DETERMINANTS OF CELLULAR L-ARGININE TRANSPORT

Margaretha Johanna Nel

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg 2012.

DECLARATION

I declare that this is my own unaided work. It is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. The work contained in this thesis has not been submitted for any other degree or examination in this University or any other University.

Margaretha Johanna Nel .....

on this 6th day of September 2012.

I certify that the studies contained in this thesis have the approval of the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa. The Ethics number is M120154.

.....

on this sixth day of September 2012.

.....	.....
Geoffrey P Candy (supervisor)	Angela J Woodiwiss (supervisor)
6th SEPTEMBER 2012	6th Sept 2012
Date	Date

ii

In memory of my loving parents

Frederik Petrus Nel

1928-1994

and

Margaretha Johanna Nel (Visser)

1929-1997

to whom I will always be grateful and who are sorely missed.

“Aan my eerbare ouers wie geleef het ten spyte van omstandighede, ten koste van hulself, ter wille van my en my broer wie baie na aan my hart is”

PRESENTATIONS ARISING FROM THIS STUDY

Nel MJ., Woodiwiss AJ., Van Zyl RL., Candy GP. 2006. *In Situ* measurement of nitric oxide (NO) in human endothelial and endothelial like cell lines. The 34th Annual Meeting of the Surgical Research Society of Southern Africa. **(Awarded the Aventis Thrombosis Research Prize for best oral presentation in vascular surgery).**

Nel MJ., Woodiwiss AJ., Van Zyl RL., Candy GP. 2007a. Arginine uptake and nitric oxide production in an endothelial cell line. The 15th Biennial Congress of the Southern African Hypertension Society.

Nel MJ., Woodiwiss AJ., Van Zyl RL., Candy GP. 2007b. Arginine uptake and nitric oxide production in an endothelial cell line. The 35th Annual Meeting of the Surgical Research Society of Southern Africa.

Nel MJ., Woodiwiss AJ., Van Zyl RL., Candy GP. 2008a. Arginine uptake by human endothelial cells. The 36th Annual Meeting of the Surgical Research Society of Southern Africa.

Nel MJ., Woodiwiss AJ., Van Zyl RL., Candy GP. 2008b. Effect of anti-hypertensive drugs on y^+L arginine transport. The 36th Annual Meeting of the Surgical Research Society of Southern Africa.

Nel MJ., Woodiwiss AJ., Van Zyl RL., Candy GP. 2009a. Elucidation of arginine uptake by endothelial cells. The 37th Annual Meeting of the Surgical Research Society of Southern Africa.

Nel MJ., Woodiwiss AJ., Van Zyl RL., Candy GP. 2009b. The effects of arginine and homocyst(e)ine on nitric oxide production in ECV_{304} and HUVEC vascular endothelial cells. The 37th Annual Meeting of the Surgical Research Society of Southern Africa.

Nel MJ., Woodiwiss AJ., Candy GP. 2010a. Effect of homocyst(e)ine on cellular arginine uptake. The 16th Biennial congress of the Southern African Hypertension Society. **(Awarded the Lionel Opie Prize for best pre-clinical oral presentation in hypertension research).**

Nel MJ., Woodiwiss AJ., Candy GP. 2010b. Effect of anti-hypertensive drugs on cellular arginine transport. The 16th Biennial congress of the Southern African Hypertension Society. **(Awarded the Lionel Opie Prize for best pre-clinical oral presentation in hypertension research).**

Nel MJ., Candy GP., Woodiwiss AJ. 2010. General kinetic modelling of amino acid uptake by two or more transporters into cells. Faculty of Health Sciences Research Day and The 38th Annual Meeting of the Surgical Research Society of Southern Africa.

Nel MJ., Woodiwiss AJ., Van Zyl RL., Candy GP. 2010a. Effect of homocystine on cellular arginine uptake. **Selected for an oral presentation** at the 23rd Scientific Meeting of the International Society of Hypertension. Vancouver, Canada. **(Elected as Research Fellow and Member of the ISH with membership number: 15236)**

Nel MJ., Woodiwiss AJ., Van Zyl RL., Candy GP. 2010b. Effect of anti-hypertensive drugs on cellular arginine transport. The 23rd Scientific Meeting of the International Society of Hypertension. Vancouver, Canada. **(Poster and oral presentation). (Awarded the ISH International Forum Poster Prize in Basic Science for the Africa Region).**

Nel MJ., Woodiwiss AJ., Candy GP. 2012a. Modelling of cellular arginine uptake by more than one transporter. The 17th Biennial congress of the Southern African Hypertension Society.

