Clinical and Experimental Pharmacology and Physiology (2009) **36**, 249–255 doi: 10.1111/j.1440-1681.2008.05123.x

INVITED REVIEW

ROLE OF L-ARGININE IN NITRIC OXIDE PRODUCTION IN HEALTH AND HYPERTENSION

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SUMMARY

1. L-Arginine is the substrate for vascular nitric oxide (NO) formation. Under normal physiological conditions, intracellular L-arginine levels far exceed the K_m of NO synthase for L-arginine. However, endogenous NO formation is dependent on extracellular L-arginine concentrations, giving rise to the concept of the 'L-arginine paradox'.

2. Nitric oxide production in epithelial and endothelial cells is closely coupled to cellular L-arginine uptake, indicating that L-arginine transport mechanisms play a major role in the regulation of NO-dependent function.

3. Consistent with the data in endothelial and epithelial cells are functional data indicating that exogenous L-arginine can increase renal vascular and tubular NO bioavailability and thereby influence kidney perfusion, function and arterial pressure. The integrated effect of increased cellular L-arginine transport is to lower arterial pressure. Therefore, the use of L-arginine in the treatment of hypertension warrants investigation.

4. Low NO bioavailability is central to the development and maintenance of hypertension and to related endothelial dysfunction and target organ damage. We propose that L-arginine can interrupt the vicious cycle that initiates and maintains low NO in hypertension by increasing the formation of NO.

Key words: L-arginine, L-arginine uptake mechanisms, hypertension, kidney, nitric oxide.

INTRODUCTION

L-Arginine was discovered as a naturally occurring amino acid more than a century ago.[1] The more recent discovery that nitric oxide (NO),[2] derived from L-arginine, is a major endothelium-derived relaxing factor shifted the focus of interest in L-arginine towards its potential role in cardiovascular control. Nevertheless, the role of L-arginine in the long-term regulation of arterial pressure remains to be determined. There are three major pathways for L-arginine metabolism: L-arginine is metabolised to L-ornithine by arginase, to agmatine by arginine decarboxylase and to NO and citrulline by NO synthase (NOS).[3] Importantly, L-arginine is the substrate for vascular NO formation.[4–6] In humans, approximately 1% of the daily L-arginine intake is metabolised via this pathway,[1] but the degree to which vascular NO production is limited by L-arginine remains controversial.

Low NO contributes to the development and maintenance of hypertension, as well as related endothelial dysfunction and target organ damage.[7] Hence, increasing NO is a potential therapeutic target in hypertension. The amount of NO available is determined by a balance between its formation and scavenging/inactivation. Superoxide is a major culprit responsible for the latter process. The role of superoxide in hypertension has been investigated extensively and the findings of these studies have been discussed in recent reviews.[8,9] Although NO formation is an important determinant of NO bioavailability, recent studies have largely overlooked the factors that regulate NO synthesis. In this review we present the argument that L-arginine is an important determinant of endogenous NO production in health and particularly under conditions of hypertension.

L-ARGININE SYNTHESIS AND METABOLISM

Most endogenous L-arginine synthesis occurs in the kidney.[3] Approximately 85% of intestinal citrulline released from glutamine metabolism is taken up by the kidney for L-arginine production.[3] Although the proximal portions of the renal tubules in the renal cortex of the kidney can synthesise L-arginine, this amino acid has been demonstrated to be a limiting factor in NO production in the renal medulla.[10] L-Arginine reabsorption in the tubules is mediated by a carrier-mediated transport system.[11] The administration of high doses of L-arginine has been shown to result in increased urinary L-arginine excretion in humans.[12]

THE L-ARGININE PARADOX

The intracellular concentration of L-arginine (100–3800 μmol/L)[13] exceeds the Michaelis–Menten constant (K_m) of NOS for L-arginine (< 5 μmol/L).[14] However, there is strong evidence that endogenous NO production is dependent on extracellular L-arginine concentration. Increasing extracellular L-arginine concentration enhances NO-dependent vasorelaxation or other indices of endothelial function in the vasculature of experimental animals and humans with hypertension,

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Received 15 July 2008; revision 29 October 2008; accepted 2 November 2008.

