*REVIEW* —*Current Perspective*—

# Beneficial Circulatory Effect of L-Arginine

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ABSTRACT—L-Arginine is an essential amino acid for infants and growing children. This amino acid is a substrate for at least five enzymes identified in mammals, including arginase, arginine-glycine transaminase, kyotorphine synthase, nitric oxide synthase (NOS) and arginine decarboxylase. L-Arginine exerts antihypertensive and antiproliferative effects on vascular smooth muscles. NOS and arginine decarboxylase appear to be important for the effect of L-arginine on the circulatory system, since each produces nitric oxide (NO), a potent vasodilator, and agmatine, an endogenous noncatecholamine ligand for central alpha-2 adrenoceptors, from L-arginine. Several issues must be clarified before the mechanisms by which L-arginine exerts its effects on the circulatory system can be fully understood.

*Keywords*: L-Arginine, Hypertension, Atherosclerosis, Nitric oxide, Agmatine

L-Arginine is an essential amino acid for infants and growing children. Although this amino acid is normally made by the liver as a step in the synthesis of urea, children cannot produce arginine rapidly enough to support growth requirements. It is known that the administration of 30 g of arginine to humans causes insulin and growth hormone release. This dose is about five times the daily requirement of L-arginine since, based on the amino acid composition of foods, the average diet is estimated to provide 5.4 g of arginine daily. In any event, this procedure has long been used as a growth hormone secretion test in children. The mechanisms by which hormonal stimulation occurs are still unknown.

The classical enzyme catabolizing arginine is arginase, in the urea cycle, that catalyzes the conversion of L-arginine to ornithine with concomitant production of urea. Another classical example of an arginine-catalyzing enzyme is arginine-glycine transaminase, which produces guanido acetic acid, the only precursor of creatine and a major source of high energy phosphate for ATP regeneration.

Recent investigations have identified three enzymes that catalyze the conversion of arginine: kyotorphine synthase that produces kyotorphine from L-arginine and L-tyrosine; nitric oxide synthase (NOS) that produces nitric oxide (NO) from L-arginine; and arginine decarboxylase that catalyzes the conversion of L-arginine to agmatine, an endogenous agonist for brain alpha-2 adrenoceptors (1).

In addition to the aforementioned hormone release, exogenous arginine has many effects on mammalian functions including the promotion of wound healing, stimulation of the immune system and analgesia. We will focus, in this brief review, on the effect of exogenously administered arginine on the circulatory system.

Pioneering work on the effects of L-arginine on the circulatory system was reported in 1988. Sakuma et al. (2) showed that L-arginine reversed $N^G$-monomethyl arginine-induced inhibition of histamine-elicited relaxation. Palmer et al. (3) demonstrated, using a cascade bioassay, that L-arginine augments bradykinin-induced endothelium-derived relaxing factor (EDRF) formation. These observations established that L-arginine is the precursor of EDRF. The first observation of the effect of L-arginine, by itself, on the circulatory system was made by us (4). We found that the administration of 30 g L-arginine induced a small but significant reduction in blood pressure in healthy volunteers. This observation was confirmed by other investigators (5). Cardiac output was increased, and total peripheral resistance was significantly decreased (6, 7). This finding can be interpreted as follows: peripheral blood vessels were first dilated, total vascular resistance then decreased, and heart rate and cardiac output were secondarily increased. Among the plasma hormones that

may affect total peripheral resistance, norepinephrine, epinephrine, arginine vasopressin and renin were not decreased. The levels of aldosterone and atrial natriuretic peptide were slightly changed, but the values were within the normal range. The L-arginine concentration was increased to 7 mM by intravenous administration of exogenous L-arginine. Comparing the time course of blood pressure reduction with the L-arginine concentration lead to the speculation that the concentration required for hypotension is at least 0.5 mM.

In the rat, L-arginine-induced hypotension was blocked by atropine and chlorpheniramine, suggesting the involvement of acetylcholine and histamine, the release of which is known to be induced by L-arginine. Therefore, the hypotension produced by L-arginine could be due to secondary effects of acetylcholine and histamine. In humans, coadministration of the $H_1$-receptor antagonist chlorpheniramine, the $H_2$-receptor antagonist famotidine, and the muscarinic antagonist scopolamine failed to inhibit the amino acid-induced hypotension (8).

It would be reasonable to speculate as to whether the amount of the vasorelaxing substance NO is reduced in hypertensive patients. It has been shown that basal forearm blood flow is similar in hypertensive patients and controls. Blood flow and vascular resistance responses to acetylcholine were significantly reduced in hypertensive patients, whereas the response to sodium nitroprusside, an NO donor, was unchanged or enhanced in such patients (9). L-Arginine did not significantly change basal blood flow or vascular resistance in either group. However, the infusion of L-arginine significantly augmented the vasodilator response to acetylcholine in normal control subjects. In contrast, in hypertensive patients, the infusion of L-arginine did not alter the response to acetylcholine (9). These results suggest that agonist-stimulated L-arginine availability may be impaired in hypertensive subjects.