Nel MJ., Woodiwiss AJ., Candy GP. 2012b. Non-linear modeling of cationic amino acid uptake into HUVEC and ECV_{304} cells allows distinction between transporters. The 17th Biennial congress of the Southern African Hypertension Society.

Nel MJ., Woodiwiss AJ., Candy GP. 2012c. Homocystine inhibits y^+L arginine transport in human umbilical vein endothelial cells without affecting y^+ transport. The 17th Biennial congress of the Southern African Hypertension Society.

PUBLICATIONS ARISING FROM THIS STUDY

Full Publications

1) Nel MJ, Woodiwiss AJ, Candy GP. Modeling of cellular arginine uptake by more than one transporter. *J Membr Biol*. 2012; **245:**1-13.
2) Nel MJ, Woodiwiss AJ, Candy GP. Homocystine inhibits y^+L arginine transport in human umbilical vein endothelial cells without affecting y^+ transport. To be submitted to *J Membr Biol*. September/October 2012.

Abstracts published

1) Nel MJ, Woodiwiss AJ, Van Zyl RL, Candy GP. Arginine uptake and nitric oxide production in an endothelial cell line. *Cardiovasc J SA* 2007; 18:23.
2) Nel MJ, Woodiwiss AJ, Candy GP. Effect of homocystine on cellular arginine uptake. *Cardiovasc J SA* 2010; 21:8.
3) Nel MJ, Woodiwiss AJ, Candy GP. Effect of antihypertensive drugs on cellular arginine uptake. *Cardiovasc J SA* 2010; 21:8.

Abstracts accepted for presentation at the SA Hypertension Society meeting from 2-5 March 2012, to be published in 2012

1) Nel MJ., Woodiwiss AJ., Candy GP. 2012a. Modelling of cellular arginine uptake by more than one transporter. *Cardiovasc J SA* 2012.
2) Nel MJ., Woodiwiss AJ., Candy GP. 2012b. Non-linear modeling of cationic amino acid uptake into HUVEC and ECV_{304} cells allows distinction between transporters. *Cardiovasc J SA* 2012.
3) Nel MJ., Woodiwiss AJ., Candy GP. 2012c. Homocystine inhibits y^+L arginine transport in human umbilical vein endothelial cells without affecting y^+ transport. *Cardiovasc J SA* 2012.

NOTE ON REFERENCING IN TEXT

In the text of this thesis, referencing indicates the first author, except in cases of the same first author publishing more than one article in one year with the same co-authors. In such cases, I have included the second and the third author for the purpose of clarity and an ‘a’ or ‘b’ after the year of publication, in the case of referring to the authors: Baylis and White & Christensen.

ABSTRACT

One of the potential causes of hypertension is endothelial dysfunction associated with a decreased production of the vasodilator nitric oxide (NO). Possible factors which may contribute to the reduced NO production include increased reactive oxygen species (eg. superoxides); increased concentrations of homocysteine; or decreased concentrations of L-arginine (cationic amino acids). L-arginine, the precursor of NO, not only increases the bioavailability of NO by increasing its production; but also by reducing the inactivation of NO by superoxides. In patients with hypertension, although fasting plasma L-arginine concentrations are elevated, L-arginine supplementation has been shown to decrease blood pressure. A possible explanation for these data may be that L-arginine uptake into cells is impaired and therefore would not be available for NO production. Indeed, studies have shown that cellular uptake of L-arginine is reduced in lymphocytes from patients with hypertension and individuals genetically predisposed to developing hypertension. However, elucidating the kinetics of L-arginine uptake into endothelial cells is fundamental to determine whether L-arginine uptake is indeed impaired.

