New Tricks From an Old Dog
Nitric Oxide–Independent Effects of Dimethylarginine Dimethylaminohydrolase

James Leiper, Patrick Vallance

The enzyme dimethylarginine dimethylaminohydrolase (DDAH) metabolizes asymmetrically methylated arginine (namely L-N monomethylarginine [L-NMMA] and asymmetrical dimethylarginine [ADMA]) residues to citrulline and methylamine.1 Considerable interest has been focused on the biology of DDAH after the discovery that asymmetrically methylated arginines (in particular ADMA) are competitive inhibitors of all 3 isoforms of nitric oxide synthase (NOS).2 The observations that plasma ADMA levels are competitive inhibitors of all 3 isoforms of nitric oxide synthase (NOS).2 The observations that plasma ADMA levels are elevated in a range of cardiovascular disorders, some of which are associated with impaired NO generation, has led to the suggestion that inhibition of NOS activity by endogenously produced ADMA represents a novel mechanism to regulate NO production in vivo.3,4 Furthermore, it has been suggested that DDAH activity might be required to maintain ADMA levels below the concentration at which NOS inhibition would occur or might act to fine tune NOS activity by maintaining tonic inhibition of NOS1–4 (Figure).

See page 1488

Mammals have 2 isoforms of DDAH, DDAH1 and 2, that appear to have similar catalytic activities but distinct tissue distributions with DDAH1 predominating in tissues that express NOS and DDAH2 predominating in highly vascularized tissues that express eNOS.5 Consistent with the proposed model of DDAH function, pharmacological inhibition of DDAH in cells in culture results in increased ADMA accumulation in the culture medium and is associated with reduced NO generation,6 whereas high-level overexpression of DDAH has the opposite effects.7 Similarly high-level global overexpression of DDAH in mice has been reported to result in lower plasma ADMA concentrations and increased NO generation.8 Despite these encouraging results in model systems where pharmacological inhibition or heterologous overexpression of DDAH produces relatively large changes in ADMA concentrations which alter NO generation, the situation in man is much less clear. The reported ranges for plasma ADMA concentration in both healthy volunteer and patient samples have been shown to differ between laboratories,9,10 and the use of time consuming analytical techniques to measure ADMA has meant that sample sizes for clinical studies have been relatively small. Recently, however, in two separate studies that analyzed plasma ADMA concentration by independent methods in patients with renal failure, both identified plasma ADMA concentration as a strong independent predictor of disease progression.11,12 Of particular interest was the finding that a 0.1 μmol/L increase in plasma ADMA was associated with a 20% increase in cardiovascular event rate. Is it plausible that such a small increase in ADMA could exert such an effect through inhibition of NO production? The IC50 of ADMA on NOS is in the order of 10 μmol/L depending on the prevailing arginine concentration. Given that arginine concentrations rarely fall below 100 μmol/L and ADMA concentrations range between 0.4 and 0.7 μmol/L, it seems unlikely that a 0.1 μmol/L increase in ADMA would have significant effects on NO generation. Although intracellular levels of ADMA might be very much higher, it is also possible that DDAH and ADMA might exert effects independent of NO. We have recently reported that pathophysiological concentrations of ADMA can regulate gene expression in human endothelial cells by what appears to be an NO-independent mechanism13 and have found that DDAH overexpression alters VEGF expression.14 In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Hasegawa et al describe the mechanism whereby DDAH regulates the expression of VEGF in primary endothelial cells in culture.14 Surprisingly, the mechanism is not via increased NO production, as had been previously assumed, but by the direct binding of DDAH2 to protein kinase A (PKA) and subsequent phosphorylation of the transcription factor Sp1. On phosphorylation Sp1 is then imported into the nucleus and activates VEGF transcription (Figure). These findings may be surprising but are not without precedent. In 2001 Tokuo et al demonstrated that DDAH1 could bind to the Ras-pathway regulator neurofibromin 1 (NF1) in vitro.14 DDAH1 binding to NF1 promoted the phosphorylation of NF1 by PKA at specific residues within the cysteine/serine rich domain. Interestingly, fibroblasts derived from NF1−− mice displayed increased DDAH activity suggesting that DDAH1 might be regulated by NF1 binding.

The observations of Hasegawa et al and Tokuo et al suggest a model in which DDAH exerts effects by 2 distinct mechanisms: metabolism of ADMA or regulation of protein function via protein–protein interactions. However, 2 key questions need to be answered before the physiological significance of the findings is clear. First, do these interactions occur in vivo, and if so what is the contribution of DDAH protein binding to the overall regulation of VEGF and NF1 signaling in physiological/pathophysiological situations? The creation and characterization of genetically modified mice that lack or overexpress specific DDAH isoforms will be required to address this issue. Second, are DDAH...
interactions restricted to PKA/NF1, or are these the first 2 examples of a more widespread phenomenon? A systematic screen for DDAH interacting proteins would be required to reveal the full extent of the ADMA-independent functions of DDAH.

In the light of the current findings it is possible that ADMA levels reflect, in part, differences in the expression level of DDAH proteins and that alterations in DDAH protein levels exert biological effects through protein–protein interactions. A better understanding of the biology of DDAH will be required to understand the relative importance of ADMA metabolism and DDAH protein binding in the regulation of cardiovascular function.

