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Review Article

Is Nitric Oxide a Hormone?

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ABSTRACT

Nitric oxide (NO) is a simple ubiquitous signaling molecule and plays important roles in almost every biological system. Recent evidences suggest that NO may act as an endocrine molecule. The aim of this review is considering available literature on endocrine roles of NO and/or its metabolites, i.e. nitrite and nitrate. Existing data suggest the idea that NO is a hormone that after production in tissues, it is stabilized and transported as nitrite and/or S-nitrosothiols in the blood to target cells. *Iran. Biomed. J. 15 (3): 59-65, 2011*

Keywords: Endocrine, Hormone, Nitric oxide (NO), Nitrate, Nitrite

HISTORY

Nitric oxide (NO) was discovered in 1772 [1]. Nitroglycerine (NG), a vasodilator acting via NO production, was synthesized in 1847 [2, 3]. The effect of NG was studied on healthy volunteers by Constantin Hering in 1849 and it was proven to cause headache [2]. Later in 1878, NG was used by William Murrell for the first time to treat angina [2]. Towards the end of 19th century, NG was established as a remedy for relief of anginal pain [2]. In 1916, Mitchell *et al.* [4] suggested that body tissues can also produce nitrate and Richard Bodo [5] in 1928 showed a dose-dependent increase of coronary flow in response to sodium nitrite administration. In 1970s, it was shown that nitrite-containing compounds stimulate guanylate cyclase and increase cyclic guanosine monophosphate (cGMP) which causes vascular relaxation and it is presumed that cGMP activation may occur via the formation of NO [2].

In 1980, Furchgott and Zawadzki [6] showed that endothelial cells are required for acetylcholine-induced relaxation of vascular bed through the endothelium-derived relaxing factor. Thereafter in 1987, it was shown that endothelium-derived relaxing factor and NO are the same or almost the same [7-9]. In 1992, NO was proclaimed as the molecule of the year [10] and in 1999, Furchgott, Ignarro, and Murad were awarded the Nobel Prize in Physiology or Medicine for studies in the NO field [1]. Due to the proven roles played by NO physiologically and pathologically, research on NO was increased rapidly and at the end of 20th century, the rate of NO publications was approximately 6,000 papers per year [1], with currently more than 100,000 references invoking NO listed in PubMed.

NO synthesis. NO is produced in all tissues [11] and the general belief is that its local production determines physiological actions [12-14].

Enzymatic and non-enzymatic NO synthesis. NO is synthesized from L-arginine by the enzymes known as NO synthase (NOS) (EC 1.14.13.39) in two separate mono-oxygenation steps; first, L-arginine is converted to N^{ω}-hydroxyarginine in a reaction requiring one O_2 and one NADPH and the presence of tetrahydrobiopterin (BH_4) and in the second step, by oxidation of N^{ω}-hydroxyarginine citrulline and NO are formed [15]. At least three NOS enzyme isoforms including neuronal, inducible, and endothelial (eNOS) have been identified and encoded by different genes [16-18]. In 1997, Ghafourifar and Richter [19] suggested the existence of mitochondrial NOS and in 1994, Lundberg and colleagues [20] and Benjamin and colleagues [21] demonstrated NOS-independent NO formation. Non-enzymatic NO production by one-

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electron reduction of nitrite, a blood and tissue NO reservoir [22], seems to be ubiquitous and greatly accelerated under hypoxic conditions [23]. This finding changes the general belief that nitrate and nitrite are waste products of NO [24].

Rate of NO formation. The rate of NO formation differs between species, 0.33-0.85 μmol/kg/h in Wistar rats [25], 7.68 ± 1.47 in C57/B16 mice [26] and 0.9 μmol/kg/h [26] or about 1 mmol/day [27, 28] in humans. The storage form of NO in tissues is not so high [28] and in conditions such as hypoxia, ischemia, or injury in which the L-arginine/NOS pathway is impaired, consumption of serum NO_x (nitrite + nitrate = NO_x) seems to produce NO [29, 30].

