Regulation of Amino Acid and Glucose Transporters in Endothelial and Smooth Muscle Cells

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Mann, Giovanni E., David L. Yudilevich, and Luis Sobrevia. Regulation of Amino Acid and Glucose Transporters in Endothelial and Smooth Muscle Cells. Physiol Rev 83: 183–252, 2003; 10.1152/physrev.00022.2002.—While transport processes for amino acids and glucose have long been known to be expressed in the luminal and abluminal...
membranes of the endothelium comprising the blood-brain and blood-retinal barriers, it is only within the last decades that endothelial and smooth muscle cells derived from peripheral vascular beds have been recognized to rapidly transport and metabolize these nutrients. This review focuses principally on the mechanisms regulating amino acid and glucose transporters in vascular endothelial cells, although we also summarize recent advances in the understanding of the mechanisms controlling membrane transport activity and expression in vascular smooth muscle cells. We compare the specificity, ionic dependence, and kinetic properties of amino acid and glucose transport systems identified in endothelial cells derived from cerebral, retinal, and peripheral vascular beds and review the regulation of transport by vasoactive agonists, nitric oxide (NO), substrate deprivation, hypoxia, hyperglycemia, diabetes, insulin, steroid hormones, and development. In view of the importance of NO as a modulator of vascular tone under basal conditions and in disease and chronic inflammation, we critically review the evidence that transport of L-arginine and glucose in endothelial and smooth muscle cells is modulated by bacterial endotoxin, proinflammatory cytokines, and atherogenic lipids. The recent colocalization of the cationic amino acid transporter CAT-1 (system y\(^{-}\)), nitric oxide synthase (eNOS), and caveolin-1 in endothelial plasmalemmal caveolae provides a novel mechanism for the regulation of NO production by L-arginine delivery and circulating hormones such insulin and 17\(\beta\)-estradiol.

**I. INTRODUCTION**

The endothelial lining of blood vessels provides a barrier for the exchange of nutrients and is itself actively involved in the local control of vascular homeostasis. Blood-borne and tissue-derived mediators act on endothelial cells, stimulating the synthesis and release of soluble vasoactive factors and the expression of surface adhesion molecules for circulating leukocytes (reviewed in Refs. 124, 223, 464), while the actin- and myosin-based contractile cytoskeleton in endothelium regulates responses to changes in blood flow and shear stress (see Ref. 514). Endothelium-dependent vascular relaxation is markedly impaired in diseases such as diabetes mellitus, atherosclerosis, hypertension, and preeclampsia. Because disease-induced alterations in plasma levels of L-arginine and related amino acids, glucose, and insulin modulate vascular relaxation, it is surprising that only recent studies have examined regulation of transport and metabolism of amino acids and glucose in vascular endothelial and smooth muscle cells. During the last two decades, the L-arginine-nitric oxide signaling pathway has emerged as one of the key second messenger systems involved in the regulation of vascular tone and permeability (reviewed in Refs. 285, 326, 412). The discovery that L-arginine is the physiological precursor for nitric oxide (NO) biosynthesis precipitated research into the role of circulating and intracellular arginine in the regulation of vascular function in health and disease. In 1998 the Nobel Prize in Physiology and Medicine was awarded to Robert Furchgott, Louis Ignarro, and Ferid Murad for their contribution to the discovery of NO as a key signaling molecule (for commentaries, see Refs. 274, 643).

This review aims to highlight the mechanisms regulating amino acid and glucose transporters in endothelial cells derived from peripheral vascular beds and the blood-brain and blood-retinal barriers. Although nutrient transport and metabolism by the brain endothelium (and to a lesser extent retinal and corneal endothelium) have been reviewed previously (see Refs. 75, 76, 455, 544), the recent advances in our understanding of transport processes in endothelial cells derived from peripheral vascular beds provide a basis for comparing the specificity, kinetics, and regulation of amino acid and glucose transport. We have focused principally on transport processes in endothelial cells but have also reviewed the available literature for vascular smooth muscle cells, in view of the modulation of smooth muscle tone by endothelium-derived mediators and proinflammatory cytokines. Because there are excellent reviews on the molecular biology of amino acid (51, 120, 125, 128, 130, 131, 162, 163, 186, 256, 301, 371, 375, 449, 450, 625) and glucose (22, 23, 95, 106, 294, 324, 414, 445, 516, 594) transporters, we have chosen to focus this review on the regulation of nutrient transport in vascular endothelial and smooth muscle cells.

Endothelial cell metabolism and the general characteristics of amino acid and glucose transport systems (and recently cloned transporters, see Table 1) expressed in mammalian cells are intended only as a brief overview. These sections, however, provide the basis for a detailed comparison of the selectivity, ionic dependence, and kinetic properties of amino acid and glucose transporters in endothelial cells derived from the blood-brain barrier, blood-retinal barrier, and peripheral vascular beds, such as fetal umbilical vein, placenta, aorta, lung, heart, and adrenal gland. Regulation of endothelial cell amino acid and glucose transport by vasoactive agonists, NO, insulin, hypoxia, substrate deprivation, and development is reviewed subsequently, highlighting where possible differences in the responses of endothelial cells derived from cerebral and peripheral circulations. In view of the importance of NO as a modulator of vascular tone in inflammation, we have critically evaluated the evidence that transport of amino acids and glucose in endothelial and smooth muscle cells is modulated by bacterial...
endotoxin, proinflammatory cytokines, and oxidatively modified low-density lipoproteins. This review aims to provide a comprehensive overview of the mechanisms regulating the activity and expression of amino acid and glucose transporters in vascular cells. With the increasing advances in the molecular identification and understanding of the function of amino acid and glucose transporters in other mammalian tissues, we believe that vascular physiology merits similar research initiatives.

Summary of the classical nomenclature for the majority of amino acid transport systems and the nomenclature adopted for recently cloned amino acid transport proteins. The substrate specificity, ionic dependency, and Michaelis constant (K_m) values have been compiled from the cited references and reviews. We have not cited all relevant references, since detailed reviews on the molecular biology of amino acid transporters have been published (see Refs. 120, 125, 128, 129, 162, 163, 301, 371, 375, 449, 625). Although several of the classical transport systems have been described in vascular endothelial and smooth muscle cells, only limited data are available on the molecular characteristics of these amino acid transporters in vascular endothelial and smooth muscle cells. Lowercase nomenclature for the classical transport systems may not exhibit kinetic properties described in other peripheral cell types. Lowercase nomenclature for the classical transport systems have been described in endothelial cells, only limited data are available on the molecular characteristics of these amino acid transporters have been published (see Refs. 120, 125, 128, 129, 162, 163, 301, 371, 375, 449, 625). Although several of the classical transport systems have been described in endothelial cells, only limited data are available on the molecular characteristics of these amino acid transporters in vascular endothelial and smooth muscle cells.