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diabetes mellitus or hypercholesterolaemia.[6,15–23] In addition, the shear stress-induced release of NO in cultured endothelial cells is dependent upon cellular L-arginine uptake.[24,25] The reliance of NO generation and NO-dependent function on the extracellular concentration of L-arginine, despite high intracellular L-arginine concentrations, has given rise to the concept of the 'arginine paradox'. Several mechanisms have been proposed to explain the arginine paradox. One possibility is the compartmentalization of L-arginine in the cytoplasm. Endothelial NOS (eNOS) and the cationic amino acid transporter (CAT) 1 (the predominant arginine transporter) are both located in the plasma membrane of endothelial cells and may not be able to readily access intracellular L-arginine pools.[26] Conversely, extracellular L-arginine is readily accessible to CAT1. The close proximity of eNOS to CAT1 may indicate that eNOS preferentially uses L-arginine transported from the extracellular fluid by CAT1. The presence of endogenous NOS inhibitors, such as asymmetric dimethylarginine (ADMA), has also been shown to reduce the sensitivity of NOS to L-arginine.[27] The addition of extracellular L-arginine may overcome the inhibition of these endogenous compounds.[27] Each of these proposed mechanisms potentially plays a role in the L-arginine paradox and underlies the beneficial effects of L-arginine supplementation in hypertension.

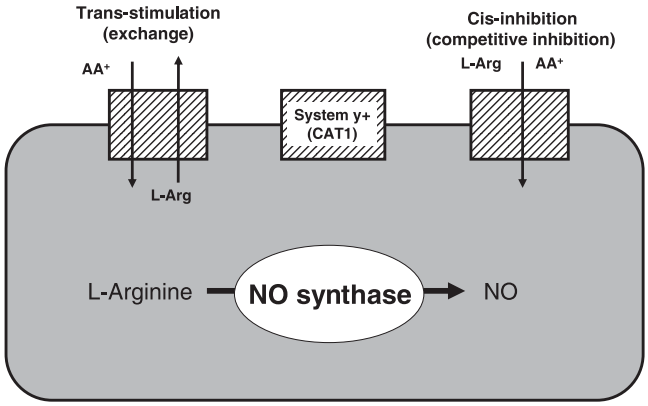

Fig. 1 Model of L-arginine (L-Arg) uptake mechanisms in rat inner medullary collecting duct cells. Cellular uptake of L-arginine and other cationic amino acids (AA^+) is mediated by system y^+ transporters encoded as cationic amino acid transporter (CAT) 1. L-Arginine transport can be altered by competitive inhibition for uptake with other cationic amino acids or by trans-stimulation. Cellular efflux of L-arginine can be stimulated by trans-stimulation mechanisms in which L-arginine is expelled in exchange for the entry of another cationic amino acid. NO, nitric oxide.

CELLULAR L-ARGININE TRANSPORTERS

Specialized transport channels in the plasma membrane are required for extracellular L-arginine to enter the cell. Differentially expressed transport systems (y^+, y^+L) are responsible for transporting not only L-arginine, but also other cationic and neutral amino acids across the plasma membrane of the cell.[28] Because other amino acids can compete with L-arginine for cellular uptake, the extracellular concentration of L-arginine and other cationic and neutral amino acids can affect L-arginine transport. In endothelial cells, L-arginine uptake is mediated by both y^+ and y^+L transport mechanisms,[28] whereas y^+ transport mechanisms appear to be most important in epithelial cells.[10] System y^+ transporters selectively mediate the cellular transport of cationic amino acids, including L-arginine; in contrast, system y^+L transports both cationic and neutral amino acids.[28] System y^+ is a family of cationic transporters encoded as CAT1, CAT2A, CAT2B and CAT3.[29–32] The y^+L mechanism observed in endothelial cells is associated with CD/984F2 heavy chain.[33] The y^+ and y^+L systems can transport cationic amino acids independently of sodium, but y^+L transporters depend on sodium for the uptake of neutral amino acids.[28] Both the y^+ and y^+L systems demonstrate competitive inhibition as well as trans-stimulation (exchange). In trans-stimulation, the addition of cationic or neutral amino acids to the extracellular space enhances L-arginine efflux from the cells.[28] Taken together, these data indicate that the extracellular concentration of L-arginine, as well as that of other cationic and neutral amino acids, can affect cellular L-arginine transport.