NO metabolites. Rates of formation and clearance of NO determine its steady state concentration [28]. The NO half-life *in vivo* in the circulation is most likely shorter than 0.1 s [31]. The major breakdown product of NO in aqueous solution, free of biological material, is nitrite [28, 32] while in the presence of sufficient amount of O_2 it is nitrate [28]. In plasma, NO is oxidized almost completely to nitrite, where it remains stable for several hours [28]. In whole blood, nitrite is rapidly converted to nitrate [28], and NO reacts with oxyhemoglobin to produce methemoglobin and nitrate [32]. In blood, NO is taken up by red blood cells and converted to nitrate, a major metabolic pathway for endogenously produced NO [32]. In the body, 90% NO is converted to nitrate, which is the main stable end product of NO formation *in vivo* [25, 33]. The half-lives of nitrate and nitrites in circulation are 5-8 h and 110 s, respectively [34, 35]. Because of short half-life of NO itself, most often its serum metabolites are measured as a surrogate for NO production [12, 36, 37]. Serum/plasma NO_x levels, the most suitable method to assess NO synthesis *in vivo* [32], are highly correlated with endogenous NO production [38]. Nitrate concentration in the blood is a major factor in determining the nitrate and nitrite levels of the rest of the body [27]. Reference values for serum NO_x concentration have been reported in both adults [39] and pediatrics [40].

Physiological roles of NO. NO is known as a ubiquitous, an omnipresent and a pleiotropic signaling molecule [18, 41, 42] and plays important roles in almost every biological system [17, 43]. It is a potent vasodilator and considered as a key regulator in vascular homeostasis by inhibition of platelet activation, inhibition of vascular smooth muscle proliferation, and control of blood pressure [44-46]. Neural transmission [47-50], memory [44], apoptosis [51], reproduction [52], lipolysis [53], regulation of energy balance [42], and host defense [48] are among other physiological processes where NO plays some roles. NO regulates hormone release in the hypothalamic-pituitary axis [49] and inhibits prolactin secretion [54] and may play a role in catecholamine release and steroidogenesis in adrenal gland [48] and can regulate actions of insulin and carbohydrate metabolism [55, 56]. NO also has a role in thyrocyte/thyroid function and sodium nitroprusside, a precursor of NO, increases cGMP in human thyrocytes [54, 57]. Estrogens increase the synthesis and release of NO through stimulation of eNOS gene expression [58] and it has been suggested that enhancement of NO activity is a mechanism for attenuation arterial hypertension in females [54, 59].

NO and metabolic/endocrine disorders. Today, it has been revealed that many diseases are associated with altered NO homeostasis [1]. Endothelial dysfunction, which is related to all cardiovascular risk factors, now has become synonymous with reduced biological activity of NO and is considered as a hallmark of cardiovascular disease [1, 46]. Several studies have shown an association between serum/plasma NO_x levels as an index for NO generation [12, 31, 60] and eNOS activity [34, 61], and different diseases including diabetes and dysglycemia [62-67], thyroid disorders [68], metabolic syndrome [63, 69-71], hypertension [72-74], heart failure [75] cardiovascular disease [11], and obesity [39, 76, 77]. Defects in endogenous synthesis and bioavailability of NO have been proposed as a common underlying molecular mechanism linking metabolic and cardiovascular disease [78] and serum NO_x level has been loaded with other metabolic syndrome components in the cluster analysis, suggesting a unifying role in the clustering of MetS components [71]. NO deficiency, which is related to hyperinsulinemia, affects glucose and lipid metabolism and seems to be a link between metabolic and cardiovascular disease [54]. In addition, it has been shown that excessive NO production impairs β cell function causing death [79]; while inducible NOS is involved in muscle insulin resistance [80] lack of eNOS causes insulin resistance [81]. Impairment of NO synthesis may represent a central defect causing metabolic abnormalities associated with insulin resistance [82]. All three isoforms of NOS are targets for thyroid hormones [83]. In human hyperthyroidism, increased NO production plays a role in vasodilation and abnormal vascular tone [84]. One study has reported impaired NO production in newborns with primary congenital hypothyroidism [85]. NOS activity is upregulated in hyperthyroid rats and may participate in cardiovascular manifestation of the disease, while in hypothyroid rats, the tissue response to NOS activity is heterogeneous [86].

Protective effects of nitrite and nitrate. It has been shown that 3-day dietary supplementation with sodium nitrate (0.1 mmol/kg/day) could reduce significantly diastolic blood pressure in non-smoking healthy volunteers [87]. Recently, a large cohort study of 52,693 patients from 14 countries with acute coronary syndrome, of whom 20% were on chronic nitrate, demonstrated that chronic nitrate therapy (medication routinely took at home and started at least 7 days prior to index event) was associated with reduced severity of myocardial injury in response to acute coronary events [88]. The result showed that the proportion of these subjects with ST-segment elevation myocardial infarction was 41% in nitrate-naïve patients compared to only 18% in nitrate users and conversely a higher percent of nitrate users (82%) presented with non-ST-segment elevation acute coronary syndrome compared to 59% in nitrate-naïve patients [88].