**TABLE 1.** Mammalian amino acid transport systems and identified transporters

<table>
<thead>
<tr>
<th>Transport System</th>
<th>Associated Protein or cDNA</th>
<th>Amino Acid Selectivity</th>
<th>Ionic Dependency</th>
<th>K_m, μM</th>
<th>Reference Nos. Relating to Transport and/or cDNA</th>
</tr>
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<tbody>
<tr>
<td><strong>CAT-associated transport</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y^+</td>
<td>CAT-1</td>
<td>Cationic</td>
<td>No</td>
<td>70–250</td>
<td>125, 128, 120, 162, 230, 300, 318, 450, 632</td>
</tr>
<tr>
<td>CAT-2A or CAT-2/2a</td>
<td>Cationic</td>
<td>No</td>
<td>2,150–5,200</td>
<td>125, 128, 120, 162, 230, 300, 308, 370, 371, 450</td>
<td></td>
</tr>
<tr>
<td>y^+</td>
<td>CAT-2B or CAT-2/2β</td>
<td>Cationic</td>
<td>No</td>
<td>38–380</td>
<td>120, 162, 300, 308, 371</td>
</tr>
<tr>
<td>y^-</td>
<td>CAT-3</td>
<td>Cationic</td>
<td>No</td>
<td>40–120</td>
<td>270, 291</td>
</tr>
<tr>
<td>y^-</td>
<td>CAT-4</td>
<td>Cationic</td>
<td>No</td>
<td>450–910</td>
<td>612</td>
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<tr>
<td><strong>Heterodimeric glycoprotein (4F2hc, CD98)-associated transport</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y^+L</td>
<td>y^+LAT1</td>
<td>Neutral/cationic</td>
<td>Na^+ (neutral)</td>
<td>340</td>
<td>73, 120, 162, 163, 304, 467, 625</td>
</tr>
<tr>
<td>y^-L</td>
<td>y^-LAT2</td>
<td>Neutral/cationic</td>
<td>Na^+ (neutral)</td>
<td>6–10</td>
<td>50, 73, 86, 120, 156, 162, 163, 192, 400, 450, 625, 659</td>
</tr>
<tr>
<td>L</td>
<td>LAT1/LAT2</td>
<td>Large neutral</td>
<td>No</td>
<td>30–300</td>
<td>87, 100, 120, 123, 296, 312, 389, 419, 450, 469, 473, 518, 625</td>
</tr>
<tr>
<td>xc</td>
<td>xCT</td>
<td>Cystine/glutamate</td>
<td>No</td>
<td>40–92</td>
<td>24–26, 39, 93, 120, 158–160, 349, 450, 450, 508–510, 539, 573, 625</td>
</tr>
<tr>
<td>asc</td>
<td>Asc-1</td>
<td>Small neutral</td>
<td>No</td>
<td>9–23</td>
<td>120, 208, 423, 625</td>
</tr>
<tr>
<td><strong>rBAT-associated transport</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b^0^-</td>
<td>b^0^- AT</td>
<td>Neutral/cationic</td>
<td>cystine</td>
<td>No</td>
<td>88</td>
</tr>
<tr>
<td><strong>Unknown heavy chain-associated transport</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>asc</td>
<td>Asc-2</td>
<td>Small neutral</td>
<td>No</td>
<td>2.9</td>
<td>110, 388</td>
</tr>
<tr>
<td>A</td>
<td>ATA 1/2/3 (SAT 1/2/3 or SA 1/2/3)</td>
<td>Neutral/N-Me group</td>
<td>Na^+</td>
<td>&gt;200</td>
<td>4, 123, 215, 236, 249–251, 284, 351, 489, 531, 570, 571, 608, 631, 658</td>
</tr>
<tr>
<td>N</td>
<td>SN 1/2/3</td>
<td>Glu, Asn, His</td>
<td>Na^+ /H^+</td>
<td>150–1,600</td>
<td>115, 123, 193, 234, 315, 372, 421, 422</td>
</tr>
<tr>
<td>ASC</td>
<td>ASCT 1/2*</td>
<td>Neutral</td>
<td>Na^-</td>
<td>9–464</td>
<td>16, 84, 268, 316, 524, 601, 665</td>
</tr>
<tr>
<td>B^0^-</td>
<td>ATB^0^-</td>
<td>Neutral/cationic</td>
<td>Na^+ /Cl^-</td>
<td>23–140</td>
<td>252, 309, 540, 590, 609, 611</td>
</tr>
<tr>
<td>Pro</td>
<td>PROT*</td>
<td>Proline</td>
<td>Na^- /Cl^-</td>
<td>6</td>
<td>123, 490, 725, 613</td>
</tr>
<tr>
<td>Gly</td>
<td>GLYT 1/2*</td>
<td>Glycine</td>
<td>Na^- /Cl^-</td>
<td>17</td>
<td>235, 295, 354</td>
</tr>
<tr>
<td>Gly</td>
<td>GLYT1a-c*</td>
<td>Glycine</td>
<td>Na^- /Cl^-</td>
<td>70–90</td>
<td>320</td>
</tr>
<tr>
<td>Beta-like</td>
<td>mTAUT*</td>
<td>β-Amino acids</td>
<td>Na^- /Cl^-</td>
<td>3–13</td>
<td>353, 477, 484, 620</td>
</tr>
<tr>
<td>GAT-1–3</td>
<td>GABA and or β-aminobutyric acid</td>
<td>Na^- /Cl^-</td>
<td>1–20</td>
<td>450</td>
<td></td>
</tr>
<tr>
<td>GBT-1</td>
<td>EAAT1–5*</td>
<td>Glu and Asp</td>
<td>Na^-</td>
<td>18–97</td>
<td>16, 375, 527</td>
</tr>
<tr>
<td>X_{AG}</td>
<td>Glu, Asp</td>
<td>Na^-</td>
<td>−200</td>
<td>213, 374</td>
<td></td>
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<tr>
<td>X_{AG}</td>
<td>Glu</td>
<td>No</td>
<td>−230</td>
<td>123, 454</td>
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Summary of the classical nomenclature for the majority of amino acid transport systems and the nomenclature adopted for recently cloned amino acid transport proteins. The substrate specificity, ionic dependency, and Michaelis constant (K_m) values have been compiled from the cited references and reviews. We have not cited all relevant references, since detailed reviews on the molecular biology of amino acid transporters have been published (see Refs. 120, 125, 128, 129, 162, 163, 301, 371, 375, 449, 625). Although several of the classical transport systems have been described in endothelial cells, only limited data are available on the molecular characteristics of these amino acid transporters in vascular endothelial and smooth muscle cells. Lowercase nomenclature for the classical transport systems denotes Na^- independence: Ala, alanine; Ser, serine; Cys, cysteine; Glu, glutamate; Asn, asparagine; His, histidine; Glu, glutamate; Asp, aspartate.
II. ENDOTHELIAL AND SMOOTH MUSCLE CELL METABOLISM

A. Role of Amino Acid Metabolism in Endothelial Cell NO Synthesis

Microvascular endothelial cells are able to use certain amino acids as fuels for oxidative phosphorylation (332). In rat coronary microvascular endothelial cells deprived of glucose, L-glutamate and L-glutamine are rapidly oxidized, whereas oxidation rates for L-asparagine, L-alanine, L-isoleucine, and L-arginine are intermediate. The negligible oxidation of L-valine and L-tyrosine suggests that coronary endothelial cells may lack specific enzymes required for the degradation of these amino acids. Amino acids with the highest rates of oxidation (glutamate, glutamine, alanine, asparagine) are degraded by no more than three intermediate steps before entering the Krebs cycle, and catabolism of these substrates has been characterized in bovine pulmonary artery endothelial cells (344). Bovine coronary venular endothelial cells metabolize L-glutamine to ammonia, L-glutamate, and L-aspartate (398), and the high activity of glutaminase in bovine pulmonary artery endothelial cells (~20-fold higher than in lymphocytes) indicates that L-glutamine provides an important respiratory fuel (395, 556). However, as cultured endothelial cells may lack a fully functional urea cycle, it seems unlikely that L-glutamine can be metabolized via L-ornithine to L-citrulline and L-arginine (see Fig. 2 in Ref. 654), confirming the lack of an effect of extracellular L-glutamine on intracellular L-arginine levels (398).

A comparative study of L-glutamine metabolism in bovine coronary venular, bovine aortic, human mesenteric, and human umbilical vein endothelial cells has confirmed that CO₂ is the major metabolic product of glutamine-derived L-glutamate (648), highlighting the importance of glutamine as an energy substrate in different endothelial cell types. Although formation of L-glutamate and ammonia from L-glutamine are similar in microand macrovascular endothelial cells, Wu et al. (648) concluded that glutamate dehydrogenase was inhibited by ammonia generated from L-glutamate by mitochondrial phosphate-dependent glutaminase. Their study also identified a novel pathway for L-ornithine synthesis from L-glutamine via pyrroline-5-carboxylate synthase, but unlike intestinal epithelial cells, glutamine-derived L-ornithine was not converted into L-citrulline and L-arginine due to the absence of carbamoylphosphate synthase-1 (see Fig. 2 in Ref. 654).

NO is a labile vasodilator synthesized in endothelial cells from the semiessential cationic amino acid L-arginine (reviewed in Refs. 285, 412). The metabolism of L-arginine by mammalian cells has been reviewed in detail (see Refs. 30, 654), and in endothelial cells a constitutive, Ca²⁺/calmodulin (CaM)-sensitive NO synthase (eNOS) metabolizes L-arginine to NO and the neutral amino acid L-citrulline (451). eNOS is present in membrane caveolae and the cytosol and requires tetrahydrobiopterin (BH₄), NADPH, flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN) as additional cofactors for its activity (see Refs. 326, 521). Vasoactive agonists normally elevate intracellular Ca²⁺ in endothelial cells (292, reviewed in Ref. 403) with binding of Ca²⁺/CaM to eNOS stimulating NO production, while fluid shear stress leads to phosphorylation of the serine/threonine protein kinase Akt (protein kinase B) in a phosphatidylinositol (PI) 3-kinase-dependent manner and activation of eNOS at basal intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) levels (see Refs. 144, 160, 202, 209).

Figure 1 depicts the described transport systems mediating cationic amino acid influx in vascular endothelial (and those known for smooth muscle) cells and further illustrates the effects of agonist-induced increases in intracellular Ca²⁺ and NO on cross-talk between endothelial and smooth muscle cells. We envisage system y⁺ as the primary carrier mediating facilitated transport of L-arginine, in which the negative membrane potential leads to an accumulation of cationic amino acids within the cell. Intracellular L-arginine concentrations in cultured endothelial cells range between 0.1 and 0.8 mM, although concentrations up to ~2–4 mM have been measured in freshly isolated endothelial cells (42, 43, 227, 398). Figure 2 compares amino acid concentrations in bovine aortic and human umbilical vein endothelial cells in culture and highlights the differential effect of L-arginine deprivation on intracellular concentrations of L-arginine, L-lysine, L-ornithine, and L-citrulline (see sect. VII A).

Within endothelial cells, recycling of L-citrulline to L-arginine occurs at a rate of ~0.7–1.9 nmol L-arginine·1⁰⁶ cells⁻¹·h⁻¹, with L-citrulline converted into L-arginine via argininosuccinate synthase in the presence of aspartate and Mg-ATP and argininosuccinate lyase (255, 406, 522, 652; see Fig. 3). Although Sessa et al. (522) reported that L-glutamine (0.2 mM) inhibited recycling of L-citrulline to L-arginine in L-arginine-depleted bovine aortic endothelial cells, Wu and Meininger (652) noted that L-glutamine only reduced L-arginine synthesis from L-citrulline in human mesenteric, but not bovine aortic or coronary venular endothelial cells. These discrepancies have not been resolved, and differences in cell culture conditions and/or experimental protocols (e.g., microcarrier cultures vs. static monolayers) seem the most likely explanation.

Agonist-stimulated NO production is also sensitive to inhibition by L-glutamine [inhibitory constant (Kᵢ) ~50–100 μM] (15, 255, 522), and the initial studies by Hecker et al. (255) attributed this inhibition to a direct action of L-glutamine on the citrulline-arginine cycle in endothelial cells. Arnal et al. (15) subsequently reported that the effect of L-glutamine on NO release from bovine aortic
endothelial cells was dependent on the stimulus used, e.g., L-glutamine inhibited NO release in cells challenged with bradykinin but slightly enhanced NO release in the presence of the calcium ionophore A23187. In contrast, another study in bovine coronary venular and aortic endothelial cells reported that L-glutamine inhibits A23187-stimulated NO release (398). Although there are no immediate explanations for the discrepancy in the effects of L-glutamine on A23187-stimulated NO production in bovine aortic endothelial cells, it is worth emphasizing that L-glutamine does not inhibit L-arginine synthesis from either L-citrulline or argininosuccinate in endothelial cell lysates, whereas inhibition of the citrulline-arginine cycle (398, 652). This same group reported that L-citrulline transport was inhibited competitively by L-glutamine (0.2–1 mM) but unaffected by 0.5 mM L-arginine, L-alanine, L-glutamate, or L-lysine. Our studies in J774 murine macrophages established that L-citrulline was transported via a saturable [Michaelis constant ($K_m$) = 0.16 mM, maximum binding velocity ($V_{max}$) = 32 pmol/µg protein−1·min−1], pH insensitive, neutral amino acid carrier insensitive to inhibition by L-arginine or the cationic NOS inhibitor Nω-monomethyl-L-arginine (L-NMMA) (41, 45). We further reported that, unlike transport of L-arginine and L-NMMA, kinetics of L-citrulline transport were not altered by bacterial lipopolysaccharide (LPS), and recycling of L-citrulline to L-arginine could only sustain limited NO production (41).