In patients with essential hypertension (4) and secondary hypertension, including renovascular hypertension, and primary aldosteronism, we have demonstrated that L-arginine causes marked reductions in blood pressure, far exceeding those seen in healthy controls, as well as increasing the heart rate (7, 10). Plasma aldosterone was significantly reduced by L-arginine administration, despite plasma renin activity not being reduced. Therefore, the site at which L-arginine exerts its inhibitory effects on aldosterone level is different from that of the regulatory site for renin. Arginine vasopressin and atrial natriuretic peptide were significantly increased and remained elevated even after blood pressure had normalized.

The hypotensive effect of L-arginine has been utilized during surgical procedures for dissecting aneurysms in elderly hypertensive patients (11). The authors concluded that prompt reduction of blood pressure and rapid recovery after cessation of infusion of L-arginine merit the use of this amino acid for intraoperative blood pressure control.

We investigated whether L-arginine-induced hypotension involves NO release. NO is oxidized to $NO_2^-$ and $NO_3^-$ in vivo. NO activates guanylate cyclase and stimulates the production of cyclic GMP. Therefore, we measured $NO_2^-/NO_3^-$ and plasma cyclic GMP as an index of in vivo NO production. The blood pressure reduction was accompanied by increases in plasma concentrations of cyclic GMP and L-citrulline, the latter being a byproduct of NO formation from L-arginine. Furthermore, urinary output of $NO_2^-/NO_3^-$ was significantly increased (6, 7). The only known endogenous source of $NO_2^-/NO_3^-$ is the L-arginine-NO pathway. These results appear to be consistent with the interpretation that exogenous administration of L-arginine increases NO production in vivo. Provided that the effect of L-arginine involves endogenous NO release, these data suggest that even in hypertensive patients the L-arginine-NO pathway remains operative when sufficient quantities of the L-arginine substrate are available.

According to research on the local effect of L-arginine on blood flow in the human anterior brachial artery, not only a high dose of L-arginine but also a comparable dose of D-arginine caused vasodilatation. Since D-arginine is not a substrate for NOS, a high dose of L-arginine causes vasodilatation independently of NO production (12). This report suggests that some effects of L-arginine are not enantiomer specific. On the other hand, an L-arginine, but not a D-arginine, infusion of up to 60 mg/min caused enantiomer-specific vasodilatation, and the authors suggested that the infusion of L-arginine leads to NO production (13).

A low dose (0.15 g/kg) of L-arginine administered to dogs caused no change in systemic blood pressure, but did significantly increase renal blood flow (14) and reduce peripheral resistance (15). When rats are kept in a hypoxic state for 3 weeks, pulmonary vessel responsiveness to relaxing substances such as acetylcholine diminishes. L-Arginine administration, in this model, restored normal responses to acetylcholine (16). In newborn pulmonary hypertensive lambs, L-arginine has been shown to reduce pulmonary artery pressure (17). This amino acid does not affect blood pressure in normal lambs. These observations suggest that even in the absence of systemic hypotension, the peripheral vasculature can dilate and increase blood flow.

It was reported that intraperitoreal injection (9–100 mg/kg) of L-arginine, but not D-arginine, for 4 days produced a hypotensive effect in spontaneously hypertensive rats (18). This is the lowest dose ever used successful-

ly for reducing blood pressure, but this observation may require further confirmation. Higher doses (25–250 mg) of L-arginine, but not D-arginine, administered to Dahl/Rapp salt-sensitive hypertensive rats reduced blood pressure to normal control levels. In contrast to the aforementioned results, the same authors reported that an identical regimen failed to produce hypotension in spontaneously hypertensive rats (19).

In hypercholesterolemic patients, relaxation of coronary arteries in response to acetylcholine is diminished and infusion of L-arginine remarkably restores this response (20). The diminished responsiveness of limb arteries (21) and brain basal arteries (22), both from rabbits fed cholesterol, were restored by L-arginine infusion into the arteries. However, L-arginine supplementation of the incubation buffer bathing the thoracic aortae taken from cholesterol-treated rabbits failed to restore normal responsiveness to acetylcholine (23).

L-Arginine diminishes the intimal thickening induced by high cholesterol (24). Whether the plasma concentration of L-arginine is reduced in hypercholesterolemic patients remains controversial. L-Arginine inhibits balloon catheter-induced intimal hyperplasia (25) and restores laser beam-induced endothelial dysfunction in rat brain arterioles (26). These observations suggest a preventive or ameliorative effect of L-arginine on endothelial injury and intimal thickening.

These effects are best understood by hypothesizing the involvement of NO in the effects of L-arginine. NO may play an important role in maintaining the normal quiescent state of vascular smooth muscle, since NO (27) inhibits the proliferation of vascular smooth muscle cells. These findings suggest that decreased NO release from injured endothelium causes disinhibition of the proliferation of vascular smooth muscle cells.