L-ARGININE UPTAKE MECHANISMS IN EPITHELIAL CELLS

Considerable experimental data support an important role for cellular L-arginine uptake mechanisms in the production of NO in renal epithelial cells.[10] In the isolated perfused thick ascending limb, increased extracellular L-arginine concentration resulted in the NO-dependent inhibition of chloride absorption.[34] Other studies have demonstrated that L-arginine uptake is important for NO-mediated effects on the renal tubuloglomerular feedback response.[35] Although cells in the proximal portions of the nephron are capable of generating L-arginine from L-citrulline by the sequential action of argininosuccinate synthase and lyase,[36,37] cell types in the distal portions of the nephron, particularly the inner medullary collecting duct (IMCD), have an extremely low L-arginine synthetic capacity. We performed a series of studies to examine the importance of L-arginine transport in the IMCD.

Initial experiments in freshly isolated IMCD cells indicated that L-arginine uptake is saturable, sodium independent and exhibits biphasic kinetics.[10,38] L-Arginine uptake was inhibited by the addition of the cationic amino acids to the cell media, but was not altered by neutral amino acids or specific substrates for other amino acid transport systems.[10] These findings are consistent with the hypothesis that L-arginine uptake is mediated by a y^+ mechanism in the IMCD. Subsequent studies identified the transporter in the IMCD as CAT1.[10] Further experiments then demonstrated that NO production in isolated IMCD is directly coupled to cellular L-arginine uptake.[10] Extracellular administration of cationic amino acids that compete with L-arginine for cellular uptake (L-lysine, L-ornithine or L-homoarginine) resulted in a reduction in NO production in freshly isolated IMCD; administration of excess L-arginine increased NO content.[4,10] These observations provide direct evidence that extracellular L-arginine is an important determinant of endogenous NO formation in epithelial cells of the renal medulla and have led us to propose a model that links cellular L-arginine transport by a CAT1-mediated mechanism to NO production in the IMCD (Fig. 1).

L-ARGININE UPTAKE MECHANISMS IN ENDOTHELIAL CELLS

The studies described above indicate that L-arginine uptake is important in NO production in epithelial cells. Subsequent studies were performed to determine whether these same manipulations could alter NO formation in cultured endothelial cells and in the renal vasculature.

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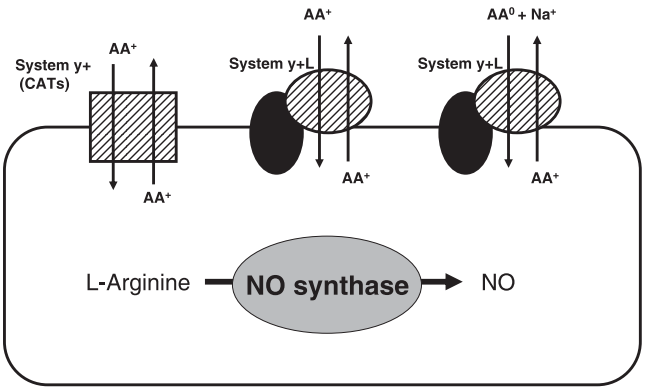

Fig. 2 Model of L-arginine uptake mechanisms in endothelial cells. Cellular uptake of L-arginine and other cationic amino acids (AA^+) is mediated by system y^+ and system y^+L transporters (a heterodimer). L-Arginine transport can be inhibited by competitive inhibition with other cationic amino acids (system y^+ and y^+L) or by neutral amino acids (system y^+L). Cellular efflux of L-arginine can be stimulated by trans-stimulation mechanisms in which L-arginine is removed from the cell in exchange for the entry of another amino acid. AA^0, neutral amino acids; NO, nitric oxide.

The addition of excess L-arginine stimulated NO production, whereas the addition of other cationic or neutral amino acids reduced NO in cultured endothelial cells.[28] Anionic amino acids did not affect NO in these cells and the addition of excess L-arginine reversed the effects of the neutral and cationic amino acids. Further experiments with short interference (si) RNA inhibition of the y^+ transporter (CAT1) or the y^+L transporter (CD98/4F2hc) produced similar results.[28] These data are consistent with the hypothesis that the modulation of cellular L-arginine transport, either by competitive inhibition of uptake or stimulation of efflux (trans-stimulation) can alter L-arginine availability and thus NO production in endothelial cells.