In view of the fact that responses to endothelium-dependent vasodilators in vivo are often less than those observed in vitro, Arnal et al. (15) suggested that studies with isolated arterial rings or cultured endothelial cells should consider supplementing incubation media with L-glutamine. More recent studies in bovine coronary venular endothelial cells have established a role for glutamine:fructose-6-phosphate amidotransferase (GFAT, EC 2.6.1.16), the rate-limiting enzyme in the synthesis of hexosamine from glutamine and fructose-6-phosphate, in the modulation of the L-arginine-NO pathway by L-glutamine (649, 650). Increased glucose flux through the hexosamine biosynthetic pathway results in the generation of glucosamine-6-phosphate from fructose-6-phosphate by GFAT. The inhibition of NO generation by L-glutamine or its metabolite glucosamine was attributed to an inhibition of the pentose cycle and decreased availability of cellular NADPH. Neither L-glutamine nor glucosamine had any effect on radiolabeled L-arginine uptake or intracellular
concentrations of L-arginine, BH$_4$, and Ca$^{2+}$. A note of caution in the interpretation of the above findings is that GFAT activity is markedly elevated in cultured endothelial cells compared with freshly isolated cells, suggesting that cell differentiation or in vitro culture conditions may upregulate GFAT expression (650). This provides a plausible explanation for the notable discrepancy between the significant expression of GFAT in human cultured mesenteric microvascular and umbilical vein endothelial cells (650) and the lack of immunohistochemical staining for GFAT in the endothelium of human blood vessels (428).

In summary, inhibition of L-citrulline transport by L-glutamine and inhibitory actions of L-glutamine (and its metabolite glucosamine) on the pentose cycle can modulate the NO generation in cultured endothelial cells. Due to the unusually high activity of GFAT in cultured endothelial cells, future studies should determine whether L-glutamine effectively inhibits the L-arginine-NO pathway in different vascular beds in vivo.

Methylated arginines are excreted into the urine and accumulate in the plasma of patients with renal insufficiency or hypercholesterolemia, with asymmetric dimethyl-arginine (ADMA) significantly attenuating endothelium-dependent relaxation (see Refs. 136, 290, 367, 368, 604). ADMA and L-NMMA can be metabolized via dimethylarginine dimethylaminohydrolase (DDHA) to L-citrulline (439), with inhibition of DDHA activity in vascular disease leading to an accumulation of ADMA, normally extensively metabolized in vivo. In bovine aortic endothelial cells, L-NMMA, but not N$^\omega$-nitro-L-arginine (L-NNA), is rapidly metabolized to L-citrulline and subsequently L-arginine (255). Experiments in human umbilical and saphenous vein endothelial cells further confirmed that L-[14C]NMMA can be metabolized to L-[14C]citrulline via an enzyme with properties similar to DDHA (367). Treatment of a transformed human cell line ECV304 (which may have limited value as a human endothelial cell model) with oxidized low-density lipoprotein or tumor necrosis factor (TNF-$\alpha$) causes a time-dependent decrease in the activity of DDHA with maximal ADMA concentrations of $\sim$4 M measured in the culture medium (290). Although there is an ongoing debate concerning the validity of the ECV304 cell line as a model for endothelium (82, 569), the identical genotype of ECV304 and T24/83 bladder carcinoma cell lines argues against the use of ECV304 cells for the study of endothelial cell biology.

Arginases are responsible for the metabolism of L-arginine into L-ornithine and urea, and at least two isoforms have been identified: arginase I, a cytosolic enzyme expressed highly in the liver, and arginase II, a mitochon-
Arginase I preferentially directs L-ornithine to polyamine biosynthesis via ornithine decarboxylase with arginase II preferentially directing L-ornithine to L-proline and L-glutamate synthesis via ornithine aminotransferase. Arginase isoforms are expressed in activated macrophages and vascular smooth muscle cells (113, 179, 633), and unstimulated rat aortic and porcine coronary arteriolar endothelial cells express arginase I constitutively (94, 666). Activation of rat aortic endothelial cells with bacterial LPS induces arginase II and the inducible Ca<sup>2+</sup>/CaM-insensitive isoform of NOS (iNOS) (for review, see Ref. 326). Under these conditions, urea production appears to be inhibited as a result of intracellular $N^\omega$-hydroxy-L-arginine accumulation following $N^\omega$-hydroxylation of L-arginine with insertion of one oxygen atom from dioxygen and consumption of two electrons from NADPH (409, 410, see Fig. 3). The inhibition of arginase by $N^\omega$-hydroxy-L-arginine in endothelial cells expressing iNOS was implicated as a mechanism for sustaining intracellular L-arginine concentrations during sustained production of NO (94).

Recent studies with bovine venular endothelial cells have shown that L-glutamine and its metabolite glucosamine can reduce intracellular NADPH levels and thereby inhibit NO synthesis (see Ref. 649). L-NMMA, $N^\omega$-monomethyl-L-arginine; ADMA, asymmetrical dimethyl-L-arginine.

FIG. 3. Metabolism of L-arginine in endothelial cells. An important step in L-arginine metabolism in endothelial cells is the formation of the labile gas nitric oxide (NO) via constitutive endothelial nitric oxide synthase (eNOS) (see Refs. 255, 285, 326, 412, 521). eNOS utilizes L-arginine (EC<sub>50</sub> ~ 6 μM), molecular oxygen, and reduced nicotinamide adenine dinucleotide (NADH) as cosubstrates and tetrahydrobiopterin (B(H)<sub>4</sub>), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and protoporphyrin IX haem as cofactors. L-Arginine is mono-oxygenated and converted to $N^\omega$-hydroxyarginine by the enzyme arginine $N^\omega$-hydroxylase (HDX) in the presence of B(H)<sub>4</sub>, nicotinamide adenine dinucleotide phosphate (NADPH), H<sup>+</sup>, and O<sub>2</sub> (see Refs. 330, 410). $N^\omega$-hydroxyarginine, but not L-arginine, can potentiate NO synthesis in endothelial cells (reviewed in Ref. 521), suggesting that the L-arginine mono-oxidation step (probably synthesis of B(H)<sub>4</sub>) may be rate limiting the overall reaction. L-Citrulline can be recycled into L-arginine via argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASSL). ASSL catalyzes the conversion of argininosuccinate into L-arginine and fumarate, which is recycled through the Krebs cycle into L-aspartic acid. Hecker et al. (255) have also described conversion of L-citrulline into L-arginine via a transamination reaction. Recycling of L-citrulline into L-arginine is dependent on availability of exogenous L-citrulline and is sensitive to inhibition by L-glutamine ($K_i$ ~ 50–100 μM), with L-glutamine (L-Gln) reported to decrease L-arginine synthesis from L-citrulline by inhibiting membrane transport of L-citrulline (see Refs. 522, 652, 654). More recent studies in bovine venular endothelial cells have shown that L-glutamine and its metabolite glucosamine can reduce intracellular NADPH levels and thereby inhibit NO synthesis (see Ref. 649). L-NMMA, $N^\omega$-monomethyl-L-arginine; ADMA, asymmetrical dimethyl-L-arginine.
laxation of porcine coronary arterial rings challenged with adenosine or serotonin, implying that inhibition of arginase may specifically increase the availability of L-arginine for NO synthesis in coronary arterial endothelium (666). Interestingly, inhibition of NO synthesis in renal mesangial cells is not associated with enhanced arginase activity (623), suggesting that increased availability of L-arginine is not necessarily diverted to arginase.

Due to the much higher L-arginine concentrations in tissue-culture media (~400 μM), it is possible that rates of Νω-hydroxy-L-arginine production by cultured endothelial cells are greater than in vivo, and thus more effective in inhibiting arginase. Because the $K_m$ of arginase for L-arginine is high (1–3 mM) whereas the $K_m$ of NO synthase isoforms for L-arginine is relatively low (~3–10 μM) (see Refs. 94, 470, 653), arginase may not necessarily limit the availability of intracellular L-arginine for NO production in all endothelial cell types. Intracellular L-arginine levels in endothelial cells are maintained within the range of the $K_m$ for arginase, and this together with efficient recycling of L-citrulline to L-arginine and the prevailing membrane potential may explain the high intracellular L-arginine levels (42, 43, 227, 398).

Accumulating evidence now suggests that supply of L-arginine for NO synthesis may be derived from a membrane-associated compartment distinct from the bulk intracellular amino acid pool (132, 244, 396, 647), e.g., near invaginations of the plasma membrane referred to a caveola or “lipid rafts” (reviewed in Refs. 210, 536). Colocalization of eNOS and the cationic amino acid transport system Y$^+$ in caveola (see sect. χ) may explain the “arginine paradox,” concerning the discrepancy in the sensitivity of eNOS to extracellular L-arginine in cell-free systems ($K_m$ of NO synthase for L-arginine in the low micromolar range, see Ref. 470) and studies in vivo where L-arginine supply seems to be rate-limiting for NO synthesis in hypercholesterolemia (148, 224, see also Ref. 136) despite high intracellular and circulating levels of L-arginine. To our knowledge there is no evidence that arginase and eNOS are colocalized in plasmalemmal caveola, although a recent study has identified eNOS and arginino-succinate synthase in the caveolar fraction of bovine aortic endothelial cells (see Ref. 201, discussed in sect. χ).

