Metabolism of methylarginines by human vasculature; implications for the regulation of nitric oxide synthesis

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1 The metabolism of methylarginines by human cultured endothelial cells and human saphenous vein was studied *in vitro*. The human endothelial cell line (SGHEC-7), primary cultures of human umbilical vein endothelial cells (HUVEC) and human saphenous vein were incubated with [¹⁴C]-monomethyl-L-arginine ([¹⁴C]-L-NMMA) and the cytosolic extract analysed by high performance liquid chromatography (h.p.l.c.) with on-line radioisotope detection.

2 SGHEC-7, HUVEC and human saphenous vein metabolized [¹⁴C]-L-NMMA to a compound which co-eluted with [¹⁴C]-citrulline. A second metabolite which co-eluted with [¹⁴C]-arginine was evident on the radiochromatograms of HUVEC cytosol and saphenous vein extracts.

3 The intracellular levels of [¹⁴C]-L-NMMA and [¹⁴C]-citrulline in SGHEC-7 cells incubated with [¹⁴C]-L-NMMA (0.5 µCi ml⁻¹; 8.9 µM) for 1 h were 113 ± 22 and 67.6 ± 6.2 pmol mg⁻¹ cell protein respectively (*n* = 7). Co-incubation with N^GN^G-dimethyl-L-arginine (ADMA; 100 µM) but not N^GN^G-dimethyl-L-arginine (SDMA; 100 µM) reduced the intracellular level of [¹⁴C]-citrulline to 26.3 ± 3.7 pmol mg⁻¹ cell protein (*P* < 0.01; *n* = 3) without reducing the intracellular level of [¹⁴C]-L-NMMA.

4 The intracellular levels of [¹⁴C]-citrulline in SGHEC-7 cells incubated with [¹⁴C]-L-NMMA for 1 h were reduced following co-incubation with N^G-nitro-L-arginine methylester (L-NAME; 1 mM), N^G-nitro-L-arginine (L-NOARG; 1 mM) and L-canavanine (1 mM) to 47.1 ± 6.2, 24.7 ± 3.6 and 12.5 ± 2.8% of control levels (*P* < 0.001; *n* = 9). ADMA (1 mM; *n* = 3) reduced intracellular [¹⁴C]-citrulline levels to 4 ± 4% of control (*P* < 0.01) but SDMA (1 mM; *n* = 3) had no effect.

5 The accumulation of endogenously synthesized ADMA in the culture supernatant of SGHEC-7 cells was increased by co-incubation with L-NMMA (1 mM) from 1.98 ± 0.08 to 2.74 ± 0.36 nmol mg⁻¹ cell protein, an increase of 40%.

6 These results demonstrate that human vasculature possesses an enzyme which has similar properties to dimethylarginase; human endothelial cells and human saphenous vein metabolize L-NMMA to citrulline via a process inhibited by ADMA but not SDMA. The increase in endothelium-derived ADMA following co-incubation with L-NMMA is consistent with competition between ADMA and L-NMMA for dimethylarginase. Inhibition of this enzyme might increase the intracellular concentration of ADMA, an endogenously produced compound that inhibits nitric oxide synthesis.

Keywords: Nitric oxide; endothelial cells; methylarginines; dimethylarginase; human vasculature

Introduction

Nitric oxide (NO) is a ubiquitous intra- and intercellular messenger, synthesized from L-arginine by a family of NO synthase isoenzymes. It is involved in the control of vascular tone (Vallance *et al.*, 1989) and blood pressure (Rees *et al.*, 1989), platelet function (Radomski *et al.*, 1987), neurotransmission (Garthwaite *et al.*, 1988) and host defence (Moncada *et al.*, 1991). Guanidino-substituted arginine derivatives including N^G-monomethyl-L-arginine (L-NMMA) (Rees *et al.*, 1990) inhibit the L-arginine:NO pathway and are useful tools with which to probe the role of NO *in vitro* and *in vivo* (Moncada *et al.*, 1991). L-NMMA causes arteriolar vasoconstriction in animals (Gardiner *et al.*, 1990) and man (Vallance *et al.*, 1989), inhibits non-adrenergic non-cholinergic neurotransmission (Gillespie *et al.*, 1989; Kim *et al.*, 1991) and diminishes the host response to leishmania (Liew *et al.*, 1990).