Previous studies have shown that the uptake of cationic amino acids into endothelial cells is mediated by the high affinity/low rate y^+L transporter and the low affinity/high rate y^+ transporter. However, data on the kinetics, the relative contribution and physiological importance of the individual transporters in cells expressing more than one transporter, are inconsistent; as most studies determining the uptake of radiolabelled amino acids have assumed Michaelis-Menten kinetics and have calculated constants from Lineweaver-Burk reciprocal plots and Eadie-Hofstee plots. Another approach was therefore required to overcome the limitations and assumptions made in these studies. My first aim was therefore to determine the kinetics of L-arginine uptake into endothelial cells using a general non-linear approach, which allows initial rates of uptake by more than one transporter to be determined and importantly includes the actual concentrations of

both the trace radiolabelled and unlabelled amino acid in the model. Furthermore, using this approach no assumptions are made regarding the type of inhibition and the concentrations of inhibitors (or activators) could be included in the model. As the model was additive, the theoretical contribution of uptake by each transporter could be modelled.

The present study used raw, rather than transformed data, in non-linear regression analysis to characterize the kinetics of L-arginine uptake into cells. I modelled the initial high affinity/low capacity and low affinity/high capacity uptake of trace L-[^{3}H]arginine by two transporters into ECV_{304} and umbilical cord vein endothelial cells in the presence of a range of unlabelled L-arginine and modifiers using GraphPad Prism. The contribution of uptake by individual transporters was modelled and showed that leucine inhibited the individual transporters differently and that the inhibition was not necessarily competitive. *N*-ethylmaleimide inhibited only y^+ transport and 2-amino-bicyclo-[2,2,1]-heptane-2-carboxylic acid may be a potential inhibitor of y^+L transport. Only the absence of sodium reduced L-arginine uptake by y^+L transport and reduced the K_m', whereas reducing sodium decreased L-arginine uptake by y^+ transport without affecting the K_m'. This non-linear modelling approach allows more than one transporter to be modelled, overcomes many of the assumptions made in reported studies and by using raw, rather than transformed data, avoids the errors inherent in methods deriving constants from the linearization of the uptake processes following Michaelian kinetics. The results of this study therefore provide explanations for discrepancies in the literature and suggest that this modelling approach better characterises the kinetics of amino acid uptake into cells.

Having elucidated the kinetics of L-arginine uptake into endothelial cells, I was then equipped to explore possible factors which could impair L-arginine uptake in hypertension. In this regard, although increases in total plasma homocysteine were thought to play a role in hypertension; large prospective clinical trials to reduce total plasma homocysteine by vitamin$B_{6/12}$/folate supplementation, have

failed to show beneficial effects on vascular outcomes. The effects of homocysteine on the vasculature were attributed to the reactive free sulphydryl group; however only a fraction (1.5 – 4%) of total plasma homocysteine is actually present as the free reduced sulphydryl (-SH or thiol) form. In comparison, free oxidized homocysteine, present as the disulphide, homocystine and the mixed disulphide (with cysteine) accounts for 20 – 30% of total plasma homocysteine. In the absence of a clear mechanism by which homocysteine causes vascular disease, one of the other species making up the total homocysteine may be contributing to vascular disease through a different mechanism which may not involve the free sulphydryl group.

Earlier studies demonstrated (in isolated nephrons) that the homocysteine disulphide, homocystine, shared the same membrane transporter as L-arginine (the precursor of NO), and competed for uptake with L-arginine. These studies may suggest that increased homocystine concentrations, by inhibiting L-arginine transport, and hence reducing intracellular L-arginine concentrations, may impact on NO production in other cell types. Therefore, the second aim of my study was to determine the effects of homocystine on cellular L-arginine uptake and hence on NO production.

The uptake of labelled L-[^{3}H]arginine was measured in confluent, L-arginine depleted HUVEC and ECV_{304} cells with unlabelled L-arginine, without or with homocystine and modifiers. The kinetic constants were determined in Graphpad Prism using a described non-linear model of uptake for two transporters acting simultaneously. The NO specific fluorescent DAF-2 dye was used to detect NO production by the cells. Elevated physiological concentrations of 2.5µM homocystine significantly inhibited L-arginine uptake by 90% by $y^{+}L$ transport in both HUVEC ($p<0.0005$) and in ECV_{304} cells ($p<0.05$). Homocystine reduced the K_{ma} of $y^{+}L$ transport in HUVEC (<0.0001) affecting uptake in a competitive-like manner. Pre-incubation of the ECV_{304} cells with L-arginine was able to reverse this inhibition by homocystine. In contrast, homocystine increased uptake by y^{+}

transport in HUVEC ($p<0.01$). Under the experimental conditions used, effects of homocystine on the rate of NO production could not be shown. By demonstrating that homocystine nearly abolishes L-arginine uptake by y^+L transport in both HUVEC and ECV_{304} cells, these data provide a mechanism as to how homocystine may affect L-arginine concentrations. These data would support studies to determine the association between homocystine concentrations and cardiovascular disease.