B. Glucose Metabolism in Endothelial Cells

Glucose is actively metabolized in endothelial cells (219) and sustains anaerobic and aerobic metabolism (i.e., ~20–50 nmol ATP·mg protein$^{-1}$·min$^{-1}$, see Refs. 332, 402). In the presence of 5 mM d-glucose, catabolism of amino acids, palmitate, and lactate is reduced significantly, with oxidation rates for L-glutamine, L-alanine, and L-arginine decreased significantly (332). In rat coronary microvascular endothelial cells, >98% of incorporated glucose is metabolized to lactate (332). At physiological concentrations of glucose, the contribution of the hexose monophosphate pathway accounts for ~1.2% of glucose metabolism and the Krebs cycle for only ~0.04%, suggesting that in microvascular endothelial cells almost all of the energy obtained from catabolism of glucose is generated glycolytically. At lower glucose concentrations (~1 mM), oxidation of glucose via the Krebs cycle is higher. Thus oxidative metabolism in endothelial cells is inhibited at physiological concentrations of glucose, demonstrating that endothelial cells express the Crabtree effect (i.e., an inhibitory effect of glucose on mitochondrial respiration, Ref. 332).

Endothelial cells synthesize ATP primarily via glycolysis with a relatively low O$_2$ consumption (152, 167, 402). Studies employing calorimetry and $^{31}$P nuclear magnetic resonance have shown that porcine aortic endothelial cells deprived of glucose for 2 h exhibit a marked loss of nucleoside triphosphates and inhibition of protein synthesis, yet are capable of metabolizing endogenous triglycerides for de novo purine synthesis, recovering most of their adenine nucleotides following readministration of glucose (151). Thus endothelial cells are able to withstand prolonged periods of substrate deprivation and can adapt to hypoxia (see sect. νιΒ) due to their low energy demand and high glycolytic activity (150, 151, 402). Recent evidence in human umbilical vein endothelial cells suggests that fatty acids can also serve as an energy fuel (154). Stimulation of AMP-activated protein kinase (AMPK) in human umbilical vein endothelial cells by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR, 2 mM) results in a decrease in malonyl coenzyme (CoA) levels and the activity of acetyl CoA carboxylase, increased oxidation of palmitate, and decreased glucose uptake and glycolysis (154). Despite a predicted decrease in the rate of ATP production, ATP levels increased by ~35%, reflecting potentially increased ATP generation from fatty acid oxidation. However, oxidation of fatty acids in umbilical vein endothelial cells only accounts for ~25% of the calculated ATP production in cells incubated with 5 mM glucose. As discussed by Dagher et al. (154), further studies are required to determine whether this reflects an underestimate for ATP generation from fatty acid oxidation and/or and actual decrease in cellular ATP utilization.

The effects of elevated glucose on endothelial cell function are often cell specific (see Refs. 298, 333, 334, 376). The cytosol of endothelial cells is reduced by accumulation of NADH and transformation of pyruvic acid to lactate, as described in microvascular endothelial cells from bovine corpus cavernosum (167) and brain microvessels (263). In some, but not all, endothelial cell types, the polyol pathway can reduce glucose to sorbitol via aldose reductase (AR), which has an extremely low affinity for glucose ($K_m$ ~100 mM) but is activated by glucose itself or glucose-6-phosphate (reviewed in Refs.
Conversion of glucose to sorbitol by aldose reductase forms NADP⁺ and may compete with other NADPH-requiring reactions such as conversion of oxidized glutathione (GSSG) to reduced (GSH) glutathione (17, 306). Kashiwagi et al. (306) emphasized that glucose-induced activation of the polyol pathway in endothelial cells may not be directly responsible for the associated decrease in NADPH content, but rather that activation of the pentose phosphate pathway and NADP supply to the GSH redox cycle is impaired by H₂O₂ generated in cells exposed to high glucose. There is lack of consensus concerning the importance of the polyol pathway in glucose-mediated endothelial dysfunction, e.g., sorbitol fails to accumulate in canine retinal capillary endothelial cells exposed to 30 mM glucose (511) while advanced glycation end products increase AR mRNA and protein in human dermal microvascular cells (420).

Elevated glucose also increases the generation of superoxide anions known to react with NO to form peroxynitrite, which upon decomposition generates a strong oxidant with reactivity similar to hydroxyl radicals (47). Human endothelial cells exposed to hyperglycemia in established diabetes mellitus (see sect. vnC) are more sensitive to reactive oxygen species, since intracellular levels of glutathione, vitamin E, superoxide dismutase, catalase, and ascorbic acid are reduced significantly (reviewed in Refs. 173, 240, 644).

C. Smooth Muscle Cell Metabolism

Vascular smooth muscle cells have a high rate of glycolysis, relying to a large extent on glycolytically generated ATP to sustain a variety of cell functions. Vascular smooth muscle metabolism and the influence of contraction on the metabolic fate of glucose and fatty acids have been studied extensively (see Refs. 6, 34–37, 242, 243). The glycogen content of vascular smooth muscle ranges from 1 to 13.9 μmol/g, and the rapid depletion of glycogen reserves in the absence of additional substrates raises doubts whether glycogen is an important oxidative substrate for vascular smooth muscle. Allen and Hardin (6), using pig carotid arteries, concluded that glycogen contributed minimally (~10%) to substrate oxidation in vascular smooth muscle whilst oxidation of glucose comprised ~40–50% of the total substrates entering the tricarboxylic acid cycle. Other than glucose, vascular smooth muscle cells utilize several different substrates including short- or medium-chain fatty acids such as acetate and octanoate. Supply of mitochondrial substrates is thought to inhibit phosphofructokinase via elevated citrate levels, resulting in an inhibition of carbohydrate metabolism. During sustained isometric contraction of arterial smooth muscle induced by KCl, oxidation of fatty acid substrates increases whilst glucose metabolism declines progressively (34). Acetate, unlike octanoate, is not a major substrate in resting arterial muscle, yet KCl-induced contractions increase oxidation of both acetate and octanoate. Interestingly, norepinephrine-induced contraction is associated with a decrease in glucose uptake by vascular smooth muscle cells (34).

Adenosine modulates oxidative metabolism in cardiac and vascular smooth muscle by increasing O₂ consumption and the concentration of high-energy phosphate and adenine nucleotides (31). In contrast to the stimulatory effects of adenosine on glucose uptake in cardiac muscle, adenosine has no effect on glucose uptake or oxidation of glucose and octanoate in porcine carotid artery smooth muscle (35). Vascular smooth muscle isolated from porcine cerebral microvessels can simultaneously utilize fructose (a glycolytic intermediate) for gluconeogenesis and glucose for glycolysis, suggesting that exogenous fructose does not mix with fructose derived from glucose metabolism (356). These authors hypothesized that, because intermediates of glycolysis and gluconeogenesis appear not to mix freely within the cytoplasm of cerebral vascular smooth muscle cells, specific membrane microdomains containing glucose and dicarboxylate transporters may account for metabolite channeling, where “intermediates are transferred from one enzyme to another without complete equilibration with the surrounding medium” (448). Thus localization of glucose transporters and glycolytic enzymes to plasma-membrane caveolae could allow direct entry of exogenous glucose to the glycolytic pathway (see Fig. 7 in Ref. 356).

Recent studies in rat aortic smooth muscle cells have established that metabolism of L-arginine is modulated by physiologically relevant cyclic stretch (179). Exposure of aortic smooth muscle cells to cyclic stretch (~10% at 1 Hz for 72 h) resulted in a stimulation of L-arginine transport and metabolism via the induction of the CAT-2 transporter and arginase I. Cyclic stretch increased L-arginine metabolism to L-proline by concomitantly inhibiting ornithine decarboxylase (ODC) activity and polyamine biosynthesis. The implication of these studies is that hemodynamic stretch stimulates collagen synthesis in vascular smooth muscle cells by regulating transport and metabolism of cationic amino acids, leading potentially to a stabilization of vascular lesions in disease.

III. GENERAL CHARACTERISTICS OF MAMMALIAN AMINO ACID AND GLUCOSE TRANSPORT

A. Amino Acid Transport Systems

As reviewed by Christensen (123), multiple transport systems mediate the influx of cationic, neutral, sulfonic, and anionic amino acids across the plasma membrane of
mammalian cells. Molecular cloning approaches have led to the identification of Na\(^+\)-dependent and Na\(^+\)-independent amino acid transporters, and Table 1 summarizes that nomenclature (see citations) and the classical nomenclature used to designate these different amino acid transport systems (reviewed in Refs. 123, 662). The ionic dependency and \(K_m\) corresponding to the different transport systems were compiled from the cited publications and do not necessarily reflect transport properties of all cell types.

The different carrier proteins mediating transport of cationic amino acids include the Na\(^+\)-independent systems \(y^+\), \(y^{-}\), \(b^+\), \(b^{-}\), and Na\(^+\)-dependent systems \(b^0^+\), \(b^0^-\). System \(b^+\), originally described in mouse blastocysts, is highly specific for cationic amino acids (610), whereas the other systems can also transport neutral amino acids. System \(y^+\) is the principal cationic amino acid transport system expressed in NO producing cells (41, 67, 130, 131, 177, 429, 486) and thus most likely plays a key role in regulating L-arginine supply for NOS. Although there is limited information on ASCT, PROT, GLYT, TAUT, and EAAT associated amino acid transport in vascular cells (see below and Table 1), recent evidence indicates that bovine aortic endothelial cells express a taurine transporter sharing a high degree of sequence homology with that of the mTAUT cDNA isolated from brain (see Ref. 477).