Certain nitric oxide synthase inhibitors occur endogenously (Vallance *et al.*, 1992); L-NMMA and N^GN^G-dimethyl-L-arginine (asymmetric dimethylarginine; ADMA) are found as components of polypeptides (Nakajima *et al.*, 1971; Kaki-moto *et al.*, 1975), occur as free amino acids in brain and

other tissues (Ueno *et al.*, 1992), circulate in plasma (Park *et al.*, 1988; Vallance *et al.*, 1992) and are excreted in the urine (Vallance *et al.*, 1992). Recently it has been demonstrated that human endothelial cells synthesize methylarginines and the predominant form is ADMA, which is produced in quantities that may affect NO synthesis (Fickling *et al.*, 1993). However, the mechanisms determining the intracellular levels of ADMA are not known.

ADMA appears to be synthesized by methylation of arginine residues in proteins and subsequent hydrolysis of the proteins (Cantoni, 1975). Thus the amounts of ADMA produced by endothelial cells may reflect rates of protein methylation and degradation. The removal of ADMA from the cell might occur by metabolism or extrusion. Rat kidney contains dimethylarginase (previously known as dimethylarginine dimethylaminohydrolase) (Ogawa *et al.*, 1989), which metabolizes ADMA and L-NMMA to citrulline, and indirect evidence suggests that bovine endothelial cells may contain a similar enzyme (Hecker *et al.*, 1990a). If this enzyme is present in cells which make ADMA, its activity might determine the intracellular levels of this NO synthase inhibitor. In the present study we have examined the metabolism of methylarginines in human cultured endothelial cells and human saphenous vein and have studied the effects of known inhibitors of NO synthase on this process.

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Methods

Endothelial cell culture

SV40-transfected human umbilical vein endothelial cells (SGHEC-7) were cultured as previously described (Fickling *et al.*, 1992) in medium M199:RPMI 1640 in a ratio of 1:1 supplemented with $2.5 \mu\text{g ml}^{-1}$ endothelial cell growth supplement, 0.09 mg ml^{-1} heparin, 2.5% (v/v) foetal calf serum and 2.5% (v/v) newborn calf serum. Cells were grown as monolayers in 9 cm petri dishes and used between passage 16 and 22. Primary cultures of human umbilical vein endothelial cells (HUVEC) were isolated with collagenase (0.5 mg ml^{-1}) as previously described (Jaffe *et al.*, 1973) and grown on gelatin-coated 9 cm petri dishes in SGHEC-7 culture medium (as above) modified to include 10% (v/v) foetal calf serum, 10% (v/v) newborn calf serum and $20 \mu\text{g ml}^{-1}$ endothelial cell growth supplement. In initial studies, HUVEC and SGHEC-7 cells were grown to confluence and then depleted of arginine by incubation in arginine- and serum-free medium for 24 h before use since this manoeuvre has been reported to increase the metabolism of L-NMMA in bovine endothelial cells (Hecker *et al.*, 1990a). In subsequent experiments SGHEC-7 cells were seeded in 6 well plates for 24 h before use in standard SGHEC-7 medium since in these studies incubation in arginine-free medium did not alter the metabolism of [^{14}C]-L-NMMA by these cells (unpublished observations).