Thus, there are several lines of convincing evidence for the antihypertensive and antiatherogenic effects of L-arginine. The mechanisms do not, however, appear to be straightforward. Among five L-arginine catalyzing enzymes, two pathways are potentially related to the regulation of blood pressure. One is NOS and the other is an agmatine forming enzyme (Fig. 1).

The intracellular concentration of L-arginine is presumed to be about 0.1 mM. The $K_m$ value of constitutive NOS for L-arginine is 0.01 mM. The constitutive NOS is thus theoretically saturated for endogenous L-arginine. Therefore, it may be difficult to explain why exogenously added L-arginine increases NO production and affects blood pressure. One possible explanation might be the presence of endogenous NOS inhibitor. For example, in patients with chronic renal failure, the plasma concentration of the endogenous NOS inhibitor $N^G,N^G$-dimethylarginine, is increased to a level that is sufficient to inhibit NOS (28). Therefore, this endogenous inhibitor is a potential factor in the hypertension associated with chronic renal failure. The presence of endogenous NOS inhibitors

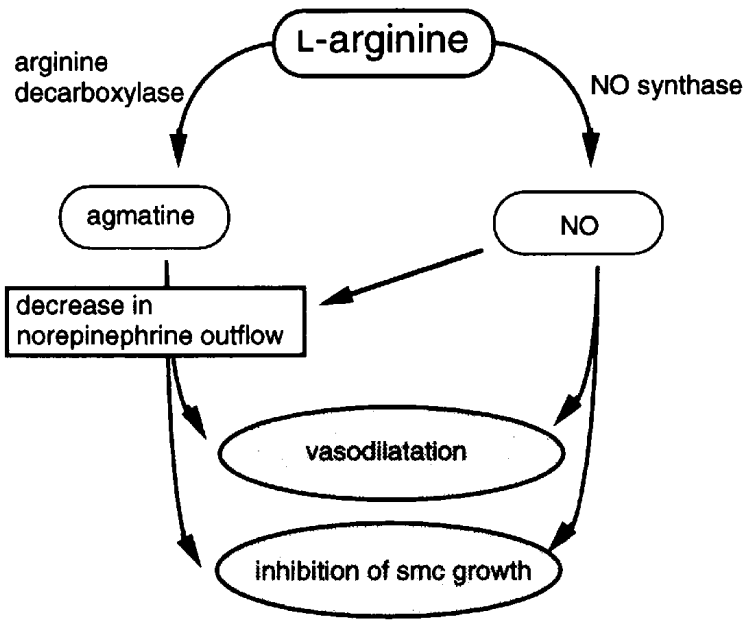


**Fig. 1.** Simplified hypothetical pathway by which the exogenously added L-arginine that affects the circulatory system is converted. Although L-arginine is the precursor of agmatine and NO, it remains to be established whether exogenously added L-arginine augments the formation of agmatine and NO. In particular, the physiological and pathophysiological significance of the L-arginine-agmatine pathway has not yet been established. NO synthase includes constitutive and inducible forms. smc: smooth muscle cells. For details, see the text.

causes a shift in the dose-response curve of L-arginine to the right so that more L-arginine is needed to produce the same effect. Under normal physiological conditions, however, this may not be the case.

Another interpretation involves the inducible type of NO synthase (iNOS). NOS is broadly divided to constitutive and inducible forms. The constitutive NOS form is found in endothelial cells, platelets and megakaryoblastic cells, bronchial epithelial cells, the cerebellum, parasympathetic nerves and the macula densa. On the other hand, iNOS is widely distributed in macrophages, vascular endothelium and smooth muscles, cardiac myocytes, kidney mesangial cells, chondrocytes, glial cells (astrocytes and microglial cells) and Kupffer cells. Recent evidence indicates that "inducible" NOS is expressed even in the "basal" state. Since the $K_m$ value of L-arginine for inducible NOS is one order of magnitude larger than that for constitutive NOS, exogenously added L-arginine is an efficient substrate for iNOS.

As to the hypotensive and antiproliferative effects of L-arginine, another interpretation is also possible. Agmatine, an endogenous noncatecholamine alpha-2 agonist derived from L-arginine, may be involved in the antihypertensive effect of L-arginine. It is well known that alpha-2 agonists act on the nucleus tractus solitarius in the brain stem, thereby decreasing the peripheral sympathetic outflow of norepinephrine. It is interesting to note that NO acts on the central nervous system so as to diminish sympathetic discharge (29). Catecholamines are known to stimulate the growth of vascular smooth muscle cells (30). The agmatine pathway has, however, been insufficiently studied and merits further elucidation. In particular, there is no evidence for agmatine generation from exogenous L-arginine.

In summary, L-arginine exerts antihypertensive and antiproliferative effects on vascular smooth muscles. The underlying mechanisms may involve NOS and arginine decarboxylase, resulting in the formation of NO and agmatine, respectively. Yet there are several issues remaining to be clarified before the mode of action underlying the circulatory effects of L-arginine is fully understood.

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