Experiments in the isolated perfused kidney were recently performed to translate the observations made in cultured endothelial cells to the intact kidney.[28] The addition of competing cationic and neutral amino acids to the renal arterial perfusate decreased NO release and increased vascular resistance in the isolated perfused kidney, whereas anionic amino acids had no effect on this relationship.[28] Further experiments then demonstrated that these responses are endothelium dependent, NOS dependent and reversed with excess L-arginine.[28] These data obtained from the renal vasculature are consistent with the findings reported above in cultured endothelial cells demonstrating that L-arginine uptake and efflux by y^+ and y^+L transporters can be modulated by cationic and/or neutral amino acids and alter NO bioavailability and NOS-dependent function.

Based on the studies outlined above, we propose the model in Fig. 2 to explain the effects of modulation of L-arginine transport on NO production in endothelial cells. L-Arginine enters cells by either a y^+ (CAT1) or y^+L (CD98/4F2hc) transport mechanism. L-Arginine transport can be modulated by other cationic amino acids through the y^+ or y^+L mechanism or by neutral amino acids through the y^+L transport mechanism. Uptake can be altered by competitive inhibition and efflux altered by trans-stimulation. Together, effects on L-arginine uptake and efflux alter the availability of L-arginine to serve as a substrate for NOS in the production of NO. As such, we propose that modulation of these transporters can have a profound effect on NO-dependent function.

FUNCTIONAL SIGNIFICANCE OF L-ARGININE UPTAKE MECHANISMS

Functional studies were then performed to evaluate the importance of CAT1 in the production of NO and in the regulation of NO-dependent function in the renal medulla of anaesthetized and conscious rats.[4,5] Acute infusion of L-arginine into the renal medullary interstitial space of anaesthetized rats increased renal medullary interstitial NO concentration. In contrast, infusion of cationic amino acids that compete with L-arginine for cellular uptake (L-lysine or L-ornithine) or administration of the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) decreased NO concentration in the medulla.[4] Furthermore, the effects of L-lysine or L-ornithine were reversed by excess L-arginine. Subsequent studies demonstrated that blood flow in the renal medulla was decreased during medullary interstitial infusion of L-lysine or L-ornithine, increased during infusion of L-arginine and decreased by L-NAME.[4] Studies were then performed to evaluate the long-term effect of inhibition of CAT1 in the renal medulla. The chronic infusion of L-lysine or L-ornithine directly into the renal medullary interstitial space of conscious rats for several days led to a sustained reduction in NO in the renal medulla and an increase in arterial blood pressure; both effects were blunted by coadministration of L-arginine.[5] To document these effects with a mechanistically different inhibitor of L-arginine uptake, we demonstrated that antisense inhibition of CAT1 in the renal medulla led to a sustained reduction in CAT1 immunoreactive protein, a significant decrease in NO in the medullary interstitial space and the development of systemic hypertension.[5] The hypertension in this model is proposed to be mediated by a decrease in medullary blood flow and/or alterations in sodium transport in epithelial cells (i.e. IMCD). Although the precise mechanisms of hypertension in this model remain to be fully elucidated, the data demonstrate that the *in vitro* observations described above have functional *in vivo* correlates.

ROLE OF L-ARGININE UPTAKE MECHANISMS IN THE REGULATION OF FLUID AND ELECTROLYTE HOMEOSTASIS AND ARTERIAL BLOOD PRESSURE

Based on our *in vitro* and *in vivo* studies of renal L-arginine uptake mechanisms, NO production and NO-dependent function, we propose the following mechanisms to explain the effects of increased L-arginine uptake on renal vascular and tubular function in the cortex and medulla (Fig. 3). Increased extracellular L-arginine leads to increased NO production in both endothelial and IMCD cells. As demonstrated in thick ascending limb cells,[34] the increase in NO in epithelial cells, including the IMCD, results in decreased sodium reabsorption, which leads to an increase in sodium (and water) excretion, a reduction in extracellular and plasma volume and a reduction in mean arterial blood pressure. Similarly, an elevation in L-arginine uptake in endothelial cells can lead to a reduction in arterial blood pressure by directly altering vascular resistance or by increasing sodium and water excretion. A fall in renal vascular resistance will be associated with increased sodium and water excretion due to an elevation in the filtered load or indirect effects on sodium transport due to changes in the renal medullary osmotic gradient (medullary washout) or increased medullary interstitial fluid pressure secondary to changes in blood flow of the vasa recta capillaries in the renal medulla.[39] The integrated effect of an elevation