Metabolism of [^{14}C]-L-NMMA and [^{14}C]-citrulline by HUVEC and SGHEC-7 cells

Confluent 9 cm dishes of HUVEC and SGHEC-7 were washed twice with 10 ml of warm (37°C) Krebs solution of the following composition (mM): NaCl 131, KCl 5.5, CaCl_2 2.5, MgCl_2 1, NaHCO_3 25, NaH_2PO_4 1, glucose 5.5, HEPES 20. Cells were then incubated at 37°C or kept on ice for 1 h in 2 ml of Krebs solution containing either [^{14}C]-L-NMMA ($0.5 \mu\text{Ci ml}^{-1}$) or [^{14}C]-citrulline ($0.25 \mu\text{Ci ml}^{-1}$). After 1 h the cells were washed twice with 10 ml of cold (4°C) Krebs solution; washing with Krebs containing 10 mM L-NMMA confirmed that non-specific binding was negligible (unpublished observations). Cells were scraped from the dish in 1.5 ml of methanol/water (90/10; v/v), freeze-thawed 3 times and centrifuged for 10 min at 10,000 g in a bench top micro-centrifuge. The precipitate was kept for protein estimation by the Bio-Rad protein assay with bovine serum albumin as a standard. The supernatant was evaporated at 140°C under nitrogen, following which the residue was resuspended in distilled water for analysis using high performance liquid chromatography (h.p.l.c.). To determine substrate specificity incubations with [^{14}C]-L-NMMA were carried out in the presence of ADMA ($100 \mu\text{M}$) or N^GN^G -dimethyl-L-arginine (symmetric dimethylarginine; SDMA; $100 \mu\text{M}$).

Effect of NO synthase inhibitors on the metabolism of [^{14}C]-L-NMMA by SGHEC-7 cells

SGHEC-7 cells seeded in 6 well plates (5×10^5 cells per well) were washed with Krebs (37°C) and incubated in $300 \mu\text{l}$ of Krebs solution containing [^{14}C]-L-NMMA ($0.4 \mu\text{Ci ml}^{-1}$) alone or [^{14}C]-L-NMMA ($0.4 \mu\text{Ci ml}^{-1}$) and N^G -nitro-L-arginine (L-NOARG; 1 mM), N^G -nitro-L-arginine methylester (L-NAME; $1 \mu\text{M}$ –1 mM), L-canavanine (1 mM), ADMA (1 mM) or SDMA (1 mM). After 1 h the cells were washed with 2 ml cold Krebs and extracted as above.

Incubation of human saphenous vein with [^{14}C]-L-NMMA

Saphenous vein was obtained from patients undergoing cardiac surgery and used immediately. Each segment was weighed and incubated for 2 h at 37°C in 1 ml of Krebs

containing $0.1 \mu\text{Ci}$ of [^{14}C]-L-NMMA. After 2 h the supernatant was centrifuged at 10,000 g for 10 min and analysed by h.p.l.c. Vein segments were mechanically homogenized in 4 ml of methanol/water (Ultra Turrax, Janke and Kunkel, Staufen, Germany) and centrifuged at 10,000 g as above. The supernatant was aspirated and evaporated to dryness under nitrogen. The residue was dissolved in distilled water for analysis by h.p.l.c.

Effect of L-NMMA on ADMA production by endothelial cells

SGHEC-7 cells were grown to confluence in 9 cm dishes and incubated for 72 h in culture medium containing 1 mM L-NMMA. The medium was aspirated, centrifuged at 10,000 g for 10 min and partial purification of dimethylarginines achieved by passage through a Bond Elut SCX column and elution with ammonia/methanol as described previously (Valance *et al.*, 1992). ADMA levels were measured by h.p.l.c. and cell protein was measured as above.

H.p.l.c. analysis

Separation of amino acids was achieved on an ODS C_{18} h.p.l.c. column using an ion pair based mobile phase containing 0.025 M phosphoric acid buffer (pH 5.0), 0.01 M hexane sulphonic acid and 1% (v/v) acetonitrile. Flow was maintained at 1 ml min^{-1} and amino acids detected by u.v. absorbance at 200 nm and/or ^{14}C scintillation counting using an on-line solid scintillator (Beckman System Gold, Beckman Instruments (UK) Ltd, High Wycombe, Bucks).