Lastly, although angiotensin-converting enzyme inhibitors (ACEI's, as well as angiotensin II receptor antagonists) but not other classes of antihypertensive agents, have been shown to decrease oxidative stress and increase NO availability independent of blood pressure lowering effects, the mechanism is not clear. The ability of ACEI's to decrease oxidative stress and enhance NO production has been attributed in part to the sulfhydryl groups present in some, but not all, ACEI's. Hence the mechanisms of the effects of ACEI's on NO production warrant further investigation, as it is possible that L-arginine transporters may play a role by enhancing L-arginine uptake into cells, and thereby increasing NO production.

ACKNOWLEDGEMENTS

The work for this thesis was carried out in the Department of Surgery at the University of the Witwatersrand Medical School. I am grateful to the Percy Fox Foundation; the Iris Hodges Cardiovascular Research Grant; the Faculty of Health Sciences Faculty Research Committee and the National Research Foundation as well as the Department of Surgery, for generous financial assistance.

I am indebted to my supervisors, Prof GP Candy and Prof AJ Woodiwiss for support, patience and encouragement. To Prof Candy, for his thorough understanding of kinetics and the mathematical modelling of data, and especially to Prof AJ Woodiwiss, for her immense enthusiasm, literary wisdom and scientific writer's talent, and who kept me optimistic during difficult times.

Thank you to Prof E Buchmann, his staff and patients at the maternity unit of the Chris Hani Baragwanath Hospital for permission and assistance in collecting umbilical cords for endothelial cell isolation.

I am grateful to the chemical- and metallurgic engineers, Prof's D Glasser and D Hildebrandt for helpful discussions on kinetic modelling, Dr Craig Griffiths for technical advice on kinetic data analysis. A special thank you to Mrs Genade for all her patience, computer art and special skills, which made the illustrations possible.

Thank you to my friends, especially Hennie and Antonio, to Prof Veller and colleagues, for their understanding, encouragement and support. A special heartfelt thank you to my brother Frik and his family, Tannie Sarie and my 'adopted' parents "Pa Hennie en Ma Bubbles" who nursed me back to health with encouraging words, healthy meals, and lots of love when times got tough. To the little people with fur who were a big part of my life, Falkor, Wollie, Gizmo, Candy, and the two who are still with me, Fifi and Tammy, for their devotion, companionship and unconditional love even though 'walkies' often got cancelled. I could not have completed this thesis without your love.

Last but definitely not least, to my Maker for giving me this opportunity, the perseverance, health and strength to achieve what seemed impossible.

