



# Nitric oxide synthase partial uncoupling as a key switching mechanism for the NO/ONOO– cycle

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**Summary** Short-term stressors, capable of increasing nitric oxide levels, act to initiate cases of illnesses including chronic fatigue syndrome, multiple chemical sensitivity, fibromyalgia and posttraumatic stress disorder. These stressors, acting primarily through the nitric oxide product, peroxynitrite, are thought to initiate a complex vicious cycle mechanism, known as the NO/ONOO– cycle that is responsible for chronic illness. The complexity of the NO/ONOO– cycle raises the question as to whether the mechanism that switches on this cycle is this complex cycle itself or whether a simpler mechanism is the primary switch. It is proposed here that the switch involves a combination of two variable switches, the increase of nitric oxide synthase (NOS) activity and the partial uncoupling of the NOS activity, with uncoupling caused by a tetrahydrobiopterin (BH4) deficiency. NOS uncoupling causes the NOS enzymes to produce superoxide, the other precursor of peroxynitrite, in place of nitric oxide. Thus partial uncoupling will cause NOS proteins to act like peroxynitrite synthases, leading, in turn to increased NF-κB activity. Peroxynitrite is known to oxidize BH4, and consequently partial uncoupling may initiate a vicious cycle, propagating the partial uncoupling over time. The combination of high NOS activity and BH4 depletion will lead to a potential vicious cycle that may be expected to switch on the larger NO/ONOO– cycle, thus producing the symptoms and signs of chronic illness. The role of peroxynitrite in the NO/ONOO– cycle also implies that such uncoupling is part of the chronic phase cycle mechanism such that agents that lower uncoupling will be useful in treatment.

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## The NO/ONOO– cycle as the cause of multiple types of chronic illness

Chronic fatigue syndrome, multiple chemical sensitivity, fibromyalgia and post-traumatic stress disorder show multiple overlaps. Cases of each have many symptoms in common and they show substan-

tial comorbidities with each other (reviewed in 1–3). Cases of each are often initiated by several short-term stressors that are summarized in [Table 1](#). Each of the 13 stressors listed in [Table 1](#) are known to be able to initiate a sequence of events leading to increases in nitric oxide (NO) synthesis [1–6], with most of these demonstrated to produce such NO increases. How might a short-term increase in nitric oxide lead to such chronic illness? Presumably by initiating a biochemical vicious cycle [1–4] recently called the NO/ONOO– cycle ([Fig. 1](#)). The

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**Table 1** Short-term stressors initiating illnesses

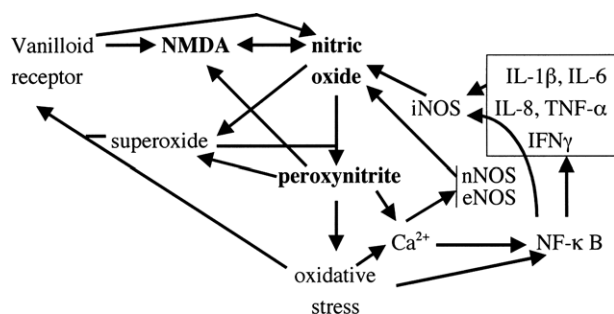
Disease/illness	Stressors
Multiple chemical sensitivity (MCS)	Volatile organic solvents; organophosphorus/carbamate pesticides and other toxicants; organochlorine pesticides; pyrethroid pesticides
Chronic fatigue syndrome (CFS)	
Fibromyalgia <sup>b</sup>	Physical trauma; viral infection; bacterial infection; severe psychological stress
Post-traumatic stress disorder (PTSD)	Severe psychological stress; head trauma

Each of these short-term stressors is known to initiate a sequence of events that are predicted and in most cases known to lead to increases in NO.

<sup>a</sup> Ionizing radiation is included here because of its role in initiating post-radiation syndrome, a CFS-like illness.

<sup>b</sup> Cases of fibromyalgia are occur secondarily to autoimmune diseases including rheumatoid arthritis and lupus. These are not included in the table because they are long-term stressors, but they also lead to increases in NO in the tissues being attacked.

name of this cycle is based on the structures of nitric oxide (NO) and its oxidant product peroxynitrite (ONOO<sup>-</sup>) but is pronounced no, on no!