Materials

The following reagents were used: medium M199 and RPMI 1640 (Gibco Ltd, Paisley, Scotland), foetal calf serum (Advanced Protein Products, UK), new born calf serum (Imperial Laboratories, Salisbury, Wilts), endothelial cell growth supplement and heparin (Sigma Chemical Co, Poole, Dorset), arginine-free media (Integra Biosciences, Northumberland), bovine serum albumin, L-canavanine, L-NAME and L-NOARG (Sigma), ADMA, SDMA, L-NMMA and [^{14}C]-L-NMMA ($56 \mu\text{Ci } \mu\text{mol}^{-1}$; a kind gift from Drs Hodson and Moncada, Wellcome Research Laboratories, Beckenham, Kent), [^{14}C]-citrulline ($55.6 \mu\text{Ci } \mu\text{mol}^{-1}$; NEN Research Products, Stevenage, Herts), acetonitrile and hexane sulphonic acid (Romil, Loughborough, Leicestershire). Bio-Rad protein assay was obtained from Bio-Rad Laboratories Inc, Hemel Hempstead, Herts. Bond Elut columns were obtained from Jones Chromatography Ltd, Hengoed, Mid Glamorgan. H.p.l.c. columns were obtained from Phase Separation, Queensferry, Wales.

Calculations and statistics

The area under each radiochromatogram peak was calculated by computerized integration following calibration with synthetic standards. Results are expressed as mol mg^{-1} cell protein (cultured cell studies) or mol g^{-1} wet tissue (saphenous vein studies) except for those undertaken in 6-well plates which are expressed as $\text{mol per million cells}$. All data are expressed as the mean $\pm$ s.e.mean and compared by Student's *t* test for paired or unpaired observations as appropriate, where $P < 0.05$ is considered significant.

Results

The radiochromatogram of [^{14}C]-L-NMMA consisted of a single peak (Figure 1) with no evidence of impurities either on the ^{14}C or u.v. chromatograms.

Metabolism of [^{14}C]-L-NMMA by endothelial cells

The radiochromatograms of the cytosolic extract of SGHEC-7 cells contained 2 peaks, one that co-eluted with [^{14}C]-L-NMMA and the other that co-eluted with [^{14}C]-citrulline (Figure 1). Analysis of the Krebs incubate confirmed a similar pattern (data not shown). The intracellular level of [^{14}C]-L-NMMA was 113 ± 22 pmol mg^{-1} protein and of [^{14}C]-citrulline was 67.6 ± 6.2 pmol mg^{-1} protein ($n = 7$; Figure 2). Incubation of SGHEC-7 cells on ice reduced the amount of [^{14}C]-citrulline to 2.5 ± 0.3 pmol mg^{-1} protein ($>95\%$ inhibition; $P < 0.001$; $n = 4$; Figure 1) but did not alter the amount of [^{14}C]-L-NMMA (87.8 ± 9.4 pmol mg^{-1} protein; $P > 0.3$; $n = 4$). Co-incubation with ADMA ($100 \mu\text{M}$) reduced [^{14}C]-

Figure 1 Representative radiochromatograms of: (a) [^{14}C]-L-NMMA (LN) standard ($0.01 \mu\text{Ci}$); (b) SGHEC-7 cytosolic extract incubated for 1 h in [^{14}C]-L-NMMA ($0.5 \mu\text{Ci ml}^{-1}$) at 37°C ; L-NMMA (LN), citrulline (Cit). (c) SGHEC-7 cytosolic extract incubated for 1 h in [^{14}C]-L-NMMA ($0.5 \mu\text{Ci ml}^{-1}$) at 4°C ; L-NMMA (LN), citrulline (Cit). For other abbreviations, see text.