CONTENTS **Page**

TITLE PAGE i

DECLARATION ii

DEDICATION iii

PRESENTATIONS ARISING FROM THIS STUDY iv

PUBLICATIONS ARISING FROM THIS STUDY vi

NOTE ON REFERENCING IN TEXT vii

ABSTRACT viii

ACKNOWLEDGEMENTS xii

LIST OF CONTENTS xiii

LIST OF ABBREVIATIONS xx

LIST OF FIGURES xxvi

LIST OF TABLES xxviii

CHAPTER 1 – GENERAL INTRODUCTION 1

1.1 The endothelium. 2

1.1.1 Endothelial function. 3

1.1.1.1 Normal endothelial function. 3

1.1.1.2 Role of endothelial dysfunction in hypertension. 3

1.1.1.3 Mechanisms of endothelial dysfunction. 4

1.2 Role of nitric oxide in hypertension. 7

1.2.1 Characteristics of nitric oxide. 7

1.2.2 Production of nitric oxide. 7

1.2.3 Synthesis of nitric oxide. 8

1.2.4 Metabolism of nitric oxide. 9

1.2.5 Functions of nitric oxide. 10

1.2.5.1 General functions of nitric oxide. 10

1.2.5.2 Functions of nitric oxide in cardiovascular disease and hypertension 11

1.2.6 Deficiencies of nitric oxide and the consequences thereof. 12

1.2.7 Role of arginine supplementation on endothelial function and nitric oxide production. 13

1.3 Role of homocysteine in cardiovascular disease and hypertension. 14

1.3.1 Introduction to homocysteine. 14

1.3.2 Characteristics of homocysteine. 15

1.3.3 Metabolism of homocysteine. 16

1.3.4 Plasma concentrations of homocysteine. 19

1.3.5 Effects of increased plasma homocysteine concentration. 20

1.3.6 Intervention to correct hyper/homocysteinaemia and homocystinuria. 23

1.4 Role of arginine in cardiovascular disease and hypertension. 27

1.4.1 Characteristics of arginine. 27

1.4.2 Metabolism of arginine. 29

1.4.3 Plasma concentrations of arginine. 31

1.4.3.1 Endogenously produced arginine. 32

1.4.4 The arginine paradox. 32

1.4.5 Functions of arginine. 34

1.4.6 Deficiency of arginine. 34

1.4.7 Interventions to increase arginine concentration. 35

1.4.7.1 Infusion of arginine in humans. 36

1.4.7.2 Oral administration of arginine. 37

1.4.7.3 Safety of arginine supplementation in humans. 38

1.4.7.4 Administration of citrulline. 38

1.4.8 Transport of cellular amino acids. 39

1.5 Role of cationic amino acid transporters in hypertension. 41

1.5.1 Introduction. 41

1.5.2 The y^+ and y^+L transporters. 41

1.5.3 Genes coding for y^+ and y^+L transporters. 44

1.5.4 Characterisation of y^+ and y^+L transporters. 44

1.5.4.1 Transporter characterization by sodium dependency. 45

1.5.4.2 Transporter characterization by the y^+ inhibitor *N*-ethylmaleimide. 47

1.5.4.3 Transporter characterization by the y^+L inhibitor 2-amino-2-nor-bornane-carboxylic acid. 50

1.5.4.4 Transporter characterization by the y^+L inhibitor leucine. 50

1.5.5 Brief introduction to arginine transport and the effects thereof. 51

1.5.5.1 Role of high versus low affinity transporter systems in arginine transport. 51

1.5.5.2 Contribution of transporters y^+ and y^+L in cellular arginine uptake. 52

1.5.5.3 Trans-stimulation and efflux of arginine 53

1.5.6 Nitric oxide and nitric oxide synthase regulation by y^+ and y^+L transporters. 56

1.5.6.1 Role of y^+ versus y^+L transport of arginine for cellular nitric oxide production. 56

1.5.6.2 Membrane transporters, arginine pools and nitric oxide production 57

1.5.6.3 Arginine transport in vascular endothelial cells and vascular smooth muscle cells. 58

1.5.6.4 Arginine transport and nitric oxide production. 59

1.5.6.5 Homocysteine transport and effects on arginine transport and hence nitric oxide production. 59

1.5.7 Role of amino acid transporters in cardiovascular diseases. 62

1.5.8 Explanations of discrepant findings. 64

1.6 Role of antihypertensive agents in endothelial dysfunction and in hypertension. 67

1.7 Objectives and aims of my studies. 70

1.7.1 Summary of problem statements 70

