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DDAH says NO to ADMA

John P. Cooke[1] and **Yohannes T. Ghebremariam**[1]
[1]Stanford Cardiovascular Institute, Stanford, California, USA.

Endothelium-derived nitric oxide (NO) is vasoprotective, as it enhances endothelial cell survival and proliferation, inhibits the excessive proliferation of vascular smooth muscle cells, and suppresses the adhesion of platelets and inflammatory cells to the vessel wall [1]. Substantial evidence from pre-clinical studies and human research indicates that impairment of the endothelial NO synthase (NOS) pathway accelerates vascular disease, and increases the risk for major adverse cardiovascular events [2–5]. Impairment of the NOS pathway is multifactorial, but it is increasingly apparent that circulating inhibitors of NOS play an important role. Asymmetric dimethylarginine (ADMA) and monomethyl-L-arginine (MMA) [6] are endogenous competitive inhibitors of NOS. Most human studies have focused on ADMA, as it is the more prevalent species in human plasma. Plasma ADMA is elevated in patients with cardiovascular disease or with risk factors, and it contributes to vascular resistance and stiffness [7, 8]. Notably, several large studies have shown that plasma ADMA is an independent biomarker for cardiovascular morbidity and total mortality [4, 5, 9]. Accordingly, endogenous mechanisms that regulate ADMA are deserving of further scientific attention.

Synthesis and Metabolism of ADMA

Protein-arginine methyltransferases (PRMTs) methylate arginine residues on histone and other nuclear proteins [10–12]. When these proteins are hydrolyzed, free methylarginines are released, including ADMA, MMA, and symmetric dimethylarginine (SDMA; this latter methylarginine does not inhibit NOS) (Figure). These may be expelled from the cell by the cationic (CAT) transporter to be secreted in the urine, which is the primary route for SDMA clearance. However, the majority of ADMA and MMA (~80%) is degraded within the cell by dimethylarginine dimethylaminohydrolase (DDAH) [13–16]. The activity of DDAH is reduced by oxidative stress that is associated with cardiovascular disease [17–19], causing ADMA levels to become elevated in these conditions [13, 20]. By contrast, global overexpression of DDAH1 in transgenic mice reduces ADMA levels and increases NO production [21–23]. These DDAH-1 overexpressing mice manifest reduced vascular resistance, increased insulin sensitivity and enhanced endothelial regeneration [21–23]; and they are resistant to vascular lesion formation induced by endothelial denudation, vascular inflammation, or hypercholesterolemia [22, 24, 25]. These observations are consistent with an essential role of DDAH1 in maintaining vascular homeostasis.

Global DDAH1 Deletion: Accumulation of ADMA and Loss of Homeostasis

In this issue of ATVB, Hu and colleagues [26] provide strong evidence that, of the two DDAH isoforms (DDAH1 and DDAH2), DDAH1 is largely responsible for the degradation of ADMA. They generated a murine model of global DDAH1 knockout (DDAH1$^{-/-}$) by

targeting exon4 of the DDAH1 gene. The DDAH1$^{-/-}$ mice displayed normal developmental features while showing negligible tissue DDAH activity in several tissues. The abrogation of DDAH enzymatic activity (assessed using isotope-labeled ADMA or MMA) was surprising, since expression of the DDAH-2 isoenzyme was unaffected. The expression of endothelial NOS (eNOS), PRMTs 1 and 3, and CAT were unaffected also. With the loss of DDAH activity there were significant elevations in tissue and plasma ADMA and MMA.

Isolated aortic rings from the DDAH1$^{-/-}$ mice manifested impaired endothelium dependent vasodilation in response to acetylcholine, consistent with ADMA-induced suppression of NOS. These animals also exhibited a significant increase in blood pressure, reminiscent of the hemodynamic abnormality in eNOS knockout mice [27]. The elevation in BP was reversed by infusion of L-arginine consistent with the competitive inhibition of NOS by ADMA.

This study confirms the importance of DDAH in regulating NO synthesis, by its degradation of the endogenous NOS inhibitors ADMA and MMA. Furthermore, this study suggests that a specific isoenzyme, DDAH1, is primarily responsible for metabolism of the methylarginines, and that DDAH2 cannot compensate for the loss of DDAH1.

Future Directions

Although the study of Hu and coworkers [26] complements previous studies using the endothelial-specific DDAH1 knockout and heterozygous DDAH1 deficient mice [28, 29], it also raises some interesting questions. Firstly, there is a discrepancy between this study and a previous one which suggested that the global DDAH-1 knockout was lethal [29]. It is possible that in the previous study (in which exon1 of DDAH was targeted), the deletion might have adversely affected another genomic region necessary for embryogenesis.