1. System \(y^+\)

Cationic amino acid transport activity was initially assigned to the classical Na\(^+\)-independent amino acid transport system \(y^+\) (639–641). Cationic amino acid transporter (CAT) proteins were among the first amino acid carriers identified in mammalian cells and are classified as members of the solute carrier family 7 (SLC7). In the early 1990s, expression of the ecotropic MuLV receptor in Xenopus oocytes demonstrated that this receptor mediated Na\(^+\)-independent transport of cationic amino acids (318, 632). The receptor was renamed mCAT-1 for mouse cationic amino acid transporter and is an integral membrane protein with 14 putative transmembrane domains and intracellular NH\(_2\) and COOH termini. Transport of L-arginine, L-lysine, and L-ornithine via CAT-1 (system \(y^+\)) is pH independent, sensitive to trans-stimulation, and saturable at circulating plasma concentrations (~0.1–0.2 mM). Because CAT-1 is sensitive to changes in membrane potential (97, 307, 547), hyperpolarization induced by vasoactive agonists increases the driving force for cationic amino acid transport in endothelial and other cell types (see sects. \(vB\) and \(vA\)). The voltage dependence of other human CAT transporter isoforms has recently been investigated in Xenopus oocytes and confirmed that L-arginine-induced currents were usually larger in CAT-2A compared with CAT-2B (splice variants of CAT-2, see below) expressing oocytes (425). With the exception of the liver, system \(y^+\) transport activity is expressed ubiquitously, and the majority of studies in endothelial and smooth muscle cells have established that transport of L-arginine and cationic L-arginine analogs is mediated predominantly by a Na\(^+\)-independent system with characteristics resembling system \(y^+\) (see sects. IV and VI–IX).

Four additional related cationic transport proteins, designated CAT-2A, CAT-2B, CAT-3, and CAT-4, have now been identified in different mammalian species (see Refs. 128–131, 162, 307, 370, 450, 555). CAT-1, -2A, and -2B are glycosylated, suggesting that these carriers are located in the plasma membrane, with CAT-2A and -2B splice variants differing only in a stretch of 42 amino acids (128, 129, 371). CAT-2A is predominantly expressed in liver, whereas CAT-2B is usually induced under inflammatory conditions in a variety of cells including T cells, macrophages, lung, and testis (see Table 6 and Refs. 46, 178, 429, 486, 524). CAT-2A is a low-affinity carrier for cationic amino acids and, unlike CAT-1, is relatively insensitive to trans-stimulation. CAT-3 isolated from mouse and rat brain mediates Na\(^+\)-independent transport of cationic amino acids, although it is worth noting that 1) the substrate specificity differs from that for other CAT isoforms and 2) the \(K_m\) for CAT-3-mediated transport in oocytes is ~100-fold lower than that for CAT-1 (270, 291). In mouse and rat, CAT-3-mediated L-arginine transport is inhibited by other cationic amino acids, as well as L-citrulline, L-methionine, L-cysteine, L-aspartate, and L-glutamate, but not homoserine, and interestingly the recognition of neutral amino acids by CAT-3 is Na\(^+\) independent (270, 291). Closs and colleagues (612) recently succeeded in cloning a cDNA encoding human CAT-3, which was found to be glycosylated and targeted principally to the plasma membrane in human cells and oocytes. Unlike mouse and rat brain CAT-3 (270, 291), human CAT-3 is not neuron specific, exhibits a high selectivity for cationic amino acids, and does not transport L-citrulline, L-methionine, L-cysteine, or L-glutamate (612). The discrepancies in the specificity of the CAT-3 transporters remain to be resolved. Although Sperandeo et al. (555) identified a cDNA (designated CAT-4) in human placenta with 41–42% sequence identity to members of the CAT family, recent evidence indicates a lack of cationic amino acid transport activity in Xenopus oocytes or glioblastoma cells (U373 MG cell line) overexpressing CAT-4 (645). Whether CAT-3 and CAT-4 play a functional role in endothelial and/or smooth muscle cell cationic amino acid transport remains to be investigated.

Heterodimeric amino acid transporters, a subfamily of SLC7, are comprised of two subunits, a heavy chain (rBAT or 4F2hc) and an associated light chain linked by a disulfide bridge (126, 163, 387, 614, 625). The heavy subunit may be necessary for trafficking of the complex to the membrane, whereas the light chain may catalyze
transport (186, 419, 467). To date, seven different light chain cDNAs have been identified, namely, LAT1, LAT2 (encoding system L), y\(^+\)LAT-1, and y\(^+\)LAT-2 (system y\(^+\)) L, xCT (system x\(_c\)'), Asc-1 (system asc), and b\(^+\)AT (system b\(^+\))\(^+\), with all but the latter associating with 4F2hc (reviewed in Refs. 163, 450, 614, 625).

2. System y\(^+\)L

In 1992 Devés et al. (164) were the first to describe system y\(^+\)L activity in erythrocytes. System y\(^+\)L-like activity has subsequently been described in intestine, placenta, lymphocytes, and platelets, and it is worth noting that this carrier exhibits a much higher affinity for cationic amino acids (\(K_m\) for lysine \(\sim 10 \mu M\)) than any other cationic amino acid transport system (reviewed in Ref. 163). System y\(^+\)L is stereoselective, electroneutral, and selective to trans-stimulation and mediates high-affinity, Na\(^+\)-independent cationic amino acid transport, whereas the affinity of this carrier for neutral amino acids decreases significantly following substitution of Na\(^+\) by K\(^+\) (164; reviewed in Ref. 163). The affinity of system y\(^+\)L for neutral amino acids differs, with L-leucine, L-methionine, L-isoleucine, and L-glutamine exhibiting higher affinities than L-alanine, L-serine, or L-cysteine. Moreover, in human erythrocytes, N-ethylmaleimide (200 \(\mu M\)) appears to be a relatively selective inhibitor of y\(^+\) activity, permitting the resolution of cationic amino acid fluxes via the low-capacity system y\(^+\)L and higher capacity system y\(^+\)L (164, 401).

Expression of the ubiquitous transmembrane protein 4F2hc (also named CD98) in Xenopus oocytes induces amino acid transport activity resembling system y\(^+\)L (50, 52, 186, 636). These studies further demonstrated that association of 4F2hc with a membrane oocyte protein was required for the expression of system y\(^+\)L transport activity, providing evidence for a heterodimeric structure of an amino acid carrier (reviewed in Refs. 120, 163, 625). More recent studies have established that 4F2hc associates with y\(^+\)LAT-1 and y\(^+\)LAT-2 (\(\sim 56\) kDa) to induce system y\(^+\)L transport activity (see Table 1). The apparent affinity of L-arginine in the presence of Na\(^+\) for y\(^+\)LAT-1 is approximately twofold lower than that reported by Devés and colleagues for human erythrocytes, suggesting that y\(^+\)LAT-2 rather than y\(^+\)LAT-1 is related to the red cell system y\(^+\)L (467). Both y\(^+\)LAT-1 and y\(^+\)LAT-2 mediate transport of dibasic amino acids in the absence of Na\(^+\) and neutral amino acids in the presence of Na\(^+\) (see Ref. 625). These transporters are likely to be involved in interorgan and intracellular transfer of amino acids. It has been suggested that y\(^+\)LAT-2 is a glutamine (leucine)/arginine exchanger, which could play a role in the Na\(^+\)-dependent uptake of L-leucine and L-glutamine into neurons, as well as in the supply of L-arginine for certain brain cells (86). As discussed in section \(nB2\), y\(^+\)LAT-1 and y\(^+\)LAT-2 are expressed in human umbilical vein endothelium (503).

3. System L

The classical Na\(^+\)-independent transport system L is most reactive with branched chain and aromatic neutral amino acids and is often characterized using the selective nonmetabolizable analog 2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (BCH; see Refs. 123, 531). Transport via system L is trans-stimulated by intracellular substrates of this carrier and in some cases may be increased by lowered extracellular pH. Studies in rat glioma cells, primary astroglial cells, and lymphocytes suggested that 4F2hc serves as a necessary component for expression of system L-like transport activity (87). This same group reported that system y\(^+\)L was not involved in L-isoleucine or L-arginine transport in rat glioma cells and that overexpression of 4F2hc in Chinese hamster ovary cells was associated with increased Na\(^+\)-independent transport of L-isoleucine (88). More recently, cDNAs encoding system L-like transporters have been isolated (see Table 1). Co-expression of 4F2hc and LAT-1 or LAT-2 in oocytes induces Na\(^+\)-independent transport with a broad specificity for small and large zwitterionic amino acids and sensitivity to trans-stimulation (305, 469, 518). Interestingly, the expression of a tumor variant of LAT-1, tumor-associated gene-1/L amino acid transporter-1 (TA1/LAT-1) is dramatically upregulated in hepatocytes deprived of L-arginine, suggesting that it could act as a sensor of amino acid deprivation (100).

4. System x\(_c\)

A Na\(^+\)-independent anionic amino acid transport system designated x\(_c\)\(_{-}\) has been described in a variety of cell types, including vascular endothelial and smooth muscle cells (24–26, 39, 158–160, 539, 576, see Table 1). System x\(_c\)\(_{-}\) is insensitive to inhibition by dibasic and neutral amino acids and mediates exchange of L-cysteine for intracellular L-glutamate with a 1:1 stoichiometry. Cysteine autoxidizes to L-cystine in the extracellular fluid, and within cells L-cystine is rapidly reduced to L-cysteine, the rate-limiting precursor for GSH synthesis (see Refs. 24, 26). Transport of L-cysteine via system x\(_c\)\(_{-}\) is cis-inhibited by L-glutamate and homocysteate and markedly induced in cells exposed to oxidative stress and/or electrophilic compounds (26). Bannai and colleagues (509) recently isolated a cDNA encoding the system x\(_c\)\(_{-}\) transporter in activated murine macrophages. Expression of system x\(_c\)\(_{-}\) activity in Xenopus oocytes required two cDNA transcripts, with one identical to 4P2hc and the other a novel protein of 502 amino acids with 12 putative transmembrane domains. The designated protein xCT is a new member of a family of amino acid transporters known to form a
heteromultimeric complex with 4F2hc (reviewed in Refs. 120, 162, 163, 450, 625), and human cDNAs for system $x_c^-$ have now been isolated (39, 78, 319, 510, 529). mRNA levels and system $x_c^-$ activity are increased in macrophages exposed to LPS, electrophilic agents such as diethylmaleate (DEM), and increased oxygen, and neither AP-1 nor NF-$\kappa$B appears to be involved in the transcription of $xCT$ mRNA by LPS (508). It is known that the transcription factor Nrf2 binds to the antioxidant/electrophile response element (ARE/EpRE) in the 5'-flanking region of stress response genes, and interestingly, system $x_c^-$ activity is not induced in Nrf2-deficient macrophages (288). Recent studies have confirmed that gene expression and activity of the $xCT$ transporter is enhanced after treatment of mouse brain endothelial cells (272) and rat retinal capillary endothelial cells (595) with DEM. As in umbilical vein endothelial (407) and arterial smooth muscle cells (593), induction of L-cystine transport activity was paralleled by a concomitant increase in intracellular glutathione levels.