CHAPTER 2 – BASIC MATERIALS AND METHODS FOR CELL CULTURE, ARGININE UPTAKE AND NITRIC OXIDE PRODUCTION

2.1 Motivation for choice of endothelial cell type. 71

2.1.1 ECV_{304} cells. 71

2.1.2 EA.hy926 cells. 74

2.1.3 HUVEC. 74

2.2 Cell culture methodology. 75

2.2.1 ECV_{304} culturing. 75

2.2.2 EA.hy926 culturing. 77

2.2.3 HUVEC culturing. 78

2.3 Arginine uptake and nitric oxide determination. 79

2.3.1 Arginine uptake methodology. 84

2.3.2 Nitric oxide determination. 85

2.3.2.1 Nitric oxide standard curve. 86

2.3.2.2 Cellular nitric oxide generation. 86

2.4 Data analysis. 87

CHAPTER 3 – MODELLING OF CELLULAR ARGININE UPTAKE BY MORE THAN ONE TRANSPORTER

3.0 Abstract 88

3.1 Introduction. 88

3.2 Experimental methodology. 91

3.2.1 Determination of uptake kinetic constants. 92

3.2.2 Statistical analysis. 94

3.3 Results. 95

3.3.1 Preliminary results. 95

3.3.2 Arginine uptake by ECV_{304} and HUVEC. 95

3.3.3 Inhibition of arginine uptake by leucine. 104

3.3.4 Effect of NEM on arginine uptake in ECV_{304} cells. 106

3.3.5 Effect of BCH on arginine uptake in ECV_{304} cells. 106

3.3.6 Sodium dependence of L-arginine uptake. 108

3.4 Discussion. 108

3.4.1 Inhibitors and effector molecules. 111

3.4.2 Leucine. 112

3.4.3 NEM and BCH. 113

3.4.4 Sodium dependence of arginine transport 113

3.5 Study limitations. 114

3.6 Conclusions. 115

CHAPTER 4 – THE EFFECT OF HOMOCYSTINE ON ARGININE UPTAKE AND NITRIC OXIDE PRODUCTION

4.0 Abstract 117

4.1 Introduction. 118

4.2 Materials and methods. 121

4.2.1 Cell culture. 121

4.2.2 Arginine uptake methodology. 121

4.2.3 Detection of nitric oxide. 122

4.2.3.1 Nitric oxide detection methodology. 123

4.3 Results. 123

4.3.1 Preliminary studies 123

4.3.2 Modelling arginine uptake 123

4.3.3 Effect of homocystine on arginine uptake 124

4.3.4 Effect of pre-incubation of arginine and homocystine. 127

4.3.5 Effect of *N*-ethylmaleimide on arginine uptake in the presence of homocystine. 131

4.3.6 Effect of homocystine on nitric oxide production. 131

4.4 Discussion. 132

4.4.1. Homocystine concentrations, transport and effects on nitric oxide production. 134

4.5 Discussion of study methodology used. 136

4.6 Study limitations. 137

4.7 Conclusions. 138

CHAPTER 5 – THE EFFECT OF ANTIHYPERTENSIVE DRUGS ON ARGININE UPTAKE

5.0 Abstract. 139

5.1 Introduction. 140

5.2 Materials and methods. 142

5.3 Results. 143

5.3.1 Effects of antihypertensive drugs on initial rates of uptake of arginine. 143

5.3.2 Pre-incubation of ECV_{304} cells with antihypertensive drugs. 143

5.3.3 Effect of captopril and enalapril on nitric oxide production by ECV_{304} cells. 149

5.4 Discussion. 149

5.5 Conclusions. 154

CHAPTER 6

6.1 Summary and conclusions 158

6.2 Recommendations and future research 159

APPENDICES 160

ALPHABETICAL LIST OF REFERENCES 162

LIST OF ABBREVIATIONS

ACE	angiotensin converting enzyme
ACEI	angiotensin converting enzyme inhibitor
ACEI's	angiotensin converting enzyme inhibitors
ADMA	asymmetric dimethyl L-arginine
Ado	adenosine
AdoHcy	adenosylhomocysteine
ADP	adenosine diphosphate
AMT	2-amino-5,6-dihydro-6-methyl-4H-1,3thiazine
ApoA-1	Apolipoprotein A-1
Asp	aspartine
ATCC	American Tissue Cell Collection
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BAEC	bovine aortic endothelial cells
BASMC	bovine aortic smooth muscle cells
BCH	2-amino-2-norbornane-carboxylic acid **or** β-2-aminobicyclo(2,2,1)heptanes-2-carboxylic acid
BH_4	tetrahydrobiopterin
Bq	Becquerel
^oC	degrees centigrade
Ca^{2+}	calcium
CAT	cationic amino acid transporter (y^+)
CD62E	cluster designation 62 endothelial
cDNA	copy deoxyribonucleic acid
CHF	congestive heart failure
Ci	Curi
C.I.	Confidence Interval
Cl^-	Chloride
cGMP	cyclic-guanosine monophosphate