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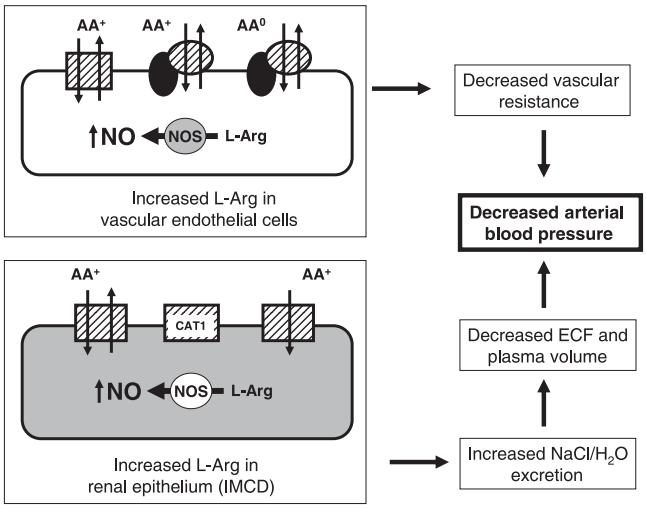

Fig. 3 Effects of increased cellular L-arginine (L-Arg) uptake in endothelial and epithelial cells on vascular resistance, renal excretory function, extracellular fluid volume and the long-term level of blood pressure. Increased nitric oxide (NO) in endothelial cells leads to decreased vascular resistance, whereas increased NO in epithelial cells reduces tubular sodium reabsorption. The integrated effect of these actions is a reduction in arterial pressure. ECF, extracellular fluid; NOS, nitric oxide synthase; AA^+, cationic amino acids; AA^0, neutral amino acids; CAT1, cationic amino acid transporter 1; IMCD, inner medullary collecting duct.

in cellular L-arginine uptake is to lower arterial blood pressure. Therefore, the use of L-arginine in the treatment of hypertension warrants investigation.

HYPERTENSION

In industrialized countries, the risk of developing hypertension during one's lifetime exceeds 90%.[40] The number of hypertensive patients is expected to increase in the future because of the progressive ageing of the population.[41] Hypertension is a major risk factor for stroke, myocardial infarction, heart failure, peripheral vascular disease and kidney failure[42] and mortality resulting from these diseases decreases with effective antihypertensive therapy.[41] Despite the availability of a wide range of antihypertensive drugs, most hypertensive patients still do not achieve optimal blood pressure regulation.[41] Thus, it is imperative to improve current treatment regimens for hypertension.

Role of the kidney in hypertension

The complex role of the kidney in long-term arterial pressure regulation is beyond the scope of the present review, but has been covered extensively elsewhere.[43–45] Alterations in the relationship between renal perfusion pressure and renal fluid and sodium excretion, the pressure diuresis/natriuresis mechanism, are suggested to play an important role in the development and maintenance of hypertension.[44,46] Renal endothelial dysfunction and reduced NO bioavailability are key contributors to the altered pressure–natriuresis relationship in the development of hypertension.[46,47] Reactive oxygen species have been shown to be major culprits in scavenging NO,[8] thereby reducing its bioavailability under conditions of hypertension. As outlined in the Introduction, there is only limited information on the factors that regulate NO formation in hypertension. L-Arginine has been shown to increase the bioavailability of NO by both increasing its formation[4,28] and reducing its inactivation by superoxide.[48,49] Thus, L-arginine supplementation has been suggested as a potential therapeutic approach in hypertension.

L-Arginine and NO bioavailability in hypertension

There is evidence that, in addition to lowering arterial pressure, antihypertensive treatments that increase NO bioavailability provide additional beneficial effects in ameliorating target organ damage in hypertension.[7] Because exogenous L-arginine can increase NO bioavailability by multiple mechanisms, L-arginine should ameliorate hypertension and particularly the related target organ damage.

Exogenous L-arginine has been shown to improve NO-dependent vasorelaxation or other indices of endothelial function in the vasculature of animals and humans with salt-sensitive hypertension. For example, chronic oral or intravenous administration of L-arginine increased urinary cGMP and nitrate excretion[50] and prevented sodium-dependent hypertension in Dahl salt-sensitive (Dahl S) rats.[50–52] These findings support the notion that L-arginine ameliorates sodium-sensitive forms of hypertension, most likely by increasing NO production. Renal medullary interstitial infusion of L-arginine into Dahl S rats abrogated the reductions in medullary blood flow and the development of hypertension induced by a high-salt diet.[53] This indicates that the antihypertensive effects of L-arginine are mediated by the kidney and that L-arginine in the renal medulla is an important determinant in the long-term regulation of arterial pressure.