**Figure 1** Vicious (NO/ONOO<sup>-</sup>) cycle diagram. Each arrow represents one or more mechanisms by which the variable at the foot of the arrow can stimulate the level of the variable at the head of the arrow. It can be seen that these arrows form a series of loops that can potentially continue to stimulate each other. An example of this would be that nitric oxide can increase peroxynitrite which can stimulate oxidative stress which can stimulate NF-κB which can increase the production of iNOS which can, in turn increase nitric oxide. This loop alone constitutes a potential vicious cycle and there are a number of other loops, diagrammed in the figure that can collectively make up a much larger vicious cycle. The challenge, according to this view, in these illnesses is to lower this whole pattern of elevations to get back into a normal range. You will note that the cycle not only includes the compounds nitric oxide, superoxide and peroxynitrite but a series of other elements, including the transcription factor NF-κB, oxidative stress, five inflammatory cytokines (in box, upper right), all three different forms of nitric oxide synthases (iNOS, nNOS and eNOS), and two neurological receptors the vanilloid receptor and the NMDA receptor. The figure and the legend are copied from the author's web site with permission.

While the cycle is centered on NO and ONOO<sup>-</sup>, it involves many other components (Fig. 1) including increased superoxide, oxidative stress, intracellular calcium, NF-κB activity, increased levels of inflammatory cytokines (upper right corner), increased activity of all three nitric oxide synthases (iNOS, nNOS and eNOS) and increased activity of two receptor systems, the vanilloid receptor and the NMDA receptor [1–6]. Although not apparent in Fig. 1, it also involves mitochondrial dysfunction produced by peroxynitrite and to a lesser extent, nitric oxide and superoxide [1–3]. The arrows shown in Fig. 1, represent 22 distinct mechanisms, 19 of which are based on well-accepted biochemistry [1], so the amount of evidence supporting these components of this cycle is very large. Three generic types of evidence support the existence of the NO/ONOO<sup>-</sup> cycle or something similar to it [1] and much evidence supports its role in this group of illnesses [1–6].

The NO/ONOO<sup>-</sup> cycle as an explanatory model of these illnesses is based on five principles [1,2], the first two of which have already been discussed:

1. Initiating stressors act to stimulate the synthesis of nitric oxide or possibly other elements of the cycle, such as superoxide.
2. The chronic nature of illness is explained by the action of the NO/ONOO<sup>-</sup> cycle.
3. The symptoms and signs of illness must be explained as being a consequence of elevation of one or more elements of the cycle.
4. The basic mechanism of the cycle is local. This is because the half lives of the compounds of the cycle, NO, ONOO<sup>-</sup> and superoxide are relatively short such that they diffuse only short distances from where they are initially synthesized and the mechanisms of the cycle act at the cellular

level. Because of this local nature, one can have one tissue impacted by NO/ONOO<sup>-</sup> cycle biochemistry but an adjacent tissue may be largely unimpacted. Variation of tissue distribution may lead, in turn, to much variation of symptoms and signs from one individual sufferer to another.

5. These illnesses are best treated by using agents that down-regulate NO/ONOO<sup>-</sup> cycle biochemistry.

Possible candidate diseases/illnesses for inclusion under the NO/ONOO<sup>-</sup> cycle paradigm should be assessed by their fit to these five principles.

The current paper explores the apparent role of nitric oxide synthase uncoupling in both the initiation of illness and in the chronic phase of illness.

### How does the NO/ONOO<sup>-</sup> cycle get turned on?

What determines whether or not the NO/ONOO<sup>-</sup> cycle gets turned on? Clearly stressors such as infection do not always initiate such illnesses such as chronic fatigue syndrome or fibromyalgia, leading one to infer that some such events switch on the NO/ONOO<sup>-</sup> cycle but others do not. One presumes that the strength of the stressor is important and there is evidence that genetic predisposition plays an important role, as does hormone balance [1–3]. Other factors that may play a role include nutritional conditions and possibly the state of the immune system [1,3]. How do these act and what constitutes the switch mechanism that turns on the NO/ONOO<sup>-</sup> cycle? Is the switch mechanism the entire NO/ONOO<sup>-</sup> cycle or is there a more compact and more easily understandable switch mechanism that may serve as the core factor that determines whether the cycle gets turned on?

The hypothesis being examined here is that there is such a compact switch mechanism and that it is centered on the partial uncoupling of the elevated nitric oxide synthase (NOS) activity.