If DDAH2 does not compensate for the loss of DDAH1, what may be its function? The literature is mixed regarding the importance of DDAH2 in the metabolism of ADMA [6, 30–32]. Overexpression of DDAH-2 improves endothelium dependent vasorelaxation and increases NO synthesis, whereas siRNA knockdown of DDAH-2 reduces NO synthesis [31–33] However, the story becomes more interesting by recent evidence that both DDAH-1 and DDAH-2 manifest protein-protein interactions that may affect the NOS pathway independently of ADMA metabolism [31, 34]

The global DDAH-1 knockout mouse of Hu and colleagues will be useful to further interrogate the role of DDAH1 deficiency in vascular disorders. In the meanwhile, the weight of the evidence indicates that DDAH-1 is a worthy therapeutic target. Agents which increase DDAH expression are known [35, 36] and one of these, an FXR agonist, is in clinical trials [37]. An alternative approach is to develop an allosteric activator of the enzyme. Although development of an allosteric activator is not a typical pharmaceutical approach, recent studies indicate that this may be an achievable aim [38, 39]. An agent that increases the expression and/or activity of DDAH-1 would be anticipated to reduce blood pressure, enhance insulin sensitivity, and reduce adverse cardiovascular outcomes.

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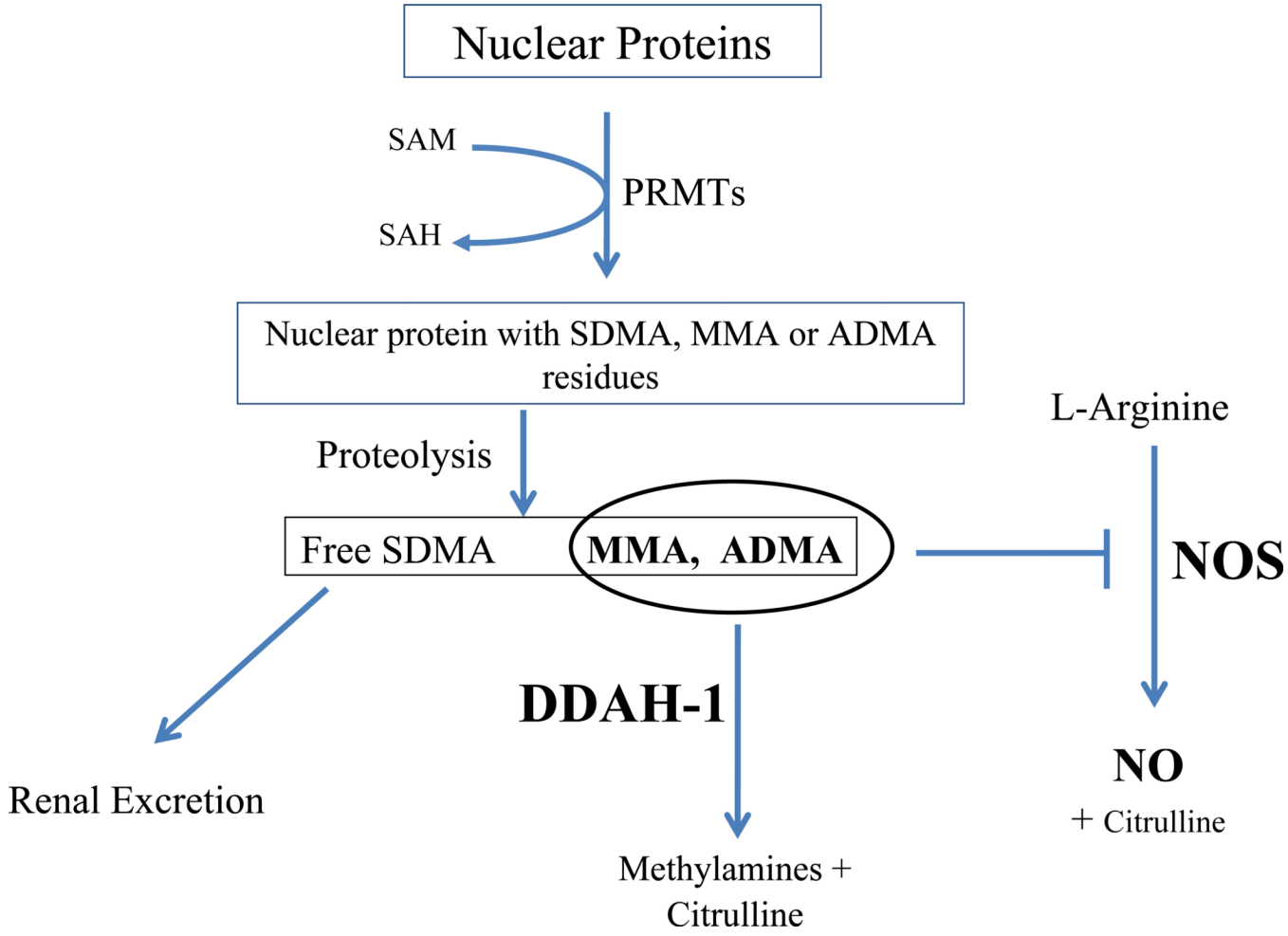

Figure.
The role of DDAH1 in the metabolism of the NOS antagonists ADMA and MMA levels. DMA = dimethylamine. PRMTs = protein arginine methyltransferases. Other abbreviations as in text.