Acute L-arginine infusion blunted angiotensin II-induced cortical vasoconstriction and prevented angiotensin II-induced medullary vasoconstriction in Dahl S rats placed on a 4% NaCl diet.[54] This indicates that when Dahl S rats are challenged with a high-salt diet, L-arginine can protect the renal medullary circulation from the ischaemic effects of angiotensin II.

Exclusive of its effects to increase NO through NOS enzymatic action, evidence indicates that L-arginine can blunt increases in urinary H_2O_2, 8-isoprostane and thromboxane B_2 excretion and decreases in plasma nitrate and nitrite levels in Dahl S rats placed on a high-salt diet.[55] Furthermore, L-arginine can blunt the upregulation of $gp91^{phox}$ and $p47^{phox}$ subunits of NADPH oxidase in the renal cortex of these rats.[55] L-Arginine can act as a scavenger of reactive oxygen species and blunt the release of oxygen free radicals from endothelial cells.[48] Most importantly, L-arginine can produce NO via a non-enzymatic pathway by reacting with hydrogen peroxide.[56] Thus, under conditions of hypertension, where there is high bioavailability of superoxide, L-arginine may increase NO formation and reduce superoxide levels by producing NO via this non-enzymatic pathway. Nitric oxide synthase is uncoupled in hypertension and, once uncoupled, NOS produces more superoxide rather than NO; this produces a vicious cycle that greatly reduces NO. Under these conditions, NO formation from L-arginine by this non-enzymatic pathway could play a central role in increasing NO formation. L-Arginine may also 'recouple' the electron transport in uncoupled NOS.[57] This would interrupt the vicious cycle that reduces NO bioavailability in hypertension. Taken together, these data indicate that L-arginine can increase NO formation in hypertension not only by the conventional pathway, but also by a number of other potential mechanisms (Fig. 4).

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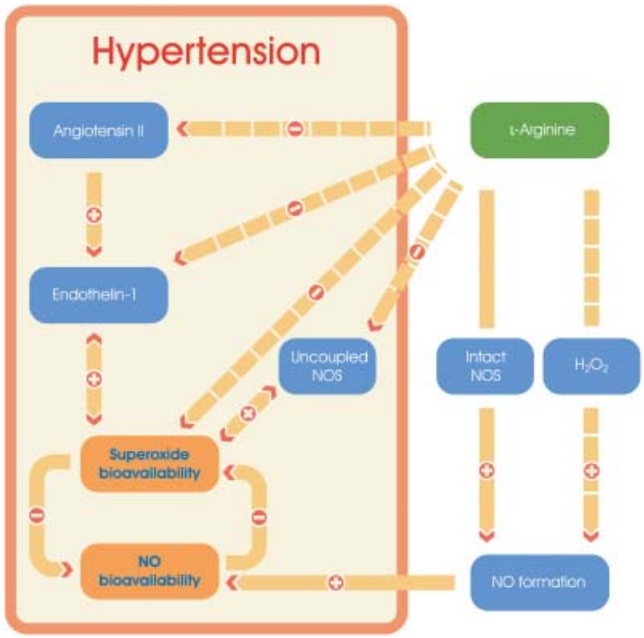

Fig. 4 Global hypothesis indicating the proposed mechanisms by which L-arginine interrupts the vicious cycle that reduces nitric oxide (NO) bioavailability in hypertension. L-Arginine reduces angiotensin II, endothelin-1 and superoxide levels and recouples 'uncoupled' nitric oxide synthase (NOS) enzyme. All these actions of L-arginine lead to an increase in NO bioavailability in hypertension. An increase in NO bioavailability, in turn, leads to a reduction in superoxide bioavailability. +, enhances; –, reduces; broken lines indicate that these pathways remain to be established.

L-Arginine and angiotensin II-induced hypertension

We recently demonstrated that L-arginine can significantly increase NO content, blunt angiotensin II-induced hypertension (by approximately 45%)[58] and prevent angiotensin II-induced renal damage in Sprague-Dawley rats.[58] This latter observation is most exciting because it provides strong support for the concept that, in addition to lowering arterial pressure, antihypertensive treatments that increase NO bioavailability can prevent target organ damage in hypertension.