The three NOS's, nNOS, iNOS and eNOS all share the property that when they are deprived of their cofactor, tetrahydrobiopterin (BH4), they become uncoupled, producing superoxide in place of nitric oxide [7–17,12,18]. Consequently, partial uncoupling of NOS activity will lead them to act like peroxynitrite (ONOO<sup>-</sup>) synthases, synthesizing the two precursors of ONOO<sup>-</sup>, NO and superoxide. Because the NOS-mediated synthesis is expected to be concentrated in cells and cellular compartments with high NOS activity, the superoxide so produced may be particularly active in reacting with NO leading to ONOO<sup>-</sup>. Most importantly,

ONOO<sup>-</sup> is active in oxidizing BH4 [16,17,12,18] and thus has the potential to propagate the partial BH4 deficiency over time, leading to a vicious cycle. The consequent chronic elevation of ONOO<sup>-</sup> will be expected to turn on the entire NO/ONOO<sup>-</sup> cycle due to the central role of ONOO<sup>-</sup> in the cycle biochemistry.

The initial elevation of superoxide levels in the initiation of NO/ONOO<sup>-</sup> cycle diseases does not have to be derived primarily from NOS uncoupling. According to this hypothesis, it simply needs to act to increase ONOO<sup>-</sup> levels and produce subsequent uncoupling by increasing ONOO<sup>-</sup>-stimulated BH4 oxidation.

NOS partial uncoupling may be expected to have a special role in activating NF-κB activity, an important element of the NO/ONOO<sup>-</sup> cycle (Fig. 1). NF-κB has an important role in the cycle but is regulated in opposite directions by NO and by ONOO<sup>-</sup>. NF-κB is activated by ONOO<sup>-</sup> and by oxidants but is inhibited by NO [1,3]. Consequently, the partial uncoupling of NOS activity and the consequent shift in the ratio of NO and ONOO<sup>-</sup> in favor of the latter may be particularly important in switching on NF-κB activity and consequently the NO/ONOO<sup>-</sup> cycle.

### Testing the hypothesis

Pathophysiological models are typically approximate representations of actual disease processes and certainly both this NOS uncoupling model and the broader NO/ONOO<sup>-</sup> cycle are expected to be approximations. Still they both make substantial testable predictions.

The most direct way of testing this hypothesis is to test the activity of BH4 in preventing these illnesses in animal models and in humans. High dose folate supplements should also be tested, because of their activity in preventing such uncoupling [1] and BH4 supplements as well as sepiapterin, which acts as a precursor of BH4, should be tested as well.

### NOS uncoupling as part of the chronic phase of illness

Because of the central role of peroxynitrite in the NO/ONOO<sup>-</sup> cycle (Fig. 1), partial uncoupling is expected to play an important role in the NO/ONOO<sup>-</sup> cycle. It is expected to act to activate NF-κB activity, as discussed above, up-regulating the cycle. It follows from this, that lowering NOS uncoupling may be expected to be a useful therapeutic target in the treatment of NO/ONOO<sup>-</sup> cycle diseases.

High dose folate treatment, which has been noted above to lower NOS uncoupling, has been reported to be effective in the treatment of chronic fatigue syndrome [19,20]. While BH4 has not been used to treat the core NO/ONOO<sup>-</sup> cycle diseases, it may be useful in the treatment of two candidate diseases for inclusion under the NO/ONOO<sup>-</sup> cycle paradigm [1]. It is reported to produce clinical improvement in autism [21–23] and to produce improved properties in cell culture models of Parkinson's disease [24,25]. So there is some evidence suggesting that lowering uncoupling may be useful in the treatment of NO/ONOO<sup>-</sup> cycle diseases but additional studies are needed.

### NOS uncoupling: must nitric oxide levels be elevated in the chronic phase of illness?

One of the consequences of NOS uncoupling in the NO/ONOO<sup>-</sup> cycle, is that even though "NOS" activity must be elevated, partial uncoupling may mean that actual nitric oxide synthesis levels may not be. There may be sufficient NOS uncoupling to lower the nitric oxide synthesis activity to a level less than or equal to that seen in controls. What does the evidence say about this possibility? The two studies on nitric oxide synthesis or levels in CFS suggest that it is elevated in this illness [1]. Two of the three studies on this in fibromyalgia also suggest that it is elevated [1,2] and the third may have been misinterpreted [1,2]. Furthermore the studies from two agents that lower nitric oxide levels, either by acting as a nitric oxide scavenger (hydroxocobalamin) [1,26], or by acting to lower nitric oxide synthesis (paroxetine, chapter 6, reference 1) are both reported to be helpful in the treatment of this group of illnesses, suggesting that nitric oxide levels may be increased and lowering such levels leads to symptomatic improvement. Thus the available evidence suggests that nitric oxide levels are usually elevated in this group of illnesses but there may still be situations where the NO/ONOO<sup>-</sup> cycle may be active but such levels are not elevated.

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