Pflugers Arch - Eur J Physiol (2002) 445:147–151
DOI 10.1007/s00424-002-0911-0

ORIGINAL ARTICLE

T. M. C. Brunini · N. B. Roberts · M. M. Yaqoob · P. F. Reis · J. C. Ellory · G. E. Mann · A. C. Mendes-Ribeiro

Activation of L-arginine transport in peripheral blood mononuclear cells in chronic renal failure

Received: 26 April 2002 / Accepted: 9 July 2002 / Published online: 29 August 2002
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Abstract Transport of LL-arginine, the precursor for nitric oxide (NO) synthesis, has been investigated in human peripheral blood mononuclear cells (PBMCs) obtained from healthy volunteers and chronic renal failure patients. Chronic renal failure patients were either on treatment by haemodialysis or continuous ambulatory peritoneal dialysis (CAPD). Saturable influx of L-arginine in PBMCs was mediated by the cationic amino acid transport systems y^+ and y^+L. Initial rates of L-arginine transport (2 μM) via system y^+ were significantly increased in chronic renal failure patients, whereas transport via system y^+L was unaffected. The increase in L-arginine transport via system y^+ was: 1.7-fold in uraemic patients on CAPD, 4.3-fold in uraemic patients pre-haemodialysis and 2.6-fold post-haemodialysis. When the intracellular PBMCs amino acid profile was analysed in chronic renal failure patients and control subjects, L-lysine and L-arginine concentrations were significantly increased in pre-haemodialysis uraemic patients and restored to normal values by haemodialysis and CAPD. The present study provides the first evidence that system y^+ mediates the increased transport of L-arginine in PBMCs from patients with chronic renal failure. The increased activity of system y^+ may provide the necessary supply of L-arginine to sustain NO synthesis in PBMCs exposed to increased levels of circulating cytokines in chronic renal failure.

Keywords Arginine transport · Dialysis · Peripheral blood mononuclear cells · System y^+ · Uraemia

T.M.C. Brunini · P.F. Reis · A.C. Mendes-Ribeiro (✉)
Departamento de Farmacologia e Psicobiologia,
Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 20551-030, Brazil
e-mail: claudio.ribeiro@kcl.ac.uk
Tel.: +55-21-22496823

T.M.C. Brunini · J.C. Ellory
University Laboratory of Physiology, South Parks Road, Oxford, OX1 3PT, UK

N.B. Roberts
Department of Clinical Chemistry, Royal Liverpool Hospital, Liverpool, L7l 8XP, UK

M.M. Yaqoob · A.C. Mendes-Ribeiro
Renal Research Laboratory, St Bartholomew's Hospital, London, EC1A 7BE, UK

G.E. Mann · A.C. Mendes-Ribeiro
Centre for Cardiovascular Biology & Medicine,
GKT School of Biomedical Sciences, King's College London, Guy's Campus, London SE1 1UL, UK

Introduction

Chronic renal failure is defined as an irreversible and long standing loss of renal function causing ill health, usually referred to as uraemia [14]. It is a clinical disorder that has systemic compensatory mechanisms, including increased sympathetic activity and up-regulation of the renin-angiotensin system, leading to peripheral vasoconstriction and water and sodium retention [14]. Furthermore, uraemic patients present with endothelial dysfunction and immune dysfunction, including an increase in circulating pro-inflammatory cytokines [12, 17, 26, 28]. Immune abnormalities in chronic renal failure appear at an early stage and are exacerbated by dialysis [12]. Accumulating evidence suggests that a disturbance of the L-arginine-nitric oxide (NO) pathway is involved in the pathophysiology of chronic renal failure, perhaps reflecting a response to the hormonal imbalance and increased production of inflammatory mediators [17, 21, 26].

NO regulates both normal physiological and pathological events [27]. In mammalian cells, NO is synthesised from the cationic amino acid L-arginine by a family of NO synthases (NOS). There are three distinct isoforms of NOS: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS) [15]. L-Arginine participates in critical metabolic pathways such as NO and polyamine synthesis, and intracellular L-arginine is derived either from protein breakdown, de novo synthesis or via membrane transport from plasma [26]. Although levels of intracellular L-arginine are well above the K_m for NOS,

synthesis of NO is critically dependent on extracellular L-arginine under conditions of inflammation [1, 2, 3, 4, 11]. In diseases, such as hypercholesterolaemia and uraemia, L-arginine transport may even be rate limiting for NO synthesis [22, 26].

