggests a role in the cell's response to hypoxia. As the oxygen concentration decreases, NO becomes a more effective inhibitor of Complex IV, so at low oxygen concentrations NO slows respiration while still enabling mitochondria to maintain a membrane potential. Thus NO could decrease oxygen consumption in cells close to hypoxia and thereby lessen the likelihood of anaerobiosis. This strategy would avoid loss of membrane potential and the consequent cell damage due to disruption to ATP synthesis and calcium homoeostasis. This oxygen-sparing effect of NO might also enable oxygen to diffuse further from capillaries and penetrate more deeply into tissues. Thus the interaction of NO with mitochondria may help prevent anaerobiosis within tissues, although these suggestions are speculative. Even so, the interplay between NO and mitochondria at low oxygen concentration suggested that an interaction between NO and hypoxia sensing by hypoxia-inducible transcription factor-1 (HIF-1) was worth exploring.

Cells respond to hypoxia by increasing expression of a range of hypoxia-inducible genes through HIF-1 binding to hypoxia response elements [8]. Most of these gene products either increase oxygen supply to the tissue or help the cell adapt to hypoxia, for example by up-regulating glycolysis [8]. The transcription factor HIF-1 is a heterodimer of HIF-1α and HIF-1β subunits. HIF-1α is unstable at high oxygen concentrations, because it is hydroxylated at Pro^{402} and/or Pro^{564} using molecular oxygen as a substrate. Hydroxylated HIF-1α is rapidly ubiquitinated and degraded by the proteasome. Thus oxygen inactivates HIF-1 and prevents transcription of hypoxia-inducible genes. As the oxygen concentration falls to levels close to hypoxia, HIF-1α is no longer hydroxylated and degraded, leading to a dramatic up-regulation of HIF-1 and the consequent expression of hypoxia-induced genes.

There were conflicting reports of NO either stabilizing or destabilizing HIF-1α at low oxygen concentrations. One of the reasons for this discrepancy may be the complicated chemistry of NO, which not only directly influences enzymes by itself binding, but also through the reactions of NO derivatives such as peroxynitrite or nitrogen dioxide. A further complication is the difficulty of delivering a controlled flux of NO within cells using extracellular NO donors, which may also have non-physiological side reactions. Mateo et al. [9], in this issue of the *Biochemical Journal*, overcame these difficulties by synthesizing authentic NO within cells using inducible NOS (iNOS). This enzyme produces NO constitutively, hence NO production is proportional to iNOS expression. To control iNOS levels, Mateo et al. [9] stably transfected cells with iNOS under the control

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of a tetracycline (tet)-inducible promoter. Thus the amount of iNOS, and consequently the steady-state flux of intracellular NO, was easily adjusted by varying the tet concentration. Using this system, the authors [9] were able to express a range of NO fluxes in cells and could then investigate the interaction of NO and HIF-1 over a range of NO concentrations. When cells were held under an atmosphere of 3 % oxygen, the concentration of HIF-1α increased as expected, because HIF-1α was no longer inactivated by hydroxylation. They then explored how increasing the NO concentration through up-regulation of iNOS affected HIF-1α stability. The effect of NO concentration on HIF-1α was biphasic, with decreased HIF-1α stability at NO concentrations up to 400 nM, but increased HIF-1α stability at NO concentrations above 1 μM. This neatly resolves the earlier discrepancies over the effects of NO on HIF-1α under low oxygen conditions and suggests that the conflicting reports may have arisen because of the difficulty of controlling the NO concentration.

The stabilization of HIF-1α at high NO concentrations was found even under normoxia, so could occur under pathophysiological conditions where high NO concentrations build up. The mechanism of stabilization was uncertain and requires further investigation: possibilities include S-nitrosylation and consequent stabilization of HIF-1α itself, or inhibition of the enzymes responsible for HIF-1α hydroxylation. The destabilization of HIF-1α at low NO concentrations is likely to be of physiological importance because it occurs at NO concentrations found *in vivo*. This effect is caused by NO inhibiting mitochondrial respiration, thereby preventing mitochondria from depleting local oxygen, enabling the continued hydroxylation and degradation of HIF-1α. This requirement for inhibition of mitochondrial respiration was confirmed by showing that low NO concentrations did not affect HIF-1α in cells lacking a functional respiratory chain, and that other respiratory-chain inhibitors also destabilized HIF-1α by preventing mitochondrial oxygen consumption.

It seems likely that the NO effect through mitochondria is a consequence of inhibiting respiration at Complex IV. This suggests that the interaction of NO with Complex IV may impinge on HIF-1 activity *in vivo*. It will be particularly interesting to see if the destabilization of HIF-1α by NO occurs *in vivo*, and whether the interaction of NO with Complex IV is modulated in order to affect HIF-1α stability. To conclude, this work [9] nicely resolves previous uncertainties about the interaction of NO with HIF-1α and demonstrates how intracellular NO can be manipulated in a controlled way. Most importantly, Mateo et al.'s [9] study indicates how NO levels could modulate HIF-1 activity through inhibition of mitochondrial respiration by NO, and this is likely to be a contributing factor to the action of HIF-1α *in vivo*. However, it is still unclear whether this interaction helps modulate HIF-1α activity or whether it is simply a secondary consequence of mitochondrial NO metabolism designed to prevent mitochondria from becoming de-energized under low oxygen conditions. Whatever the final outcome, the work of Mateo et al. [9] strengthens the suggestion that the interaction of NO with mitochondria is of physiological significance.

Received 21 October 2003; accepted 21 October 2003
Published on the Internet 24 November 2003, DOI 10.1042/BJ20031605

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