Figure 2 Intracellular levels of [^{14}C]-citrulline (a) and [^{14}C]-L-NMMA (b) following incubation of SGHEC-7 cells with [^{14}C]-L-NMMA ($0.5 \mu\text{Ci ml}^{-1}$) alone (open columns; $n = 7$), or [^{14}C]-L-NMMA with ADMA ($100 \mu\text{M}$; solid columns; $n = 3$) or SDMA ($100 \mu\text{M}$; cross hatched columns; $n = 3$). Error bars show s.e.mean and data were compared by Student's t test ($*P < 0.01$ compared with control). For abbreviations, see text.

citrulline to 26.3 ± 3.7 pmol mg^{-1} protein (60% inhibition; $P < 0.01$; $n = 3$); co-incubation with SDMA ($100 \mu\text{M}$) had no effect ($n = 3$; $P > 0.3$; Figure 2). Neither ADMA nor SDMA had any significant effect on [^{14}C]-L-NMMA levels (Figure 2).

The radiochromatograms of the cytosolic extract of HUVEC were similar to that of SGHEC-7 (Figure 3) although intracellular levels of [^{14}C]-L-NMMA were higher (361.5 ± 60.6 pmol mg^{-1} protein; $n = 4$; $P < 0.002$) and [^{14}C]-citrulline lower (23.9 ± 5.1 pmol mg^{-1} protein; $n = 4$; $P < 0.002$). In addition there was a third peak which co-eluted with [^{14}C]-arginine (14.6 ± 5.2 pmol mg^{-1} protein; $n = 4$). Incubation with [^{14}C]-citrulline confirmed that HUVEC ($n = 3$) but not SGHEC-7 ($n = 4$) metabolized [^{14}C]-citrulline to [^{14}C]-arginine (Figure 3).

Concentration of [^{14}C]-L-NMMA in endothelial cells

Assuming an endothelial cell volume of 1 pl (Baydoun *et al.*, 1990), the mean intracellular concentration of [^{14}C]-L-NMMA in SGHEC-7 cells was approximately $40 \mu\text{M}$ when the concentration of [^{14}C]-L-NMMA in the Krebs incubate was $8.9 \mu\text{M}$. The extraction efficiency for intracellular amino acids was of the order of 90% (unpublished observations), indicating that these cells concentrate [^{14}C]-L-NMMA approximately 5 fold.

Metabolism of [^{14}C]-L-NMMA by human saphenous vein

The radiochromatograms of human saphenous vein incubated with [^{14}C]-L-NMMA were composed of 3 peaks co-eluting with [^{14}C]-citrulline, [^{14}C]-arginine and [^{14}C]-L-NMMA (Figure 3; $n = 3$). The quantity of [^{14}C]-L-NMMA, [^{14}C]-citrulline and [^{14}C]-arginine was 2.2 ± 0.3 , 0.16 ± 0.05 and 0.25 ± 0.07 pmol g^{-1} tissue respectively. Analysis of the Krebs incubate showed a similar radiochromatogram pattern (data not shown).

Effect of NO synthase inhibitors on the metabolism of [^{14}C]-L-NMMA

The intracellular levels of [^{14}C]-L-NMMA and [^{14}C]-citrulline in SGHEC-7 cells grown in 6 well plates were 41.9 ± 6.3 and 42.5 ± 5.4 pmol per 10^6 cells respectively ($n = 16$). The NO synthase inhibitors, L-canavanine (1 mM), L-NAME (1 mM),

Figure 3 Representative radiochromatograms of: (a) HUVEC cytosolic extract incubated for 1 h with [^{14}C]-L-NMMA ($0.5 \mu\text{Ci ml}^{-1}$); L-NMMA (LN), citrulline (Cit), arginine (Arg); (b) HUVEC cytosolic extract incubated for 1 h with [^{14}C]-citrulline ($0.25 \mu\text{Ci ml}^{-1}$); citrulline (Cit), arginine (Arg); (c) Human saphenous vein extract incubated for 2 h in [^{14}C]-L-NMMA ($0.1 \mu\text{Ci ml}^{-1}$); L-NMMA (LN), citrulline (Cit), arginine (Arg). For other abbreviations, see text.