Four key transport systems for cationic amino acids (L-arginine, L-ysine and L-ornithine) have been described in mammalian cells: y^+, y^+L, $B^{o,+}$ and $b^{o,+}$ [13]. The transport system y^+ is selective for cationic amino acids and is Na^+ independent, whereas transport systems y^+L, $B^{o,+}$ and $b^{o,+}$ can transport cationic and neutral amino acids, but differ in their interactions with inorganic monovalent ions [13, 26]. System $b^{o,+}$ is Na^+ independent and system $B^{o,+}$ is Na^+ dependent. System y^+L transports cationic amino acids independent of sodium, and neutral amino acids only in the presence of Na^+ [13, 26]. Molecular studies have identified the proteins involved in cationic amino acids transport [10, 15]. A family of four homologous proteins mediates system y^+ activity: CAT-1, CAT-2A, CAT-2B and CAT-3 [9, 18]. The heavy chain 4F2hc interacts with the light chains, y^+LAT1 and y^+LAT2, forming a heterodimeric protein with y^+L transport activity [8, 32, 33].

We have previously reported a reduced plasma concentration and increased transport capacity for L-arginine in platelets and red blood cells from chronic renal failure patients [24, 25, 26]. Interestingly, in uraemic patients not yet on dialysis, low plasma levels of L-arginine were correlated with the degree of reduction in renal function and the increase in L-arginine transport via the y^+ system in red blood cells [26]. The activation of transport may represent a failing counter-regulatory mechanism to sustain NO synthesis in a milieu of reduced L-arginine availability [26]. We also established that L-arginine transport via system y^+L is up-regulated in platelets from uraemic patients [25].

Human peripheral blood mononuclear cells express inducible and constitutive isoforms of NO synthase [30]. As with red blood cells, transport of L-arginine in human peripheral blood mononuclear cells is mediated by systems y^+ and y^+L [5]. At a molecular level, CAT-1 and CAT-2B are responsible for system y^+ activity in these cells [13]. In the present study, we have investigated whether influx of L-arginine into human peripheral blood mononuclear cells is affected by chronic renal failure.

Materials and methods

Selection of patients with chronic renal failure

Fourteen patients with chronic renal failure on regular haemodialysis and six on continuous ambulatory peritoneal dialysis participated in this study. Ten healthy volunteers of both sexes were used as controls, and the study was conducted with approval from the Oxford and St. Bartholomew's Hospital Research Ethics Committees. The main exclusion criteria were heart failure, diabetes mellitus, infection and recent blood transfusion. Blood was drawn from healthy volunteers and chronic renal failure patients by venipuncture and collected in heparinised tubes. In chronic renal failure patients on haemodialysis, the blood was taken before and after a 4-h dialysis session. The aetiology of chronic renal failure in the patients used in this study was as follows: 8, hypertension, 5, glomerulonephritis, 2, reflux nephropathy, 2, unknown, 1, cystic disease, 1, pyelonephritis, 1, drug nephropathy.

Influx experiments in human peripheral blood mononuclear cells

Human peripheral blood mononuclear cells (PBMCs), comprised of ~85% lymphocytes and ~15% monocytes, were isolated from whole blood using a Ficoll-Sodium Metrizoate medium, followed by centrifugation [6]. The interface containing PBMCs was removed, and the cells were pelleted by centrifugation. The cell pellet was re-suspended in Krebs Ringer (mmol: NaCl 110, KCl 5, $MgCl_2$ 1.25, $CaCl_2$ 1, $NaHPO_4$ 16, Glucose 5 and NaH_2PO_4 4) and washed twice with two further centrifugation steps. The washed cell pellet, depleted of extracellular amino acids, was re-suspended in Krebs Ringer and the influx of 2 μM of L-[^{3}H]arginine or L-[^{3}H]lysine were measured over 3 min under initial rate conditions at 37°C. Total influx was divided into transport via systems y^+ and y^+L by *cis* inhibition of y^+L with 100 μM L-leucine [5]. Aliquots of the cell suspension containing tritiated L-arginine (or L-lysine) were removed at 15 s, 1 min, 2 min and 3 min, layered over oil (dibutyl phthalate plus dinonyl phthalate) and centrifuged. The amount of radioactivity associated with PBMCs was measured by scintillation counting.

Measurement of amino acid concentrations using HPLC

As described previously [24], individual amino acids were measured by reverse-phase HPLC using fluorescence of the orthophthalaldehyde derivatives (ASTED; Anachem, Luton, UK). The separation resolved all the known plasma amino acids over a 45-min analytical period. Fluorescence was measured at wavelengths 340 nm (excitation) and 440 nm (emission), using a model 4100 fluorimeter (Jasco, Thermo Separation Products, Stone, UK).