Increasing nitrate or nitrate dietary intake provides significant cardioprotection against ischemia-reperfusion (I/R) injury in mice and it has been proposed that nitrite-/nitrate-rich foods may provide protection against cardiovascular conditions characterized by ischemia [89]. It has been suggested that the nitrate-nitrite-NO pathway serves as a backup system to ensure sufficient NO generation under hypoxic condition when NOS may be malfunctioning [23].

Abundant consumption of fruits and vegetables, especially green leafy vegetables, is associated with lower risk of cardiovascular disease [24]. It has been proposed that inorganic nitrate, which is found in vegetables with a high concentrations, i.e. >2000-3000 mg/nitrate/kg [90], is the major factor in contributing to the positive health effects of vegetables via bioconversion to nitrite, NO, and nitroso-compounds [24], NO_x intake now being considered as a dietary parameter for assessing cardiovascular risk [89].

Any intervention that increases blood and tissue concentration of nitrite may provide cardioprotection against I/R injury because it serves as a NOS-independent source of NO and reacts with thiols to form S-nitrosothiols [89]. Nitrate-nitrite-NO pathway can be boosted by exogenous administration of nitrate or nitrite and this may have important therapeutic as well as nutritional implications [23]. However, additional studies are required to clarify the protective roles of nitrate, considering the medical status of subjects, concomitant use of inhibitors of endogenous nitrosation (e.g. vitamin C and E), or foods containing high levels of nitrosatable precursors (e.g. fish) [91, 92]. Some individuals, including those with high blood pressure and atherosclerosis may benefit from increased nitrate while those with esophageal dysplasia should avoid foods with high concentration of nitrate [93].

Adverse health effects of nitrate and nitrite. NO_x has generally been regarded as a harmful substance leading to the enactment of strict limits on concentration of nitrate and nitrite in drinking water [89, 94]; 50 mg/L for nitrate and 3 mg/L for nitrite. The sum of the ratios of the concentration of each to its guideline value should not exceed 1 [95]; however, some authors believe that these limits are overprotective [90], while others do not [91]. Two health issues that have been attributed to nitrate are cancers of digestive tract and infant methaemoglobinaemia [30, 94]. Quite number of epidemiological studies, carried out during the last four decades, concluded that no convincing link between nitrate and stomach cancer incidence and mortality can be established [94]; neither has any causative link between nitrite or nitrate exposure and cancer been documented [89]. Therefore, it has been suggested that none of the health claims against dietary nitrate are substantiated [90] and it is the time to double the maximum contaminant level of nitrate, based on evidence [90]. Nevertheless, it must be notified there are some disagreements on this decision [91].

Evidences for an endocrine role of NO. The first evidence for intravascular NO transport, provided by Cannon III *et al.* [96] in 2001, showed that inhaled NO can be transported in the blood and results in peripheral vasodilation. In 2002, Rassaf *et al.* [97] using intra-arterial aqueous NO solution suggested for the first time that NO is transported considerable distances in human plasma; and they criticized the ultra-short half-life of NO in blood which is reported to be 0.05 -1.8 ms [28]. The authors suggested that longer activation of the downstream signaling cascade, formation of intermediates, or reduced NO consumption under flow condition may be likely explanations that lifespan of NO in plasma lies with the seconds to minutes [97]. In 2003, Schechter and Gladwin [98] presented the hypothesis that "NO may be transported throughout the body in the manner of a hormone". In 2008, Elrod *et al.* [99] showed that eNOS-generated NO in the heart of mice, with cardiac-specific overexpression of human eNOS gene, is transported in plasma and protects the liver against I/R injury; suggesting that NO can exert endocrine activity.

NO has a short half-life in the blood that limits its transport to distant organs [31, 99]; therefore, it has been suggested that nitrite [32, 99-102] and/or S-nitrosothiol [32, 97, 103-105] are responsible for intravascular transport of NO. To support endocrine roles for NO, it has been shown that inhaled NO increases plasma levels of nitrate in healthy subjects by ≈ 50% [33], produces diuretic actions and produces extrapulmonary systemic vascular effects [101, 106, 107]. In addition, dietary nitrite supplementation

protects mice against myocardial I/R injury [89]. Recently, Carlstrom *et al.* [78], have shown that dietary nitrate supplementation in eNOS-deficient mice can partly compensate for disturbances in endogenous eNOS NO generation and attenuates features of metabolic syndrome. Finally, it has been proposed recently that a nitrite/nitrate/NO endocrine system along with NOS enzymes help to maintain NO bioavailability [108].

CONCLUSION

Existing data suggest and support this idea that NO is a hormone, which after its production in tissues, is stabilized and transported as nitrite and/or S-nitrosothiols in the blood to target cells. Therefore, further studies are required to explore the endocrine roles of NO.

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