The lung in the balance: arginine, methylated arginines, and nitric oxide

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THE LUNG IS A MAJOR SOURCE of nitric oxide (NO), be it from nitric oxide synthase (NOS) III in the endothelium of the vast pulmonary circulation, NOS II in the epithelium of the large surface area of the airways, or NOS I in the nonadrenergic noncholinergic nerves (6). NO produced in the lung has major roles in lung physiology, including airway and vascular smooth muscle relaxation, ventilation perfusion matching, neurotransmission, host defense and bacteriostasis, mucociliary clearance, and airway mucus secretion (12). It is also involved in the pathobiology of different lung diseases including asthma and pulmonary hypertension (4–7, 12). Furthermore, as a highly pathobiology of different lung diseases including asthma and pulmonary hypertension (4–7, 12). Furthermore, as a highly diffusible molecule with strong affinity to hemoglobin, NO produced in the lung is avidly taken up by the blood in the pulmonary circulation and transported throughout the body, serving physiological functions well beyond the lungs and the pulmonary circulation (8, 16, 18). The report from Bulau et al., one of the current articles in focus (Ref. 3, see p. L18 in this issue), reports another remarkable related discovery: the lung is also a major source of the endogenous NOS inhibitor asymmetric N\textsuperscript{0}\text{G}-N\textsuperscript{0}\text{G}-dimethylarginine (ADMA). This finding has significant physiological and pathological implications not only for the pulmonary circulation and the lung but also for the systemic circulation and beyond.

The discovery in the late 1980s that endothelial derived relaxing factor was NO (9, 14) shifted the attention of the scientific community on arginine from its traditional role in protein synthesis to its role as a precursor for the production of NO (19). Although NO has long been known as an atmospheric pollutant present in vehicle exhaust emissions, smog, and cigarette smoke, it was also used as a pharmacological agent known to activate guanylate cyclase and produce cGMP (1). However, the discovery that this simple gaseous molecule is endogenously produced was certainly a paradigm shift at the time that led to an exponential growth in our knowledge about NO and its role in human physiology and disease. Interestingly enough, N\textsuperscript{2}-monomethylarginine (L-NMMA), a pharmacological inhibitor of NOS that was used to study the function of NO (13), turned out to be a naturally occurring compound as well (21). Along with other methylated arginines, L-NMMA is the product of protein degradation and release of methylated arginine, bringing the story back full circle with the focus again on arginine not only as a precursor of NO but also as the precursor of methylated arginines through protein synthesis, posttranslational modification, and finally degradation.

Arginine or 2-amino-5-guanidinovaleric acid (Fig. 1) is a semiessential amino acid. Young mammals require arginine exogenously, whereas adults can synthesize it de novo. Arginine participates in various metabolic pathways including cleavage (via arginase) into urea and ornithine in the urea cycle, deamination (via arginine deaminase) to citrulline, synthesis of creatine (via arginine-glycine amidinotransferase and guanidinoacetate N-methyltransferase), synthesis of proteins (where it can be methylated via protein arginine methyltransferases [PRMT]), and as a precursor for the synthesis of NO (via NOS) (19). Although the arginine-NO pathway only represents a fraction of the total arginine metabolism, it has attracted considerable attention due to the many versatile roles that NO plays in almost all organ systems. In addition to activating guanylate cyclase resulting in smooth muscle relaxation, NO is also involved in a variety of physiological functions (12).

It is currently well accepted that the lung plays a major role in NO metabolism and is well established as a major source of NO. Interestingly, in this issue, Bulau et al. (3) demonstrate that the lung is also a major source of the NOS inhibitor and arginine analog ADMA. They demonstrated that the lung expresses the enzymes necessary for methylarginine formation (type I PRMT) as well as clearance [dimethylarginine dimethylaminohydrolase (DDAH) 1]. PRMT expression correlated with enhanced protein arginine methylation. Furthermore, bronchoalveolar lavage fluid and serum exhibited almost identical levels of ADMA/symmetric N\textsuperscript{0}\text{G}-N\textsuperscript{0}\text{G}-dimethylarginine (SDMA), suggesting that methylarginine metabolism by the lung significantly contributes to circulating levels of ADMA.

ADMA is one of three circulating endogenous methylated analogs of L-arginine (Fig. 1) that are produced as a result of proteolysis of methylated proteins. Methylation of arginine incorporated in proteins is a process of posttranslational modification of protein function (similar to phosphorylation) that is carried out by a group of enzymes known as the PRMT enzymes. Several subtypes of PRMTs have been identified as being responsible for methylation of protein arginine residues by the addition of one or two methyl groups to the guanidine nitrogen atoms of arginine. Methylated arginines are products of subsequent protein degradation. The two asymmetric methylarginines, L-NMMA and ADMA, act as false substrates and competitively inhibit NOS activity, blocking the formation of endogenous NO (19, 20). SDMA does not inhibit NOS, whereas L-NMMA is frequently used as a pharmacological inhibitor of NOS, its low circulating levels makes it unlikely to contribute significantly to NOS inhibition in vivo. This makes ADMA the major endogenous NOS inhibitor among the methylated arginines (20). Asymmetric methylarginines (L-NMMA and ADMA) can be hydrolyzed by DDAH to yield citrulline and mono- or dimethylamine (11). DDAH does not hydrolyze SDMA. By competitively inhibiting NO synthesis from L-arginine by NOS (Fig. 2), the asymmetric endogenous methylarginines can have significant biological effects encompassing all the negative effects of “NO deficiency.” Thus methylated arginines may be responsible (at least in part) for a peculiar concept in NO metabolism known as the “L-arginine paradox.” It refers to the phenomenon that exogenous arginine causes NO-mediated effects despite the fact that, at physiological state, NOS is already saturated with arginine and its activity should not be affected by increasing arginine concentration.
Both intracellular (0.1–1 mM) and extracellular (73–150 μM) arginine concentrations far exceed the apparent $K_m$ (the half-saturating arginine concentration) of all NOSs (e.g., 2.9 μM for endothelial NOS) (19). Despite this, elevating plasma arginine levels enhance systemic and vascular NO production in a dose-dependent manner suggesting some form of competitive inhibition of NOS that could be overcome with increasing arginine concentrations (19). This paradox is not fully understood, but several theories have been put forth to explain it based on our current understanding of arginine and NO metabolism (19) including: the compartmentalization of arginine in the cytoplasm (extracellular arginine may be preferentially utilized by NOS within this microenvironment); the inhibitory effects of L-citrulline (cells may need extra arginine to compete with citrulline); and competition from arginase for arginine (a substrate for both enzymes) making less arginine available for NO production by NOS. This concept could also be explained by the presence of ADMA, which competitively antagonizes arginine and which could also be overcome with increasing arginine concentrations.

Overall, it appears that the synthesis and metabolism of endogenous methylarginines are highly regulated. Imbalance in this pathway is associated with several pathobiological consequences. Since elevated plasma levels were first reported in patients with renal failure (21), ADMA has been implicated in the pathogenesis of a variety of clinical conditions such as systemic hypertension, pulmonary hypertension, stroke, diabetes, hyperlipidemia, hyperhomocyst(e)inemia, and atherosclerosis, and the list is constantly expanding (11, 17, 19, 20). More recently, ADMA has been shown to be a risk factor for cardiovascular disease (2). The lung seems to play a prominent role in this important and delicate balance. One particular lung artery disease; DM, diabetes mellitus; HTN, systemic hypertension; PAH, pulmonary arterial hypertension.

### REFERENCES