Chemicals

All chemicals were purchased from Sigma (Poole, Dorset, UK). Lymphocyte separation medium, L-[2,3-^{3}H]arginine (53 Ci/mmol) and L-[4,5-^{3}H]lysine (80 Ci/mmol) were obtained from ICN Biomedicals, UK.

Statistics

Data are expressed as mean±SEM of measurements in n different subjects. Statistical significance was determined at $P<0.05$ using the Mann-Whitney test.

Results

L-Arginine influx into PBMCs in chronic renal failure patients

Total L-arginine influx (2 M, initial rate) was enhanced in PBMCs from pre- and post-haemodialysis patients compared to controls (Fig. 1). Total L-arginine influx increased from 5.4±0.5 pmol/10^6cells/min in controls to 7.1±1.4 pmol/10^6cells/min in CAPD patients ($P<0.05$). After inhibition of system y^+L with 100 μM L-leucine, the initial rate of L-arginine influx mediated by system y^+ increased 1.7-fold in uraemic patients on CAPD, 4.3-fold

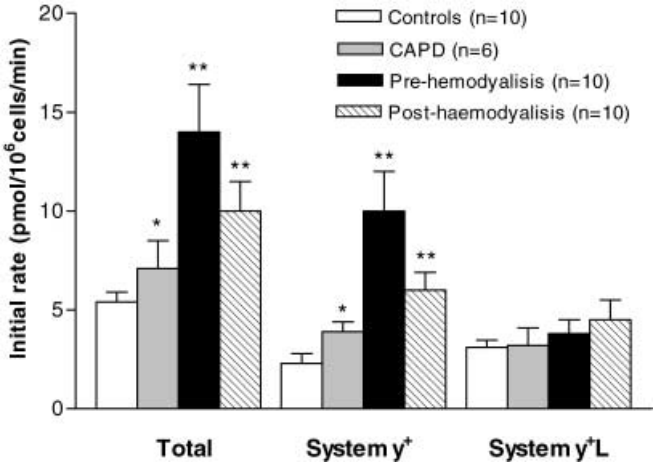

Fig. 1 Comparison of initial rates of L-arginine influx (2 μM) into peripheral blood mononuclear cells from controls and uraemic patients. Data are mean±SEM; *P<0.05, **P<0.005

Table 1 Amino acid concentrations in plasma and peripheral blood mononuclear cells (PBMCs) from controls and chronic renal failure patients on haemodialyisis (HD) or continuous ambulatory peritoneal dialysis (CAPD). Data are mean±SE of measurements in 5 controls, 5 haemodialysis and 5 CAPD patients (*Pre-HD* uraemic patients on dialysis prior to a haemodialysis session, *Post-HD* uraemic patients on dialysis after a haemodialysis session)

	Amino acid concentrations			
	Control	CAPD	Pre-HD	Post-HD
PBMCs (μM/10^6cells)				
L-Arginine	6.1±1.5	10±3	14±2.6*	6.1±0.2
L-Citrulline	0.7±0.1	2.8±1.1**	1.2±0.2**	0.3±0.01
L-Lysine	11±4	6.8±1.8	29±12*	8.6±0.8
L-Ornithine	1.5±0.4	12±3**	4.7±0.3	11±4*
Plasma (μM)				
L-Arginine	117±23	54±3**	49±26**	49±18**
L-Citrulline	50±12	76±4*	82±4*	58±18
L-Lysine	207±48	81±10*	196±70	170±42
L-Ornithine	125±42	65±7*	138±42	101±25

*P<0.05, **P<0.01

in uraemic patients pre-haemodialysis and 2.6-fold post-haemodialysis (Fig. 1). Haemodialysis partially reversed the stimulation of L-arginine transport in PBMCs. By contrast, initial rates of L-arginine transport via system y^+L were similar in PBMCs from controls, CAPD and haemodialysis patients (Fig. 1).

Plasma and PMBCs amino acid profiles in chronic renal failure patients

Table 1 summarizes plasma and intracellular PMBCs concentrations of L-arginine, L-citrulline, L-lysine and L-ornithine determined in controls and uraemic patients on haemodialysis or CAPD. The concentration of L-arginine in PBMCs was significantly increased pre-haemodialysis compared with controls and reduced by haemodialysis and CAPD. Intracellular L-lysine in PBMCs was also significantly increased in pre-haemodialysis patients compared with other groups. L-Citrulline levels in PBMCs were significantly higher in pre-haemodialysis and CAPD patients, but normalized by haemodialysis (Table 1). Plasma concentrations of L-arginine were reduced in uraemic patients on haemodialysis (pre- and post-haemodialysis) and CAPD (Table 1).