L-NOARG (1 mM; Figure 4) and ADMA (1 mM) reduced the intracellular levels of [14 C]-citrulline to 5.3 ± 1.1 ($P < 0.0001$; $n = 9$), 10.1 ± 1.5 ($P < 0.0002$; $n = 9$), 20 ± 2.6 ($P < 0.01$; $n = 9$) and 1.2 ± 1.2 ($P < 0.05$; $n = 3$) pmol per 10^6 cells respectively. Intracellular levels of [14 C]-L-NMMA were higher following incubation with L-NOARG (72.1 ± 8.8 pmol per 10^6 cells; $P < 0.01$; $n = 9$) but unaffected by L-canavanine, L-NAME (Figure 4) or ADMA. SDMA (1 mM) had no effect on the intracellular levels of either amino acid ($n = 3$; data not shown). L-NAME caused dose-dependent inhibition of [14 C]-citrulline synthesis from [14 C]-L-NMMA (Figure 5): $100 \mu\text{M}$ L-NAME caused approximately 40% inhibition of [14 C]-citrulline synthesis.

Figure 4 Intracellular levels of [14 C]-citrulline (a) and [14 C]-L-NMMA (b) following incubation of SGHEC-7 cells with [14 C]-L-NMMA ($0.4 \mu\text{Ci ml}^{-1}$) alone (open columns; $n = 16$), or [14 C]-L-NMMA with L-NOARG (1 mM; solid columns; $n = 9$), L-NAME (1 mM; cross hatched columns; $n = 9$) or L-canavanine (1 mM; stippled columns; $n = 9$). Error bars show s.e.mean and data were compared by Student's t test (* $P < 0.001$ compared with control). For abbreviations, see text.

Figure 5 The effect of L-NAME on the synthesis of [14 C]-citrulline from [14 C]-L-NMMA by SGHEC-7 cells ($n = 4$). For abbreviations, see text.

Effect of L-NMMA on ADMA production by SGHEC-7 cells

The amount of endogenous ADMA released into the culture medium by SGHEC-7 cells was increased by co-incubation with L-NMMA (1 mM). The amount of ADMA in the culture medium (10 ml) following a 72 h incubation period was $1.98 \pm 0.08 \text{ nmol mg}^{-1}$ cell protein in the absence of L-NMMA and $2.74 \pm 0.36 \text{ nmol mg}^{-1}$ cell protein in the presence of L-NMMA ($P < 0.05$; $n = 4$), an increase of approximately 40%.

Discussion

Methylarginines that inhibit nitric oxide synthesis are synthesized endogenously *in vivo* in animals (Kakimoto & Akazawa, 1970) and man (Vallance *et al.*, 1992) and *in vitro* by human endothelial cells in culture (Fickling *et al.*, 1993). Metabolism of methylarginines has been reported in the rat (Ogawa *et al.*, 1987) and the rabbit (McDermott, 1976) and indirect evidence suggests that bovine endothelial cells metabolize L-NMMA to citrulline (Hecker *et al.*, 1990a). The results of this study demonstrate the presence of an enzyme with the characteristics of dimethylarginase in human tissues. Human cultured endothelial cells and human blood vessels *in vitro* metabolized [14 C]-L-NMMA to [14 C]-citrulline and the activity of this enzyme determined the amount of ADMA released by the cells into the culture medium.

Direct evidence for a pathway for the metabolism of methylarginines is provided by these experiments using [14 C]-L-NMMA. SGHEC-7 cells, primary cultures of HUVEC and isolated human saphenous veins metabolized [14 C]-L-NMMA to [14 C]-citrulline. The metabolism of [14 C]-L-NMMA occurred at 37°C but not at 4°C and was inhibited by ADMA but not SDMA suggesting that this is an enzymatic process and that there is competition between ADMA and L-NMMA for metabolism. Similar results have been obtained with dimethylarginase isolated from rat kidney which metabolizes L-NMMA ($K_m = 0.36 \text{ mM}$) and ADMA ($K_m = 0.18 \text{ mM}$) but not SDMA (Ogawa *et al.*, 1989).