Angiotensin II increases L-arginine and L-lysine transport and CAT1 mRNA in rat aortic smooth muscle cells.[59] Thus, in angiotensin II-induced hypertension, the ability of extracellular L-arginine to increase endogenous NO production should be augmented. We propose that CAT1 is upregulated in angiotensin II-induced hypertension, which, in turn, facilitates the antihypertensive effects of L-arginine.

Previous studies have shown that the administration of L-arginine reduces endothelin-1 levels in humans.[22,60,61] The precise mechanisms by which L-arginine reduces endothelin-1 levels remain to be determined. However, this action of L-arginine is likely to be dependent, at least in part, on its ability to increase NO formation. Nitric oxide has been demonstrated to directly decrease endothelin-1 secretion and endothelin-1 gene expression.[62] Because endothelin-1 stimulates superoxide production during chronic angiotensin II infusion,[63] a reduction in endothelin-1 levels would reduce superoxide bioavailability and increase NO levels. We propose that one of the mechanisms by which L-arginine prevents renal damage and ameliorates hypertension during chronic angiotensin II infusion is via inhibition of endothelin-1 production (Fig. 4).

L-Arginine has been shown to act as an angiotensin-converting enzyme (ACE) inhibitor in humans.[64] For example, acute administration of L-arginine to healthy male subjects reduced serum ACE activity and plasma angiotensin II levels.[64] The precise mechanisms by which L-arginine inhibits ACE remain to be determined.

L-Arginine transport in hypertension

Plasma L-arginine concentration has been shown to be increased,[65] whereas L-arginine transport is decreased, in hypertension.[65–67] However, this reduction in L-arginine transport is only linked to the y^+L (and not system y^+) mechanism.[65] It is likely that L-arginine uptake by CAT1, which resembles system y^+ transport, remains intact under conditions of hypertension. Thus, CAT1 may be responsible for the beneficial effects of L-arginine supplementation observed in hypertension.

L-Arginine in clinical trials

To date, there is only scant information available on the effects of L-arginine in human essential hypertension and the results of clinical studies have been ambiguous.[41] Most clinical studies have used only a small number of patients (n = 10–20) and treatment was administered either acutely or only for several weeks. It has been shown that L-arginine can reduce arterial pressure and improve endothelial function in patients with mild to moderate hypertension.[68,69] Another study indicated that oral L-arginine acutely improved endothelium-dependent brachial artery flow-mediated dilatation in patients with essential hypertension.[18] In contrast, exogenous L-arginine augmented acetylcholine-induced forearm perfusion in normotensive subjects, but had no effect on hypertensive patients.[70] This latter study suggests that reduced bioavailability of NO observed in human hypertension is not due to reduced availability of L-arginine.[70] Overall, the findings of these studies suggest that L-arginine has the potential to improve endothelial dysfunction in patients with mild to moderate, but not severe, hypertension. Large randomized controlled trials are required to determine the effects of long-term L-arginine therapy in hypertensive patients.

CONCLUSIONS

Recent data indicate that exogenous L-arginine can increase NO in endothelial and epithelial cells. L-Arginine is also an important determinant of renal NO production, NO-dependent function and long-term arterial pressure regulation. Low bioavailability of NO is a key factor contributing to the pathogenesis of hypertension, particularly to the related target organ damage. Therefore, antihypertensive treatments that restore NO are suggested to be more beneficial than those that merely ameliorate hypertension in treating hypertensive patients. L-Arginine increases NO production, in addition to reducing its quenching by superoxide. Therefore, L-arginine has the potential to reduce arterial pressure and related kidney damage and improve NO bioavailability in hypertension. However, more studies are needed to determine the biological mechanisms underlying the antihypertensive effects of L-arginine. Large randomized controlled clinical trials are also required to assess the beneficial effects of L-arginine in human hypertension. This information should aid in the evaluation of L-arginine as an antihypertensive therapy.

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ACKNOWLEDGEMENTS

NWR is a recipient of a National Health and Medical Research Council of Australia CJ Martin fellowship (ID 384299). The authors' work reported herein was supported, in part, by National Institutes of Health (US) grants HL-29587 and DK-62803 to DLM.

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