L-Lysine influx into PBMCs in chronic renal failure patients on haemodialysis

To exclude any effects of metabolism on L-arginine transport, experiments were also performed in PBMCs using L-lysine, another cationic amino acid substrate, to discriminate transport systems y^+ and y^+L using the same experimental protocol and cohort of patients. System y^+L was inhibited by *cis* inhibition with 100 μM L-leucine. In these experiments, transport of L-lysine via system y^+ (2 μM, initial rate) was increased in cells taken from uraemic patients on pre-haemodialysis (2±0.21 pmol/10^6cells/min, P<0.05) and post-haemodialysis (2±0.24 pmol/10^6cells/min, P<0.05) compared with cells from controls (0.7±0.23 pmol/10^6cells/min). Transport of L-lysine via system y^+L was not significantly altered in uraemic cells pre- (1.2±0.25 pmol/10^6cells/min) and post-(1.5±0.2 pmol/10^6cells/min) haemodialysis compared with controls (2±0.24 pmol/10^6cells/min).

Discussion

The present findings provide the first evidence that L-arginine transport in PBMCs is significantly increased in chronic renal failure patients compared with normal subjects. As in our previous studies with red blood cells, this increase in uptake was mediated selectively by the cationic amino acid transport system y^+ [24, 25, 26]. It is interesting to note that dialysis can reduce, but fails to normalise, the activation of system y^+ in PBMCs from chronic renal failure patients. To our knowledge, there are no reports on the effects of uraemia on L-arginine transport in PBMCs.

We have shown previously that chronic renal failure is associated with reduced plasma concentrations of L-arginine [24], which may explain the activation of system y^+ in PBMCs in the present study. There is evidence that L-arginine deprivation is associated with an up-regulation of L-arginine transport in endothelial cells via system y^+ [4] and mRNA levels of CAT-1 in Fao cells [19].

The ability of bacterial lipopolyssaccharide (LPS) and cytokines to enhance the transport of L-arginine via system y^+ under conditions of increased NO production provides a unique mechanism for sustaining NO [4, 31]. Cationic amino acid transport via system y^+ is increased markedly in human T lymphocytes stimulated by phytohaemaglutinin (PHA) [5]. The same group further documented that the highest rates of NO production occurred

in the same population of lymphocytes expressing increased system y^+ activity [7]. Thus, an increase in L-arginine transport via system y^+ observed in chronic renal failure may also represent an important mechanism to sustain NO production via iNOS, as previously reported for endotoxin- and cytokine-activated murine macrophages [1, 3] and rat aortic smooth muscle cells [34].

Uraemic patients are characterized by increased circulating levels of LPS and cytokines, particularly TNF-α, which may contribute to the activation of system y^+ in PBMCs [12, 28]. Induction of L-arginine transport via system y^+ in response to cytokines and LPS seems to be secondary to a signal transduction pathway requiring DNA transcription and de novo protein synthesis [29], which may directly affect circulating mature lymphocytes and/or the precursors of lymphocytes. In this context, recent studies in human foetal endothelial cells have shown that TNF-α increases L-arginine transport via increased activity of CAT-1 and CAT-2B transporters, with negligible changes detected for L-arginine transport via system y^+L [31]. Another interesting possibility is that neurohumoral activation, present in chronic renal failure, might also result in up-regulation of system y^+ in PBMCs, since it has been demonstrated that angiotensin II activates system y^+ in vascular smooth muscle [23].

Trans stimulation is a consequence of an increased substrate concentration at the *trans* side of the cell membrane [13]. The enhanced intracellular concentration of cationic amino acids in PBMCs from pre-haemodialysis uraemic patients and the return to normal levels after a dialysis session suggest that the marked increase in transport pre-dialysis may be related, at least partially, to a *trans* stimulation.

Several reports have demonstrated that transport mediated by system y^+ (CAT-1 and CAT-2B) is affected by membrane potential [4, 10, 13]. This feature may be another explanation for increased rates of L-arginine via system y^+ in PBMCs in uraemic patients, although it is worth noting that membrane potential in different cell types from uraemic patients has been shown to be increased or decreased [16, 20].

In summary, transport of L-arginine is elevated in peripheral blood mononuclear cells, suggesting that uraemia induces an adaptive increase in the activity of the cationic transport system y^+. Activation of L-arginine transport via system y^+ in PBMCs may thus provide a mechanism by which human blood cells compensate for the reduced vascular pool of L-arginine found in this disease state. Moreover, increased rate of L-arginine transport could provide the necessary substrate supply for enhanced production of NO, since the rate of NO production is dependent upon extra cellular L-arginine in these cells [7].

Acknowledgements This work was funded in part by a project grant from the British Heart Foundation and CNPq, Brazil. We are indebted to Prof. Richard Boyd (University of Oxford) for helpful discussions.

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