cm^2	squared centimeter
cNOS	constitutive nitric oxide synthase
CO_2	carbon dioxide
cpm	counts per minute
CβS	cystathionine-β-synthase
d	deci
Da	Dalton
DA	diacetate
DAF	diamino fluorescein
DAF-DA	diamino fluorescein diacetate
DDAH	dimethylarginine dimethylamine hydrolase
DEA/NO	diethylamine NONOate sodium salt hydrate
DMEM	Dulbecco's Minimum Essential Medium
DNA	deoxyribonucleic acid
EA.hy926	human endothelial vein cells fused with human lung cancer cells (hybrid number 926)
ECV_{304}	transformed human endothelial cord vein cells (T24/83 bladder carcinoma cells)
ED_{50}	50% effective dosage
EDTA	ethylenediamine tetra acetic acid disodium salt
EDRF	endothelial derived relaxing factor
EBM-2	endothelial cell basal medium-2
EGM^{TM}-2	endothelial growth medium-2
eNOS	endothelial nitric oxide synthase
Eq.	equation
Eqs.	equations
f	femto
F	permeability ratio
FAD	flavin adenine dinucleotide
FCS	foetal calf serum
FDA	food and drug association

FGF2	fibroblast growth factor-2
Fig.	figure
Fig's.	figures
FMN	flavin mononucleotide
g	gram
GFR	glomerular filtration rate
GIT	gastrointestinal tract
Glu	glutamine
[^{3}H]	tritiated hydrogen
HCl	hydrochloric acid
HCTZ	hydrochlorothiazide
Hcy	homocysteine
HDL	high density lipoprotein
HEPES	(*N*-[2-Hydroxyl]piperazine-N^1-[2-ethanesulfonic acid]
HHF	hypothalamic hypertensive factor
HIV-1	human immunodeficiency virus-1
HOPE-2	Heart Outcomes Prevention Evaluation-2
HUVEC	human umbilical vein endothelial cells
iNOS	induced nitric oxide synthase
I_{50}	50% inhibition of uptake
K^+	potassium
k	kilo
kDa	kilo Dalton
kg	kilogram
K_D	diffusion constant
K_i	constant of inhibitor
K_{ia}	constant of inhibitor *a*
K_{ib}	constant of inhibitor *b*
K_m	Michaelis constant
K_m'	apparent Michaelis constant
K_{mi}	Michaelis constant of inhibitor

L	liter
L-[^{3}H]arginine	tritiated arginine; L-[2,3,4-^{3}H] monohydrochloride arginine
LAT	neutral amino acid transport ($y^{+}L$)
LDL	low density lipoprotein
Li^{+}	lithium
L-NAME	N^{G}-nitro-L-arginine-methylester
L-NMMA	*N*-monomethyl-L-arginine
LPS	lipopolysacharide
µ	micro
M	molar
M199	culture medium 199
m	milli
MBq	Megabecquerel
5-methyl-THF	5-methyltetra hydrofolate
Mg^{2+}	magnesium
mg	milligram
ml	milliliter
mm	millimeter
mM	milliMolar
mol	moles
mRNA	messenger ribonucleic acid
MTHFR	methyltetra hydrofolate reductase
n	nano
N_2	nitrogen
N_2O_3	dinitrogen trioxide
Na^{+}	sodium
NaCl	sodium chloride
NADPH	nicotinamide adenine dinucleotide phosphate
nCi	nano Curie
NEM	*N*-ethylmaleimide
nm	nanometer

nM	nano molar
nmol	nanomole
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOC-9	MAHMA.NONOate
NORVIT	Norwegian Vitamin Trial
NOS	nitric oxide synthase
ω	omega
O_2	oxygen
p	pico
PAEC	porcine aortic endothelial cells
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PECy5	phycoerytherin cyan 5
Pen	penicillin
PHM5	glomerular epithelium monoclonal antibody and anti-human endothelium
RDA	recommended daily allowance
rHcy	reduced homocysteine
ROS	reactive oxygen species
S	substrate
[*S*]	substrate concentration
SEM	standard error of the mean
-SH	sulfhydryl group
SHR	spontaneously hypertensive rats
Strep	streptomycin
SOD	super oxide dismutase
T½	half-life
TCA	trichloroacetic acid
tHcy	total homocysteine

tPA	tissue-type plasminogen activator
UEA-I	Ulex europaeus-I
v	rate
VISP	Vitamin Intervention of Stroke Prevention
V_{max}	maximum velocity
V_{max}'	apparent maximum velocity
V_{maxa}	maximum velocity of inhibitor *a*
V_{maxb}	maximum velocity of inhibitor *b*
vs	versus
VSMC	vascular smooth muscle cells
WKY rats	Wistar-Kyoto rats