HUVEC and saphenous vein metabolized [14 C]-citrulline to [14 C]-arginine, a property shared by bovine aortic endothelial cells (Hecker *et al.*, 1990b). This pathway is unlikely to involve the urea cycle as [14 C]-citrulline was not metabolized via [14 C]-ornithine to [14 C]-arginine. The enzyme responsible has the characteristics of arginosuccinate synthase (Hecker *et al.*, 1990b) and makes any estimate of the activity of dimethylarginase in these cells unreliable. In contrast, SGHEC-7 cells did not metabolize [14 C]-citrulline to [14 C]-arginine and the rate of production of [14 C]-citrulline from [14 C]-L-NMMA in these cells was of the order of 40 pmol per 10^6 cells h^{-1} , which represents a 50% conversion of the intracellular [14 C]-L-NMMA but only a 1% conversion of the total [14 C]-L-NMMA present. This rate of metabolism is unlikely to deplete the levels of [14 C]-L-NMMA added to cells and tissues for pharmacological studies *in vitro*, but might affect the distribution, tissue levels and profile of activity of this drug administered to experimental animals or man *in vivo* or influence the levels of endogenous methylarginines within the cells. Indeed, when exogenous L-NMMA was added to endothelial cells the amount of ADMA released into the culture medium increased by about 40% (approximately $0.5 \mu\text{M}$ to $0.7 \mu\text{M}$). The precise concentration of ADMA in the cells is not known, but the studies with [14 C]-L-NMMA suggest that methylarginines are concentrated within human endothelial cells indicating that ADMA levels inside the cell may be higher than those we have measured in the culture medium.

NO synthase also metabolizes arginine and L-NMMA to citrulline (Feldman *et al.*, 1993); however, the rate of conversion is 20 times slower for L-NMMA than for arginine (Olken & Marletta, 1993) and unlikely to account for the

activity we have seen in endothelial cells; in SGHEC-7 cells the rate of conversion of [^{14}C]-L-NMMA to [^{14}C]-citrulline was of the same order of magnitude as the rate of conversion of [^{14}C]-arginine to [^{14}C]-citrulline either in whole cells (about 100 pmol per 10^6 cells h^{-1} ; unpublished observations) or in homogenates of SGHEC-7 cells (24 pmol mg^{-1} h^{-1} ; Radomski *et al.*, 1993). Our results are most consistent with metabolism of [^{14}C]-L-NMMA to [^{14}C]-citrulline by dimethylarginase in endothelial cells.

Structural analogues of ADMA and L-NMMA inhibited the conversion of [^{14}C]-L-NMMA to [^{14}C]-citrulline. L-NAME produced a dose-dependent inhibition and at a concentration of 1 mM caused near total abolition of [^{14}C]-citrulline production. L-Canavanine and L-NOARG had similar effects. L-NOARG has previously been reported to inhibit the activity of rat isolated dimethylarginase (Ogawa *et al.*, 1989) and L-canavanine appears to inhibit NO synthase and endothelial dimethylarginase in the same concentration-range. Further studies will be required to determine whether the inhibition of metabolism of endogenous ADMA produced by these compounds contributes to their actions as NO synthase inhibitors. Therefore it would be useful to identify compounds that inhibited dimethylarginase but had no direct effect on NO synthase.

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- ADMA and SDMA are produced by human endothelial cells and ADMA is an inhibitor of NO synthase. Dimethylarginase metabolizes ADMA but not SDMA and the finding that an enzyme similar to or identical with dimethylarginase is present in human endothelial cells provides further evidence for possible physiological or pathophysiological roles for ADMA. Our results demonstrate that alterations in the activity of this enzyme affect the amount of ADMA released by endothelial cells and possibly the intracellular concentrations of this compound. The potential importance of dimethylarginase is supported by the demonstration of dimethylarginase-like activity in human saphenous vein and also the identification of dimethylarginase in the brain, aorta and immune cells of the rat by use of monoclonal antibodies (Kimoto *et al.*, 1993). Manipulation of this enzyme might provide a novel approach to the regulation of nitric oxide synthesis and would help determine the biological significance of endogenous methylated arginines in the regulation of the L-arginine:NO pathway.
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