Source of Funding
Research in the author’s laboratory is supported by the British Heart Foundation.

Disclosures
None.

References
6. MacAllister R, Parry H, Kimoto M, Ogawa T, Russell R, Hodson H, Whiteley G, Vallance P. Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase (DDAH) to citrulline (CIT) and dimethylamine (MA). ADMA is recognized as a plasma marker of increased cardiovascular risk, but it is unclear whether it ever accumulates to sufficient levels to affect NO pathways. DDAH might also exert biological effects through protein–protein interactions. DDAH1 has previously been shown to interact with neurofibromin 1 (NF1) and promote its phosphorylation (P). The physiological effect of this interaction remains to be determined (?). In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology. Hasegawa et al14 demonstrate that DDAH2 binds to protein kinase A (PKA) and promotes the phosphorylation (P) and activation of the transcription factor Sp1. Activated Sp1 increases the transcription of the vascular endothelial growth factor gene (VEGF).

The DDAH/ADMA pathway. Methylation of arginine residues (R) in proteins and subsequent proteolysis results in the liberation of free methylarginines, including asymmetrical dimethylarginine (ADMA), an inhibitor of nitric oxide synthases (NOS). ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) to citrulline (CIT) and dimethylamine (MA). ADMA is recognized as a plasma marker of increased cardiovascular risk, but it is unclear whether it ever accumulates to sufficient levels to affect NO pathways. DDAH might also exert biological effects through protein–protein interactions. DDAH1 has previously been shown to interact with neurofibromin 1 (NF1) and promote its phosphorylation (P). The physiological effect of this interaction remains to be determined (?). In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology. Hasegawa et al14 demonstrate that DDAH2 binds to protein kinase A (PKA) and promotes the phosphorylation (P) and activation of the transcription factor Sp1. Activated Sp1 increases the transcription of the vascular endothelial growth factor gene (VEGF).

The DDAH/ADMA pathway. Methylation of arginine residues (R) in proteins and subsequent proteolysis results in the liberation of free methylarginines, including asymmetrical dimethylarginine (ADMA), an inhibitor of nitric oxide synthases (NOS). ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) to citrulline (CIT) and dimethylamine (MA). ADMA is recognized as a plasma marker of increased cardiovascular risk, but it is unclear whether it ever accumulates to sufficient levels to affect NO pathways. DDAH might also exert biological effects through protein–protein interactions. DDAH1 has previously been shown to interact with neurofibromin 1 (NF1) and promote its phosphorylation (P). The physiological effect of this interaction remains to be determined (?). In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology. Hasegawa et al14 demonstrate that DDAH2 binds to protein kinase A (PKA) and promotes the phosphorylation (P) and activation of the transcription factor Sp1. Activated Sp1 increases the transcription of the vascular endothelial growth factor gene (VEGF).

The DDAH/ADMA pathway. Methylation of arginine residues (R) in proteins and subsequent proteolysis results in the liberation of free methylarginines, including asymmetrical dimethylarginine (ADMA), an inhibitor of nitric oxide synthases (NOS). ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) to citrulline (CIT) and dimethylamine (MA). ADMA is recognized as a plasma marker of increased cardiovascular risk, but it is unclear whether it ever accumulates to sufficient levels to affect NO pathways. DDAH might also exert biological effects through protein–protein interactions. DDAH1 has previously been shown to interact with neurofibromin 1 (NF1) and promote its phosphorylation (P). The physiological effect of this interaction remains to be determined (?). In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology. Hasegawa et al14 demonstrate that DDAH2 binds to protein kinase A (PKA) and promotes the phosphorylation (P) and activation of the transcription factor Sp1. Activated Sp1 increases the transcription of the vascular endothelial growth factor gene (VEGF).

The DDAH/ADMA pathway. Methylation of arginine residues (R) in proteins and subsequent proteolysis results in the liberation of free methylarginines, including asymmetrical dimethylarginine (ADMA), an inhibitor of nitric oxide synthases (NOS). ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) to citrulline (CIT) and dimethylamine (MA). ADMA is recognized as a plasma marker of increased cardiovascular risk, but it is unclear whether it ever accumulates to sufficient levels to affect NO pathways. DDAH might also exert biological effects through protein–protein interactions. DDAH1 has previously been shown to interact with neurofibromin 1 (NF1) and promote its phosphorylation (P). The physiological effect of this interaction remains to be determined (?). In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology. Hasegawa et al14 demonstrate that DDAH2 binds to protein kinase A (PKA) and promotes the phosphorylation (P) and activation of the transcription factor Sp1. Activated Sp1 increases the transcription of the vascular endothelial growth factor gene (VEGF).
New Tricks From an Old Dog: Nitric Oxide-Independent Effects of Dimethylarginine
Dimethylaminohydrolase
James Leiper and Patrick Vallance

Arterioscler Thromb Vasc Biol. 2006;26:1419-1420
doi: 10.1161/01.ATV.0000229598.55602.17
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/26/7/1419

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/