LIST OF FIGURES

CHAPTER 1

Figure 1.1 Metabolism of homocysteine and folate, homocystine transport. 17

Figure 1.2 Arginine metabolic pathway in the gastro-intestinal tract (GIT) and liver. 28

Figure 1.3 Structure of *N*-Ethylmaleimide (NEM), inhibitor of the y^+ transporter and structure of 2-amino-2-norbornanecarboxylic acid (BCH), inhibitor of the y^+L transporter. 48

Fgure 1.4 Arginine transport, intracellular arginine pools and nitric oxide production. 55

Figure 1.5 Role of y^+ and y^+L in arginine transport, effects of homocysteine and extracellular K^+ and nitric oxide production by endothelial cells. 61

CHAPTER 2

Figure 2.1 Diagram of cell culture method. 76

Figure 2.2 Diagram of arginine uptake method. 80

Figure 2.3 Diagram of nitric oxide detection method. 81

CHAPTER 3

Figure 3.1 Uptake of labelled arginine by $ECV_{304.}$ 97

Figure 3.2 Data for ECV_{304} to determine kinetic constants by non-linear modelling. 98

Figure 3.3 Data for HUVEC to determine kinetic constants by non-linear modelling. 99

Figure 3.4 Data for ECV_{304} plotted as Eadie Hofstee and Lineweaver Burk graphs to determine kinetic constants. 100

Figure 3.5 Data for HUVEC plotted as Eadie Hofstee and Lineweaver Burk graphs to determine kinetic constants. 101
Figure 3.6 Data for ECV_{304} and HUVEC plotted as Michaelis-Menten graphs to determine kinetic constants. 103
Figure 3.7 The effect of leucine on arginine uptake. 105

CHAPTER 4

Figure 4.1 Uptake of L-[^{3}H]arginine by HUVEC. 125
Figure 4.2 The effect of homocystine on the kinetic constants. 126
Figure 4.3 Michaelis-Menten plot of theoretical uptake by the individual transporters in the presence of homocystine by y^+L uptake. 128
Figure 4.4 The effect of co-incubation of homocystine with arginine on the kinetic constants of arginine uptake in ECV_{304} cells. 129
Figure 4.5 Effect of pre-incubation with NEM on arginine uptake in ECV_{304} cells. 130
Figure 4.6 Nitric oxide production by HUVEC and ECV_{304} cells. 133

CHAPTER 5

Figure 5.1 Effect of captopril on the initial rate kinetic parameters. 144
Figure 5.2 Effect of enalapril on the initial rate kinetic parameters. 146
Figure 5.3 Effect of HCTZ on the kinetic constants of L-[^{3}H]arginine uptake into ECV_{304} cells. 147
Figure 5.4 Dilution corrected uptake of L-[^{3}H]arginine by ECV_{304} cells pre-incubated with captopril and unlabelled arginine. 148
Figure 5.5 Lineweaver-Burke graphs of ECV_{304} cells pre-incubated with captopril and enalapril. 150
Figure 5.6 Effect of pre-incubation with 25μM enalapril, on arginine uptake by ECV_{304} cells. 151
Figure 5.7 Effect of captopril and enalapril on the rate of nitric oxide production by ECV_{304}. 152

LIST OF TABLES

CHAPTER 3

Table 3.1 Comparison of kinetic constants obtained from non-linear modelling of arginine uptake into ECV_{304} and HUVEC cells. 102

Table 3.2 Effect of pre-incubation of the inhibitors NEM (0.2mM) and BCH (30mM) on arginine uptake by ECV_{304} cells. 107

Table 3.3 Kinetic constants for ECV_{304} cells determined at various sodium concentrations. 109

CHAPTER 4

Table 4.1 Summary of the various forms of homocysteine measured in biological fluids. 119

CHAPTER 5

Table 5.1 Effects of captopril on the kinetic constants of arginine uptake by HUVEC. 145