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Editorial

Nitric oxide signaling comes of age: 20 years and thriving

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The lyrics of a famous tango state that "twenty years time is just nothing". While this impression may broadly apply to different circumstances, such might not be the case for nitric oxide (NO). In the year 1987 two seminal contributions coming from independent laboratories [1,2] established the identity of the elusive endothelial derived relaxing factor reported by Bob Furchgott seven years earlier [3]. These laboratories showed that the chemical nature of "endothelium-derived relaxing factor" (EDRF) was identical to NO. The idea that NO could act as an activator of soluble guanylate cyclase had been proposed even earlier [4]. These four papers, together with the demonstration of L-arginine as substrate for the generation of NO [5], defined a pathway in endothelial cells which is now considered classical: the L-arginine-NO-cGMP pathway. Of importance, a considerable amount of information had already accumulated almost at the same time and had arrived at the same conclusions in non-vascular cells such as macrophages (see [6] for review). For the past twenty years, we have both witnessed and participated in an explosion of knowledge in the NO field, leading to the comprehension of the physiological and pathophysiological roles of NO in several organs and tissues, among which the cardiovascular system is "primus inter pares".

This Spotlight Issue is devoted to classical and non-classical targets of NO signaling in the cardiovascular system. One of the most cogent analyses of NO signaling in pathways independent from cGMP was published in 1992, in which Loscalzo and colleagues proposed that *S*-nitrosylation of albumin could represent an effective mechanism to deliver NO at distal sites [7]. Studies from the laboratories of Stamler, Loscalzo and others opened a new perspective related to the potential modification of protein function by *S*-nitros(yl)ation [8]. In this current Spotlight Issue, Derakhshan et al. provide a broad view of *S*-nitros(yl)ation and its importance for NO signaling, while highlighting unresolved issues related to reactivity, specificity and selectivity of thiol groups in their reactions with NO [9]. Of interest, nitrosothiols are able to promote alternative post-translational modifications as is the case with *S*-nitrosoglutathione and its ability to induce the formation of mixed disulfides, a post-translational modification known as *S*-glutathionylation. Parallels and contrasts of this process with the pathway of *S*-nitros(yl)ation are discussed by Martínez-Ruiz and Lamas [10]. *S*-nitrosylation is known to affect many protein targets (see [11] for review), although only a few of them have been fully elucidated from a functional point of view. In this issue, two fundamental cellular processes influenced by *S*-nitrosylation are discussed in depth: signaling by small GTPases [12] and intracellular vesicle trafficking [13]. In addition, original contributions explore the role of this post-translational modification on other targets including NADPH oxidase activity [14], and TIMP-3 inhibition [15], thus exemplifying the potential diversity of cellular targets for this biochemical modification.

Since the initial molecular characterizations of NO synthase (NOS) enzymology in the early 1990's, endothelial

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NOS (eNOS) has proven to be a fascinating enzyme [16]. It is now clear that the endothelial synthesis of NO is a highly regulated process governed by a series of post-translational modifications and protein-protein interactions, as reviewed in detail by Dudzinski and Michel in this issue [17]. These post-translational modifications include acylation, phosphorylation, and *S*-nitrosylation. Central to eNOS activation by growth factors and hormones is phosphorylation of the enzyme near its C-terminus by the enzyme Akt [18,19]. This eNOS phosphorylation site is also modified by the cAMP-dependent protein kinase and the AMP-activated protein kinase, among other protein kinases [17]. A new facet of this key mode of eNOS activation is suggested by the work of Zhang et al. in an original article of this issue [20], whereby swimming exercise promotes eNOS activation through an Akt-dependent mechanism. Activation of eNOS has also been proposed as a main mechanism to explain the protective effect of certain dietary antioxidants, as discussed by Mann et al. [21]. The beneficial effects of dietary isoflavones may be mediated by NO itself as a main regulator of transcription through activation of specific factors such as the Nrf2/ARE complex [22]. Of crucial importance, the effects of NO on the fundamental hypoxia transcription factor, HIF-1α, is discussed in this issue by Brüne and Zhou [23]. Along this same conceptual avenue, NO has been proposed to represent a key regulator of cellular respiration, and the chemical basis for this role relies on its capacity to reversibly inhibit the mitochondrial complex IV, cytochrome oxidase. The impact of this inhibition on cardiovascular function is reviewed by Brown and Borutaite [24].

