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Review

# The chemistry of nitroxyl (HNO) and implications in biology

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*Abbreviations:* $BH_4$, tetrahydrobiopterin; cGMP, guanosine cyclic 3′, 5′-monophosphate; CGRP, calcitonin gene related product; cyt *c*, cytochrome *c*; deoxyMb, deoxymyoglobin; DETC, diethyldithiocarbonate; DTT, dithiothreitol; EDRF, endothelium-derived relaxing factor; EPR, electron paramagnetic spectroscopy; FAD, flavin-adenine dinucleotide; ferricyt *c*, ferricytochrome *c*; ferrocyt *c*, ferrocytochrome *c*; FMN, flavin mononucleotide; GSH, reduced glutathione; GSNO, *S*-nitrosoglutathione; Hb, hemoglobin; HbNO, nitrosohemoglobin; HNO, nitroxyl; HRP, horseradish peroxidase; $LD_{50}$, lethal dose 50%; Mb, myoglobin; MbNO, nitrosyl myoglobin; $MbO_2$, oxymyoglobin; metHb, methemoglobin; metMb, metmyoglobin; NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NHE, normal hydrogen electrode; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; $NO^-$, nitroxyl anion; $NO_2^-$, nitrite; $NO_3^-$, nitrate; NOHA, $N^G$-hydroxy-L-arginine; NOS, NO synthase; PARP, poly ATP-ribosyl polymerase; RNOS, reactive nitrogen species; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase; SOD, superoxide dismutase; TMPyP, meso-tetrakis(*N*-methylpyridinium-4-yl)porphyrinato

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**Abstract**

Over the past century, HNO research has evolved from fundamental physical examinations to elucidation of interactions in atmospheric, industrial and bacterial processes. Most recently, the HNO literature has been primarily concerned with the pharmacological effects and potential physiological functions of HNO in mammalian systems. The chemistry of HNO is inordinately complicated for a triatomic molecule. Further, the rapid self-consumption of HNO through dehydrative dimerization impedes detection and necessitates in situ production. This review provides a detailed discussion of the most common donors of HNO and of the current understanding of the aqueous chemistry of HNO and the synthesis, consumption and reactivity of HNO in a cellular environment, as ascertained with these donors. Additionally, the consequences of the molecular interactions of HNO on physiology are described, and a comparison is made to NO in terms of cellular signaling and pharmacological potential.


*Keywords:* Nitroxyl; Nitric oxide; Chemical biology; Donor compounds; Detection; Angeli's salt

## 1. Introduction

Postulation of the formation of HNO (variously called nitroxyl, nitrosyl hydride, hydrogen oxonitrate (IUPAC), nitroso hydrogen, monomeric hyponitrous acid) as an intermediate in a variety of thermal and photochemical reactions dates from the early 1900s [1,2]. As a triatomic molecule, HNO has been the subject of extensive experimental and theoretical analysis of structure, spectroscopy and chemical dynamics ([3–6] and references therein). This fundamental research evolved into investigation of the intermediacy of HNO in combustion of nitrogen-containing fuels, in atmospheric and interstellar chemistry and in bacterial denitrification. The recent HNO literature has been primarily concerned with the pharmacological effects and potential physiological functions of HNO in eukaryotes. This review focuses on the chemical mechanisms of HNO under physiological conditions and on the consequences of these reactions in mammalian biology and extends the information provided by past reviews [7–13].

The caveat must be imparted here that the production, detection and investigation of the chemistry of HNO is severely complicated by high reactivity, such that the gas phase and solution literature has been controversial at nearly every stage of experimental sophistication including recent biological investigations. This review presents the current understanding of the chemical biology of HNO, which will no doubt be modified and expanded considerably as the field matures.

A major impediment to the understanding of HNO chemistry is the rapid dehydrative dimerization of HNO, which produces nitrous oxide through the transient hyponitrous acid [14,15].

$$2\mathrm{HNO} \rightarrow [\mathrm{HONNOH}] \rightarrow \mathrm{N_2O} + \mathrm{H_2O} \quad (1)$$

This reaction has been studied both experimentally and theoretically since the 1960s, and numerous values for the rate constant have been offered (reviewed in [10,16]). The accepted value, determined by flash photolysis techniques at room temperature, is $8 \times 10^6\ \mathrm{M^{-1}\,s^{-1}}$ [17], recently revised from $2 \times 10^9\ \mathrm{M^{-1}\,s^{-1}}$ [18]. Although this reaction provides $N_2O$ as an indirect marker of HNO formation, direct detection of HNO is inherently challenging, and the in situ use of reductive techniques or donor compounds is generally required in the study of HNO.

## 2. HNO donors

HNO has been suggested to be a probable intermediate of a variety of oxidation processes involving nitrogen-containing compounds [19]. This is well illustrated by the extensive number of proposed mechanisms for HNO formation, which encompasses both inorganic and organic reactions and over a century of literature (reviewed briefly in [9,20,21]). Inorganic pathways, for example the reaction of hydrogen with nitric oxide (NO) [22,23] or aerobic photolysis of ammonia [24], have significant atmospheric importance. Processes leading to HNO elimination from organic precursors include acid-catalyzed solvolysis of *aci*-nitroalkanes (Nef reaction [25,26]), nitrosative cleavage of tertiary amines [27], retro Diels–Alder reactions [28–30] and decomposition of organophosphorous compounds [31–33].

Synthesis of HNO for experimental use has most commonly been achieved by either photochemical or thermal decomposition of organic compounds, such as nitromethane [3] or *N*-hydroxybenzensulfonamide (Piloty's acid, benzensulfohydroxamic acid) [34], or the inorganic salt $Na_2N_2O_3$ (Angeli's salt, sodium salt of trioxodinitrate, α-oxyhyponitrite, hyponitrate, *N*-nitrohydroxylamate or trioxodinitrate(N–N) (2−) (IUPAC)) [35]. Of the available HNO donors, Angeli's salt has been studied most extensively in terms of structure, thermodynamics and decomposition mechanism. Further, the majority of the available data on the aqueous solution chemistry and pharmacological effects of HNO have been obtained with Angeli's salt. The primary organic donors of HNO are Piloty's acid ($C_6H_5SO_2NHOH$) and more recently synthesized sulfohydroxamic acid derivatives, such as hydroxylamine-*N*-sulfonic acid ($HOSO_2NHOH$) [36] and *N*-hydroxymethanesulfonamide (methanesulfohydroxamic acid (MSHA), methylsulfonylhydroxylamine; $CH_3SO_2NHOH$) [37]. Nagasawa and coworkers have synthesized several series of N- and/or O-substituted analogs of