In several cell types, L-aspartate and L-glutamate are accumulated by a high-affinity Na$^+$- and K$^+$-dependent system $X_{AG}$ (213), with one report in human umbilical vein endothelial cells also describing a Na$^+$-independent anionic transporter (designated $xAG$) markedly inhibited by L-aspartate and L-glutamate (454).

5. System asc

This Na$^+$-independent, high-affinity transport system mediates entry of small neutral amino acids such as L-alanine, L-serine, L-cysteine, glycine, L-threonine, and 2-aminoisobutyric acid (AIB), with transport activity not inhibited by the system A analog MeAIB (see Refs. 208, 603). Recent molecular strategies have successfully isolated cDNAs from mouse and human brain encoding asc-type amino acid transport (208, 423). The encoded proteins were designated Asc-1 and hAsc-1 and are structurally related to the family of amino acid transporters linked via disulfide bonds to the type II membrane glycoproteins such as 4F2hc and rBAT. Functional expression of these Asc-1 transporters required 4F2hc, was not dependent on Na$^+$ or Cl$^-$, and mediated uptake of D-serine required activation of the glutamate N-methyl-D-aspartate receptor (see Refs. 208, 423, 625). The characteristics of another asc-type amino acid transporter (Asc-2) have recently been described (110). Coexpression of Asc-2 with either 4F2hc or rBAT in oocytes or COS-7 cells did not induce transport activity, whereas a fusion protein of the COOH terminus of Asc-2 with the NH$_2$ terminus of either 4F2hc or rBAT mediated L-serine uptake. This same group recently identified another transporter AGT1, structurally related to Asc-2, which as a fusion protein with either rBAT or 4F2hc exhibits a high affinity for Na$^+$-independent transport of L-aspartate and L-glutamate (388). These authors speculated that Asc-2 and AGT1 represent a new subgroup of the heterodimeric amino acid transporter family, whose members associate not with rBAT or 4F2hc but with as yet unknown heavy chains.

6. System b$^{0+}$

Detection of system b$^{0+}$ in the intestine and renal tubules has generated considerable interest, since a defect in system b$^{0+}$ in the human kidney causes inherited hyperaminoaciduria cystinuria (reviewed in Refs. 120, 162, 450, 625). Unlike system b$^{0+}$ in blastocysts (610), human b$^{0+}$ transports L-cystine as well as neutral and dibasic amino acids. The affinity of diabasic and L-cystine is severalfold higher than for neutral amino acids, with a negative membrane potential stimulating inward flux of diabasic amino acids in exchange for neutral amino acids (50). The rapid reduction of cystine to cysteine intracellularly provides the chemical gradient for L-cystine transport. Expression cloning identified rBAT (also referred to as NBAT or D2) as a potential subunit of system b$^{0+}$ (50, 52, 636), which has a predicted molecular mass of $\sim 54$ kDa and has also been detected in heart, liver, placenta, and lung (see Ref. 625). There is limited evidence that overexpression of 4F2hc leads to an interaction with the b$^{0+}$AT light chain in mammalian cells and oocytes (85, 483), although Wagner et al. (625) have questioned the physiological relevance of these findings since 4F2hc is localized predominantly on basolateral membranes whereas b$^{0+}$AT is targeted to apical membranes. Limited system b$^{0+}$ transport activity has been described in vascular endothelial cells (see Table 3).

7. System B$^{0+}$

System B$^{0+}$ represents a Na$^+$-dependent transport system identified for cationic and neutral amino acids (609). Although the substrate specificity of systems b$^{0+}$ and B$^{0+}$ is similar, the latter also accepts L-alanine, L-serine, and 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (reviewed in Refs. 162, 450). A cDNA encoding a Na$^+$/Cl$^-$-dependent carrier (designated ATB$^{0+}$) has been shown to transport cationic and neutral amino acids (540). This transporter has a sequence homology with neurotransmitter transporters and exhibits a broad specificity for neutral amino acids and a high affinity for cationic and neutral amino acids. Because cationic amino acid transport in endothelial and smooth muscle cells is predominantly Na$^+$ independent, the ATB$^{0+}$ transporter may play a negligible role in mediating $l$-arginine transport for NO synthase.

8. System A

The classical Na$^+$-dependent system A is expressed ubiquitously and is a key target for hormonal regulation,
with transport activity also upregulated in response to amino acid deprivation or hypertonic stress (reviewed in Refs. 123, 236, 531, see sects. vii and ix). Neutral amino acids with short, polar, linear, and N-methylated side chains (e.g., MeAIB) are most reactive with system A, providing a useful tool for discriminating transport from other systems. System A transport activity is reduced markedly at lowered extracellular pH and subject to trans-inhibition by intracellular substrates for this carrier. The molecular identification of the system A was elusive during the 1990s, and cDNAs encoding system A have only been isolated within the past 2 years (3, 249, 284, 351, 570, 571, 658, see Table 1). Albers et al. (3) have briefly reviewed the nomenclature for the three reported variants of system A (ATA1, ATA2, ATA3), clarifying that ATA isoforms have also been designated as SAT1/2/3 or SA1/2/3 (see Table 1). Notably, a neuronal glutamine transporter (GlnT) was one of the first members of the system A family of transporters to be cloned (608), and this same group subsequently renamed the transporter SAT1 (658). ATA1 and ATA2 have similar functional characteristics, with transport in oocytes saturable, voltage and Na\(^{+}\) dependent, pH sensitive, and inhibitable by MeAIB. Tissue expression of ATA isoforms varies, with ATA1 expressed principally in placenta and brain (631), ATA2 expressed ubiquitously in mammalian tissues including endothelial cells (4, 571, 658), and ATA3 virtually restricted to the liver (249, 571). Although expression of human ATA3 in retinal pigment epithelial cells evokes Na\(^{+}\)-coupled neutral amino acid transport (lower affinity for MeAIB than ATA1 or ATA2), ATA3 exhibits a greater affinity for cationic compared with neutral amino acids, which the authors suggested may provide an important mechanism for L-arginine transport in hepatocytes (249). In summary, the ubiquitous expression of ATA2 in mammalian tissues suggests that ATA2 may encode the classical, Na\(^{+}\)-dependent system A. In addition to adaptive increases in ATA2-mediated transport in response to amino acid deprivation and hypertonic stress (4, 351), steady-state levels of ATA2 are elevated by increased intracellular cAMP (250), a characteristic of system A in hepatocytes (reviewed in Ref. 531).

9. System ASC

System ASC is a ubiquitously expressed Na\(^{+}\)-dependent system that prefers small neutral amino acids such as L-alanine, L-serine, and L-cysteine. Transport exhibits a marked stereoselectivity and sensitivity to trans-stimulation and is unaffected by amino acid starvation (123, 316, 375). As extracellular pH is lowered, anionic amino acids such as L-glutamate may become more effective substrates and inhibitors of system ASC (602). A cDNA (ASCT1) encoding system ASC has been isolated from human hippocampal libraries, sharing ~40% sequence identity with the excitatory amino acid transport (EAAT) family of glutamate transporters (see Refs. 16, 524, 635). A second identified cDNA (ASCT2) encoding system ASC is believed to play an important role in facilitating the efflux of L-glutamine from glial cells (601). The ASCT transporters primarily mediate exchange of neutral amino acids rather than net uptake, and transport is associated with a substrate-dependent anion conductance (665).

10. System N

Classical studies in hepatocytes demonstrated that system N exhibits a preference for L-glutamine, L-aspartate, and L-histidine, an intolerance for N-methylated substrates, an insensitivity to regulation by glucagon and insulin, and a reduced rate of transport at lowered extracellular pH (see Refs. 123, 315, 531). Unlike systems A and ASC, amino acid transport via system N tolerates substitution of Li\(^{+}\) for Na\(^{+}\) as the cotransported ion. Subtypes of system N with different functional characteristics and tissue distribution patterns have been described. The subtype of system N in skeletal muscle was designated system Nm and exhibits a much weaker Li\(^{+}\) tolerance and pH sensitivity than the hepatic system N (2, 278). Two further subtypes of system N have been described in the brain, with characteristics of transport in astrocytes (418) similar to hepatocytes and the system in neurons (421) distinct from systems N and Nm. The neuronal system Nb exhibits a similar Li\(^{+}\) tolerance and pH sensitivity to system N but is inhibited by L-glutamate.

A system N subtype was recently isolated from a rat brain cDNA library and designated SN1, with expression highest in liver and much lower in kidney, heart, and brain (115). Heterologously expressed rat or human SN1 mediates Na\(^{+}\)-coupled transport of L-glutamine and other neutral amino acids, with efflux of H\(^{+}\) through the transporter resulting in intracellular alkalization. Transport mediated by rat and human SN1 appears to be electrogenic, with inward transport of two Na\(^{+}\) and an amino acid coupled to efflux of one H\(^{+}\) (193). SN1 mediated L-glutamine transport is modulated by changes in membrane potential, with depolarization resulting in a switch from uptake to efflux, as well as by transmembrane L-glutamate gradients. A new member of this gene family (designated SN2) was cloned from rat brain and a human liver cell line, with broader expression including lung and stomach (421, 422). Gu et al. (234) subsequently identified another member of system N (SN3) with sequence homology to both system A and N transporters and expression in liver, muscle, kidney, and pancreas. For an overview of the molecular advances in our understanding of L-glutamate transport, we refer readers to a review by Bode (65).
B. Glucose Transport Systems

Classical mammalian facilitative glucose transporters (GLUTs) belong to a supergene family, and four of them were initially identified as glucose transporters (GLUT1, GLUT2, GLUT3, GLUT4) and one as a fructose transporter (GLUT5). These transporters differ in their kinetic properties, sugar specificity, tissue localization, and regulation (see Refs. 22, 294, 415, 594). Three further glucose transporters have been cloned, namely, GLUT6 (352), previously referred to as GLUT9 by Doege et al. (169) and Phay et al. (468), GLUT8 (103, 171), and GLUT11 (detected exclusively in human heart and skeletal muscle and with sequence similarity to GLUT5, see Ref. 170). The distribution of GLUT transporters in mammalian cells is widespread, including endothelial cells from peripheral blood vessels and the blood-brain barrier. GLUT1, GLUT3, and GLUT4 have a higher affinity for glucose, with $K_m$ values around ~2 mM, whereas GLUT2 has a lower affinity for glucose ($K_m$ ~20 mM).