The ability of NO to interact with reactive oxygen species such as the superoxide anion allows for the formation of the non-radical, highly oxidative species peroxynitrite, which is able by itself to inhibit other mitochondrial components. Both the peroxynitrite and heme peroxidase pathways may lead to protein tyrosine nitration, a nearly irreversible post-translational modification with important functional consequences. The relevance of protein tyrosine nitration for the cardiovascular field is analyzed here by Peluffo and Radi [25].

The realization by the scientific community in the early 1990's of the fact that cGMP was the distal, highly efficient and natural effector of NO in the vascular wall and synaptic transmission fostered the research and development of new pharmaceutical targets and tools addressing the mechanisms of degradation of cGMP with selective or preferential tissue topology. This led to the discovery and commercialization of phosphodiesterase (PDE) V inhibitors, which have revolutionized the treatment of erectile dysfunction [26]. This class of drugs has now expanded its use to other therapeutic contexts such as pulmonary hypertension. Kass et al. review the importance of phosphodiesterases in cardiovascular function and their cross-talk with the NO-cGMP pathway [27]. Clearly, NO is not alone in the cast of vasoactive factors governing the delicate balance of vascular tone and derivatives of arachidonic acid such as prostanoids or leukotrienes, which are of major importance, specially during inflammation. The interaction of NO with COX-1 and -2 has been the subject of many studies (see [28] for review). In one of the original contributions within this issue, Klein et al. provide data supporting an effect of a COX-2 inhibitor on the generation of cGMP in the coronary vessels through a mechanism involving PDE inhibition [29]. In another example showing mutual interactions between the NO-cGMP pathway and that of prostanoid generation, Blanco-Rivero et al. describe how fibrates may contribute to endothelial dysfunction by inhibiting prostacyclin release in rat vessels [30].

In addition to its fundamental role as a paracrine regulator of vascular relaxation, NO also plays a major role in regulating cardiac function. Getting to the heart of the matter, Seddon et al. review the current studies on NO and cardiomyocyte signaling, emphasizing the presence of eNOS and nNOS in this cell type and their role in cardiac contractility [31]. The effects of pro-inflammatory cytokines in neonatal mouse cardiomyocytes is explored by Geoghegan-Morphet et al., showing that nNOS inhibits lipopolysaccharide-mediated TNF-α expression [32]. The importance of endogenous inhibitors of NO synthesis, such as asymmetric dimethylarginine (ADMA) in cardiomyocytes reaches a new twist in the light of the observations of Stühlinger et al. reported here [33]. These authors show that myocardial ischemia-reperfusion injury is prevented by the overexpression of DDAH, the enzyme whose impairment leads to ADMA accumulation. In studies addressing the effect of NO on cardiomyocyte mitochondrial damage, Jang et al. propose that mobilization of Zn^{2+} through a cGMP-dependent process might constitute an important mediator of the cardioprotective effect of NO [34].

One of the most striking discoveries in the field of NO signaling in the recent past is nitrite (NO_2^-). Once believed to be an inert metabolite of NO, it is in itself a biological reservoir of NO. Certain conditions such as hypoxia or ischemia may drive the reduction of NO_2^- to NO by metal-containing enzymes such as hemoglobin or myoglobin. The physiological and pathophysiological implications of this phenomenon are reviewed here by Dezfulian et al. [35]. Fluxes of NO and its metabolites in the resting coronary circulation are studied by Rogers et al., who show that there is no net loss or gain of NO from the total metabolite pool across the coronary circulation [36].

So, what has transpired in human therapy arising from NO signaling? Beyond oral nitrate vasodilators and sildenafil, there is one clear clinical setting where NO has revealed to be life-saving, and that is in the treatment of primary pulmonary hypertension of the newborn. The vast experience of Zapol, Bloch and co-authors on this matter is summarized here, suggesting the possibility that inhaled NO therapy might also be useful for other clinical conditions such as adult cardiopulmonary disease [37]. The turn of this century found the study of NO signaling still in its infancy, and the field is now reaching its adolescence in full exhibition of power. It is likely that it shall continue to evolve into new and exciting directions as it reaches maturity.

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