Nitrite reduction to nitric oxide in the vasculature

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THROUGH THE CONTINUOUS GENERATION of vasoactive substances including nitric oxide (NO), prostaglandins, and endothelin, the vessel wall is actively involved in regulating blood flow in response to tissue demand (27). Vasodilatory NO is produced by NO synthase (NOS) in endothelial cells upon shear stress and numerous agonistic substances and acts via activation of soluble guanylyl cyclase (sGC) to increase cyclic GMP (cGMP). (22). The biological effects of NO are acutely terminated by its rapid oxidation to nitrate and nitrite. Until only recently, nitrate and nitrite were considered to be physiologically inert end products of NO metabolism. However, from more recent research it is now clear that these inorganic anions can recycle back into bioactive NO in vivo (6, 17, 19). Nitrite reduction to NO was first described in the gastric lumen (2, 20), and a year later the Zweier group demonstrated the same phenomenon in the ischemic heart (31). Although pH-dependent nonenzymatic nitrite reduction (via formation of HNO2 and then N2O3) is considerable in the acidic gastric lumen and in anoxic tissues (18), this reaction is much slower under more physiological conditions. Thus an effective systemic nitrite reduction along the physiological oxygen and pH gradients would probably have to involve some kind of enzymatic activity. Indeed, this turned out to be true. Xanthine oxidase was the first mammalian enzyme shown to possess nitrite reductase activity (30), but the list of other enzymes that can catalyze this one electron reduction is now growing rapidly. Kozlov and colleagues (12) demonstrated nitrite reduction by cytochrome P-450, and enzymes of the mitochondrial respiratory chain can also perform this reaction (13). Members of the heme globin family such as hemoglobin (Hb) (3, 23), myoglobin (24, 26), and neuroglobin (M Gladwin, personal communication) all have nitrite reductase capacity. Surprisingly, even the heme oxidation is the mechanism by which the sGC inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ).

These findings add to the already complex physiology of NO-dependent vasoregulation. A continuous release of NO from endothelial NOS (eNOS) in endothelial cells (and possibly other cells) is essential for maintenance of a basal dilator tone. The clearest evidence for this is that pharmacological inhibition of NOS acutely increases blood pressure and dramatically reduces blood flow in most vascular beds (22). Classically, NO-mediated vasodilation is thought to occur in response to shear stress on the vessel wall, which stimulates calcium influx and then NOS activity. In such a case, an increase in blood flow is the signal for vasodilation, which is adequate, for example, when adapting to an increase in cardiac output. In other situations, it is the metabolic demand of the tissues that gives the signal to vasodilation, e.g., during hypoxia. From a mechanistic viewpoint, the NOS/Arginase pathway does not seem to be optimally designed for hypoxic vasodilation since molecular oxygen is a cosubstrate for NO production. In this respect, the NOS-independent pathways seem better adapted because, in most of these, hypoxia directly enhances nitrite reduction to NO (19).

It seems clear that vasodilation from nitrite reduction occurs in vivo, but the exact mechanism by which NO is formed is still a matter of debate. A major question is the primary location of nitrite reduction. Is it taking place in the blood stream or in the vessel wall? Gladwin and coworkers (3, 4, 7) have suggested the existence of an allosterically regulated nitrite reductase activity of Hb in red blood cells (RBCs). When Hb is deoxygenated along the vascular tree, it gradually changes from an effective NO scavenger to a net NO producer. The RBC then exports NO or a closely related species that diffuses to the underlying smooth muscle to elicit vasodilation. The beauty of this and earlier suggested theories of RBC-mediated control of blood flow (5, 9) is the direct coupling between Hb saturation and vasodilation. On the other hand, NO formation from nitrite can take place also in the vascular wall. Modin and colleagues (21) first demonstrated vasodilation from physiological amounts of nitrite in aortic ring preparations and suggested a role for nitrite in metabolic vasodilation (21). At that time, they proposed simple nonenzymatic acidic reduction as a mechanism for nitrite reduction, and the reaction was enhanced by the reducing agent ascorbic acid. As mentioned above, myoglobin and xanthine oxidase (both present in the human vasculature) can also catalyze nitrite reduction. The results from Zweier’s group now add sGC and possibly other heme proteins to this list. sGC is especially intriguing since this heme protein is also the “receptor” for NO-mediated vasodilation. The new data would imply an autoregulatory loop where the enzyme generates the compound that subsequently activates it. However, nitrite reduction is suggested to occur at the ferrous (Fe²⁺) heme in sGC, and, since both nitrite and NO compete for this heme, the nitrite-sGC interaction would lead to more NO but simultaneously less room for NO to bind and activate sGC. Moreover, as nitrite is reduced, the heme is oxidized to the ferric state (Fe³⁺), which makes it less responsive to NO. In fact, heme oxidation is the mechanism by which ODQ inhibits sGC activity (25). Nevertheless, both NO and nitrite are constitutively present in the vessel wall, and the interaction and regulation of NO signaling through sGC are interesting and warrant further studies. One interesting aspect

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relates to the vasodilatory actions of organic nitrates. The fact that nitroglycerin activates sGC was one of the key findings in solving the elusive nature of endothelium-derived relaxing factor (10). Organic nitrates are metabolized to both nitrite and NO in vivo. The data from Alzawahra and colleagues add another twist to the not yet fully resolved mystery of the metabolism and action of organic nitrates. In addition to pharmaceuticals, our diet also represents an important source of nitrite, mainly provided by inorganic nitrate. Ingestion of nitrate (abundant in vegetables) leads to a sustained increase in circulating nitrite (16) and NO-like bioactivity (14, 15, 29), including a reduction in blood pressure. The bioconversion of nitrate to nitrite involves commensal nitrate-reducing bacteria in the gastrointestinal tract (18), but a recent study surprisingly shows that also mammalian enzymes can catalyse nitrate reduction in vivo under normoxic conditions (8). Taken together, endogenous NO synthesis, treatment with pharmaceuticals, and our diet may all influence nitrite levels in the vessel wall and subsequently affect the formation of bioactive NO.

In aggregate, there seems to be ample evidence for nitrite reduction occurring in the circulation and in the vessel wall although its exact role in physiological regulation of blood flow is still uncertain. We clearly need further studies to fully understand how nitrite vasodilates in vivo, how the process is regulated, and where the NO formation occurs. Interestingly, one would think that at least the latter question would be settled for NOS-dependent NO generation where the enzyme is classically said to be situated in the endothelial cells. However, recent studies now show that a functional eNOS is present also in circulating RBCs (11), again demonstrating how complex these systems are.

REFERENCES