Glucose transport has been a major interest in blood-brain barrier research, and the high capacity of glucose transport into brain microvessels occurs primarily via GLUT1. The human erythrocyte glucose transporter GLUT1 can carry glucose, galactose, and mannose and is expressed in fetal tissues (239, 459). GLUT1 is also widely expressed in adult tissues but is most abundant in fibroblasts, erythrocytes, and brain endothelial cells compared with muscle, liver, and adipose tissue, suggesting that GLUT1 transport activity may be associated with developmental adaptation. In this context, children with glucose transporter protein syndrome (GLUT1 deficiency) exhibit impaired glucose transport across the blood-brain barrier, associated with infantile seizures and developmental delay (323). GLUT1 transporters in adipocytes have been shown to translocate to the plasma membrane in response to insulin (99), suggesting a regulatory mechanism and/or intracellular signaling pathway similar to GLUT4 in insulin-sensitive tissues. Interestingly, GLUT1 appears to be absent in human iris and corneal capillaries in diabetes mellitus (334).

The low-affinity glucose transporter GLUT2 has been identified in cells near the abluminal surface of liver cells, small intestine, and kidney and may facilitate glucose uptake or efflux from tissues depending on their nutritional status (reviewed in Ref. 294). To our knowledge, there are no reports of GLUT2 expression in endothelial or smooth muscle cells. Neuronal tissues have a high expression of GLUT3, which may be the main isoform involved in moving glucose into nerves and brain (417). GLUT3 seems to be more abundant in human brain tumor cells (433), and thus, as for GLUT1, its expression may be modulated by disease. In situ hybridization has detected GLUT3 mRNA and protein in the endothelium of human intraplacental microvessels, where it was proposed to play a potential role with GLUT1 in sustaining glucose supply to the developing fetus (254). However, a physiological role for GLUT3 in the placental vasculature remains questionable, since an earlier study failed to detect GLUT3 in the human syncytiotrophoblast (38).

The insulin-sensitive glucose transporter isoform GLUT4 is expressed mainly in adult skeletal muscle, cardiac muscle, and adipose tissue (reviewed in Refs. 294, 594) and has only been detected in very low abundance in the rat forebrain microvasculature using high-stringency hybridization of poly(A)$^+$ RNA (394). To our knowledge, there is no functional evidence for GLUT4 activity in the cerebral or retinal vasculature. Regulation of GLUT4 by insulin has been studied extensively in adipocytes, where it is situated in perinuclear membranes and is translocated to the plasma membrane following stimulation with insulin (see Refs. 415, 438). Gene regulation of GLUT4 expression is now recognized as an essential process in the modulation of glucose transport, particularly in diabetes and hypoxia (438).

GLUT5 has been isolated from cDNA libraries for human, rat, and rabbit intestinal epithelial cells (see Ref. 294), with mRNA detected in human kidney, small intestine, skeletal muscle, adipocytes, and microglial cells. GLUT5 is constitutively expressed in the plasma membrane of muscle and adipocytes, and its distribution is not affected by insulin treatment. Although GLUT5 has been detected in the brain microvasculature (382, 383, 584), there is no convincing evidence implicating a transport role for GLUT5 in the blood-brain or blood-retinal barriers.

GLUT6 and GLUT8 exhibit glucose transport activity and form a separate branch of the GLUT family, with marked differences from GLUT1–5. GLUT6 mRNA has been detected in brain, spleen, and leukocytes (169) and more recently in adipose tissue (352). Initial reports from Doege et al. (169) show that GLUT6 can be recognized as a sugar anion transporter with a high $K_m$, based on cytchalasin binding studies. Expression of GLUT6 and GLUT8 has been achieved in transiently transfected primary rat adipose cells, and translocation is not responsive to phorbol ester, hyperosmolality, or insulin (352), even though this isoform exhibits similarities to the insulin-responsive GLUT4. The putative GLUT6 and GLUT8 proteins have 44 and 31% sequence identity to GLUT5 and GLUT3, respectively, which could be one of the reasons why GLUT6 or GLUT8 expressed in adipocytes do not respond to translocation induced by insulin (352). GLUT11 is only expressed in human heart and skeletal muscle, with overexpression of GLUT11 cDNA in COS-7 cells resulting in a two- to threefold stimulation of glucose transport, inhibitable by an excess of fructose (170). The sequence similarity of GLUT11 and GLUT5 and sensitivity
of GLUT11 transport activity to inhibition by fructose suggests that GLUT11 may be a fructose transporter. There is no evidence that GLUT6, GLUT8, or GLUT11 is expressed or has a functional role in vascular endothelial and smooth muscle cells.

IV. AMINO ACID TRANSPORTERS IN ENDOTHELIAL CELLS

A. Blood-Brain and Blood-Retinal Barriers

Endothelial cells lining the blood-brain barrier are joined by zonulae occludens and limit the intercellular diffusion of circulating solutes such as monoamines. Free intracellular amino acid levels in the brain are related to their rates of influx across the blood-brain barrier, and synthesis of neurotransmitters such as serotonin, dopamine, and histamine are substrate limited (see Refs. 455, 544). Cerebrovascular endothelial cells express specific carriers that mediate the entry and efflux of amino acids across the luminal and abluminal membranes of the blood-brain barrier. Two Na⁺-independent amino acid transport systems have been identified in brain capillary endothelium: a transport system for large neutral (system L or a high-affinity isofrom L1) and cationic (system \(\mathbf{y}^{\mathbf{+}}\)) amino acids. Na⁺-dependent amino acid transport systems A, B\(\mathbf{v}^{\mathbf{+}}\), ASC, \(X_{\mathbf{AG}}\) and one for \(\beta\)-amino acids have also been characterized in the cerebral vasculature (see Table 2). The general consensus is that high-affinity Na⁺-dependent transporters are located principally in the abluminal membrane of the cerebral endothelium (57, 281, 328, 329, 340, 506, 587). Transport systems with similar characteristics for neutral and cationic amino acids have been identified in the blood-retinal barrier (596), and molecular and functional studies of amino acid transport in endothelial cells and pericytes derived from the retinal vasculature are limited (e.g., see Ref. 595).

1. Neutral amino acids

Direct measurements of radiolabeled amino acid uptake in anesthetized rats in vivo have been obtained using the brain uptake index (BUI) technique (441), which involves injecting a bolus of a defined saline solution containing a radiolabeled amino acid and highly diffusible internal standard (\(\text{[H]water}\)) into the left common carotid artery followed 6–15 s later by decapitation and scintillation counting of discrete brain regions. Transport of large neutral (LNAA) and small neutral amino acids was inhibited by plasma amino acids, with uptakes for L-cysteine, L-serine, L-alanine, L-proline, glycine, L-glutamate, and L-aspartate extremely low (2–8%), perhaps due to the low specific tracer activity or the limited expression of relevant transporters at the luminal surface of the brain endothelium (441, 520). Rapid indicator dilution techniques applied in the dog brain in vivo also revealed significant uptakes (17–47% relative to \(\text{\text{Na}}\)) for radiolabeled LNAA, whereas influx of small neutral, cationic, and acidic amino acids was negligible (600, 601, see Fig. 4, top panel), findings confirmed recently in the human brain in vivo (327). As in the rat brain (441), transport of LNAA in dog and human brain preparations was inhibited effectively by unlabeled competitor amino acids (327, 532, 661). The negligible uptake for L-alanine, L-serine, glycine, and L-proline led several investigators in the 1970s to conclude that the Na⁺-dependent system A was not expressed in the luminal membrane of the blood-brain barrier endothelium (57, 346, 520, 624, 661). However, under steady-state conditions LNAA and many other amino acids are accumulated by the rat brain in vivo (27). Kinetics of L-proline, L-alanine, and AIB transport have been measured in anesthetized rats using the in situ brain perfusion technique (49, 184). Transport of AIB into the frontal cortex of the perfused rat brain was Na⁺ independent and inhibited significantly by 2 mM L-phenylalanine and a mixture of 14 amino acids but unaffected by 2 mM MeAIB, confirming that AIB is a substrate for system L in the luminal membrane of the blood-brain barrier (184). However, with system L saturated by LNAA, AIB uptake from blood to brain was apparently mediated by an unsaturatable, as yet undefined, pathway. When Benrabh and Le-fauconnier (49) analyzed distribution volumes for radiolabeled alanine and AIB in the in situ-perfused rat brain, they noted that tracer uptake was followed by rapid efflux across luminal and abluminal membranes of the endothelium. Tryptophan is the only LNAA that binds reversibly to plasma proteins, and under physiological conditions, 75–90% of plasma L-tryptophan is bound to albumin. As discussed by Pardridge and Fierer (456), the tryptophan-albumin complex can dissociate and reassociate several times during passage through the brain microvasculature. Although there is some controversy whether albumin-bound tryptophan is transported across the blood-brain barrier, Smith et al. (542) argued that bound tryptophan contributes minimally to brain tryptophan influx. Earlier studies in human erythrocytes described a novel T system limited apparently to aromatic amino acids such as L- and D-tryptophan, L-tyrosine, and L-phenylalanine (495, 603), but to our knowledge there are no subsequent reports of T system-like transport activity in the blood-brain or blood-retinal barriers.

