

Numerous recent studies have used either feeding or intravenous infusion of L-arginine as a probe for examining the role of endogenous nitric oxide (NO) in various physiological and pathophysiological processes. Such studies have shown that L-arginine produces peripheral vasodilation, inhibits platelet aggregation, and acutely improves endothelium-dependent vasodilator responses. L-Arginine feeding reduces macrophage adhesion to the endothelium and prevents atherosclerosis. These effects of L-arginine seem to involve the NO synthase pathway because levels of plasma nitrate and urine cyclic GMP increase during the L-arginine administration. An implicit, and often stated, conclusion from these studies is that a deficiency of L-arginine exists, such that this amino acid is rate limiting in terms of NO synthase activity. On the surface, this line of logic seems perfectly logical. The perplexing aspect of this is that the intracellular concentration of L-arginine (several hundred μM) far exceeds the K_m of the NO synthases ($\sim 5 \mu\text{M}$) (1). When endothelial cells in culture are deprived of L-arginine, cell viability is reduced before levels are lowered to ranges nearing the K_m for NO synthase. Therefore, it seems unlikely that L-arginine could ever be rate limiting in vivo. The term "arginine paradox" has been used to refer to situations in which exogenous L-arginine administration seems to drive enzyme activity even when levels of L-arginine are available in excess.

Not all investigators have found that arginine affects NO production. In their first description of the dependence of endothelial cell NO production on L-arginine, Palmer and Moncada showed that arginine did not stimulate NO production from cells in culture unless they had been deprived of the amino acid for prolonged (24-h) periods (2). Previously, we found that in vitro treatment of aortic rings from cholesterol-fed rabbits with L-arginine did not improve relaxations to acetylcholine (3) and recently have confirmed this in another animal model and in human vessels studied in vitro.

It is perplexing that the arginine paradox is, for the most part, only observed when the amino acid is administered in vivo. If L-arginine is acting as a substrate for NO synthase, it should be as effective in vitro as in vivo. NO synthase antagonists, which are only slightly modified forms of L-arginine, gain ready access to the enzyme when administered to in vitro tissue preparations. One explanation for why L-arginine might not be effective in in vitro studies has to do with the composition of the buffers used. In a prior study, we found that L-arginine had no effect on endothelium-dependent vascular relaxations in a simple Krebs/Hepes buffers. When L-glutamine was present in concentrations similar to those in plasma, L-arginine dose-dependently enhanced endothelium-dependent vascular relaxation. This effect seemed to involve a complex interplay with L-glutamine and receptor-mediated activation of NO synthase, which was independent of intracellular L-arginine levels (1).

In this issue of *The Journal*, we learn of another mechanism whereby L-arginine might stimulate vasodilation in vivo, which has nothing to do with it serving as a substrate for NO synthase. Giugliano et al. (4) demonstrate that intravenous infusions of L-arginine stimulate insulin release (a well established phenomenon). They further demonstrate that this increase in plasma insulin is the factor responsible for the peripheral vasodilation, decrease in platelet aggregation, and decrease in blood viscosity, which others have attributed to enhanced production of NO (4). The vasodilation produced by L-arginine could be prevented by octreotide, a somatostatin analogue that inhibits insulin release. This hemodynamic effect of octreotide could be overcome by addition of insulin during the L-arginine infusion (to mimic the effect of stimulation of insulin release by the L-arginine).

Insulin has been known to produce vasodilation and increase cardiac output for several years (5). Earlier studies suggested that this only occurred in the presence of pharmacological levels of the hormone, however, more recent work indicates that insulin is capable of modulating vascular tone over physiological concentration ranges. Indeed, the current paper by Giugliano et al. (4) confirms this. Of significance, at least some of the vasoactive effects of insulin are mediated by the endogenous release of NO. For example, during euglycemic clamp studies, insulin infusion approximately doubled leg blood flow; this effect could be prevented by treatment with the NO synthase antagonist L- N^G -monomethyl arginine (6). The signaling mechanism whereby insulin activates NO synthase remains undefined.

The study by Giugliano et al. (4) leaves certain questions to be answered. It would have been helpful to show that the effect of L-arginine could have been prevented by an NO synthase antagonist, such as L- N^G -monomethyl arginine. This has been the case in other, similar types of experiments. It is not clear if this action of L-arginine working via insulin simply produces vasodilation or if it could also enhance vasodilator responses to other endothelium-dependent agents, such as muscarinic agonists. It is conceivable that it might. An increase in the basal release of NO could shift the position on a dose-response curve from a plateau region, where additional release of NO would have minimal effect, to a steep region of the dose-response curve, where release of tiny additional amounts of NO could produce marked vasodilation. Lastly, the data by Giugliano et al. (4) are likely not applicable to studies in which L-arginine feeding has been used. In feeding protocols, plasma L-arginine levels only increase by about twofold, while in the studies by Giugliano et al. (4), levels rose dramatically. Thus, effects of L-arginine feeding are unlikely caused by changes in plasma insulin.

To be fair, there are theoretical scenarios whereby L-arginine administration could directly drive activity of the endothelial NO synthase. An endogenous inhibitor of NO synthase, asymmetric dimethylarginine (ADMA) accumulates in renal failure, preeclampsia, and in the serum of cholesterol-fed rabbits. It is conceivable that it might antagonize the normal intracellular concentrations of L-arginine such that additional arginine could overcome this effect (7–9). The plasma concentrations of ADMA, even in renal failure, are low compared

with L-arginine levels. Despite the fact that it was described several years ago, a definitive role for ADMA in vivo, even in renal failure, has yet to be established. There is exciting new data showing that the endothelial NO synthase is preferentially targeted to caveolae (10). The concentration of L-arginine in such a protected microenvironment may be different than that of the entire cell. This concept is highly speculative, and it is unclear how L-arginine treatment might specifically affect caveolar concentrations.

In summary, the findings of Giugliano et al. (4) go a long way toward explaining the arginine paradox. These studies should caution investigators not to overinterpret experiments in which substrates for enzymes are infused in an intact animal or human subject in an effort to probe an enzyme's role. In their most favorable interpretation, such studies with L-arginine may allow an indirect assessment of the NO synthase system (if this is the only mechanism whereby insulin produces vasodilation), but cannot be used to reach conclusions about endogenous substrate supply for the NO synthases. Conditions of insulin resistance may make even such a limited extrapolation invalid.

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