Isolated microvessels, membrane vesicles, and endothelial cells cultured from the blood-brain and blood-retinal barriers have advanced our understanding of the polarity of amino acid transport processes in these vascular beds. Elegant studies by Betz and colleagues (53, 57) provided the first direct evidence that capillaries isolated from the cerebral cortex of rats and bovine retina only concentrated MeAIB in the presence of a Na⁺ gradient. In
<table>
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<th>$V_{max}$, pmol · μg protein$^{-1}$ · min$^{-1}$</th>
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<td>L Rat</td>
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<td>L Rat</td>
<td>60+</td>
<td>0.214</td>
<td>No</td>
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<td>L Rat</td>
<td>26†</td>
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<td>L-Tyrosine</td>
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<td>L Porcine</td>
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<td>L Rat</td>
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<td>L Rat</td>
<td>54†</td>
<td>0.012</td>
<td>No</td>
<td>In vivo brain uptake index, conscious</td>
<td>404</td>
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</tr>
<tr>
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<td>L Rat</td>
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<td>L-Glutamine</td>
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<td>L-Histidine</td>
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<td>L Rat</td>
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<td>L Rat</td>
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<td>190</td>
<td>0.016*</td>
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<tr>
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<td>L Rat</td>
<td>48</td>
<td>0.016*</td>
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<td>L Rat</td>
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<td>0.016*</td>
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<td>542</td>
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<td>L Dog</td>
<td>160</td>
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<td>624</td>
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<td>L Rat</td>
<td>290</td>
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<td>In situ brain perfusion</td>
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<td></td>
<td>L Rat</td>
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<td>No</td>
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<td>624</td>
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<td>9</td>
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<td>Capillary endothelial cells (4F2hc/LAT1)</td>
<td>312</td>
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<tr>
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<td>L Mouse</td>
<td>88</td>
<td>3.6</td>
<td>No</td>
<td>Capillary endothelial cell line MBEC4 (4F2hc/LAT1)</td>
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<td>L Rat</td>
<td>54</td>
<td>3.6</td>
<td>No</td>
<td>Capillary endothelial cell line MBEC4 (4F2hc/LAT1)</td>
<td>12</td>
<td></td>
</tr>
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</table>

*Significant differences compared to controls.
†Anesthetized.
‡Conscious.

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isolated brain capillaries, L-proline selectively inhibited Na\(^+\)-dependent MeAIB uptake (10 min) by 50–60% but had no effect on L-leucine uptake (1 min) measured in the absence of Na\(^+\). The inhibition of MeAIB and L-leucine by LNAA and L-alanine demonstrated a degree of overlap between systems A and L Betz and Goldstein (57) hypothesized that the Na\(^+\)-dependent system A was expressed in the abluminal membrane of the brain endothelium, normally in contact with brain interstitial fluid. Subsequent studies in rat isolated brain capillaries revealed that L-proline uptake (3 min) was mediated by two saturable systems (see Table 2), with the high-affinity pathway (K\(_m\) ~110 \(\mu\)M) most likely reflecting system A transport activity. Hwang et al. (281) also reported inhibition of L-proline uptake by L-leucine and BCH in rat brain capillaries, suggesting that some system L transport activity was expressed in the abluminal membrane of the endothelium. Selective expression of Na\(^+\)-dependent amino acid transporters in the abluminal membrane of the blood-brain barrier most likely explains the absence of system A in studies of amino acid transport across the luminal membrane of the blood-brain barrier in vivo.

Hjelle et al. (266) compared uptake of L-valine, L-leucine, and L-tyrosine in microvessels isolated from bovine blood-brain and blood-retinal barriers. Although the time course (0–30 min) of L-leucine uptake was similar in brain and retinal microvessels, K\(_m\) values were only reported for brain microvessels (see Table 2). In isolated brain microvessels from rats undergoing portacaval anastomosis, K\(_m\) values for saturable L-lysine, L-leucine, and L-phenylalanine transport range between 101 and 162 \(\mu\)M (104), confirming earlier findings in bovine brain microvessels. Brain microvessels isolated from rats with portacaval anastomosis transport significantly more L-leucine, L-phenylalanine, L-glutamine, L-methionine, cycloleucine, and L-tryptophan than microvessels from brains of control rats, suggesting a selective stimulation of system L kinetic analyses of L-glutamine uptake by bovine brain microvessels revealed that L-glutamine enters the brain endothelium via Na\(^+\)-dependent (system A) and Na\(^+\)-independent (system L) transporters (102). Preloading bovine brain microvessels with L-glutamine (20 mM for 20 min), in the presence of a normal Na\(^+\) gradient, accelerated influx of L-leucine, L-tyrosine, and L-tryptophan, whereas influx of MeAIB and L-lysine was unaffected. Collagenase digestion of bovine brain microvessels resulted in the loss of trans-stimulation of LNAA influx by L-glutamine, due to the inactivation of system A

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Transport System</th>
<th>Species</th>
<th>K(_m) (\mu)M</th>
<th>V(_{max}) pmol (\cdot \mu)g protein(^{-1} \cdot \text{min}^{-1})</th>
<th>Ionic Dependence</th>
<th>Preparations and Main Findings</th>
<th>Reference Nos.</th>
</tr>
</thead>
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<tr>
<td>Acivicin</td>
<td>L</td>
<td>Rat</td>
<td>667</td>
<td>No</td>
<td>In situ brain perfusion, inhibited by LNAA</td>
<td>118</td>
<td></td>
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<tr>
<td>Taurine</td>
<td>Bovine</td>
<td>12.1</td>
<td>0.14 Na(^+)/Cl(^-)</td>
<td>Na(^+)/Cl(^-)</td>
<td>Capillary endothelial cells, luminal</td>
<td>587</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bovine</td>
<td>13.6</td>
<td>0.09 Na(^+)/Cl(^-)</td>
<td>Na(^+)/Cl(^-)</td>
<td>Capillary endothelial cells, abluminal</td>
<td>587</td>
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<tr>
<td></td>
<td>Rat</td>
<td>28</td>
<td></td>
<td></td>
<td>Isolated capillaries</td>
<td>588</td>
<td></td>
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<tr>
<td></td>
<td>Rat</td>
<td>78</td>
<td></td>
<td></td>
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<td>48</td>
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<tr>
<td>(\beta)-Alanine</td>
<td>Bovine</td>
<td>25–47</td>
<td>0.13–0.23 Na(^+)/Cl(^-)</td>
<td>Na(^+)/Cl(^-)</td>
<td>Capillary endothelial cells, abluminal</td>
<td>328,329</td>
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</tr>
<tr>
<td>L-Glutamate</td>
<td>X(_{\text{Mi}})</td>
<td>Rat</td>
<td>1.9</td>
<td>Na(^+)</td>
<td>Isolated microvessels</td>
<td>279</td>
<td></td>
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<tr>
<td>L-Glutamate</td>
<td>x(_c)</td>
<td>Mouse</td>
<td>48</td>
<td>No</td>
<td>Indicator dilution, perfused choroid plexus</td>
<td>517</td>
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<tr>
<td>L-Cystine</td>
<td>x(_c)</td>
<td>Mouse</td>
<td>64</td>
<td>No</td>
<td>Brain endothelial cell line</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>x(_c)</td>
<td>Rat</td>
<td>3.6</td>
<td>Na(^+)</td>
<td>Isolated brain capillaries</td>
<td>282</td>
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<tr>
<td></td>
<td>x(_c)</td>
<td>Rat</td>
<td>9.2</td>
<td>0.075 Na(^+)</td>
<td>Rat retinal capillary endothelial cell line TR-iBRB (↑ xCT mRNA, ↔ 4F2hc by diethylmaleate)</td>
<td>505</td>
<td></td>
</tr>
</tbody>
</table>

Data have been collated from the cited references, and tabulated values for K\(_m\) and V\(_{max}\) and the ionic dependency are based on reported values. The assignment of amino acid uptake to a designated transport system is based on the reported characteristics of transport and/or our interpretation of the published data. LNAA, large neutral amino acids; system L1, designated nomenclature for high-affinity system L; MeAIB, methylaminoisobutyric acid; Sershen and Lajtha (520) argue that L-Phe can also enter blood-brain barrier endothelium via system ASC.
amino acid transport (105), highlighting the importance of controlling for the potential loss of transport activity in collagenase-digested cells. This same group reported that ammonia (0.25 mM) stimulates influx of LNAA, without altering uptake of MeAIB, L-lysine, or L-glutamate. Stimulation of LNAA influx was apparently mediated by trans-stimulation via system L following intracellular accumulation of L-glutamine (105).

Transport measurements in cultured cerebral endothelial cells have reported that glial conditioned medium enhances MeAIB transport (101), yet detection of system A activity in cultured cerebral endothelial cells may re-
fect the loss of blood-brain barrier polarity normally maintained in preparations in vivo, providing access to system A in the antiluminal surface (277). Experiments in cultured cerebral endothelial cells from bovine brain have reported very similar kinetic constants for LNAA, although the $K_m$ for L-alanine transport was considerably lower than for rat cerebral endothelial cells (see Table 2).

Further evidence for an asymmetry of amino acid transport across the blood-brain barrier was provided by transport studies in luminal and abluminal membrane vesicles prepared from the bovine blood-brain barrier. Phenylalanine uptake by luminal vesicles is mediated by a high-affinity ($K_m \sim 12 \mu M$), Na$^+$-independent transporter (designated system L1) sensitive to inhibition by L-leucine, L-tryptophan, and BCH but not MeAIB (505). In contrast, an inwardly directed Na$^+$ gradient in abluminal vesicles markedly increased the initial rate of MeAIB uptake and resulted in a small but significant increase in L-phenylalanine uptake that was abolished by 1 mM MeAIB. In this study, γ-glutamyl transpeptidase (GGT, EC 2.3.2.2) activity was used as a marker enzyme for the luminal plasmalemma and Na$^+$-K$^+$-ATPase as a marker for the abluminal membrane of the blood-brain barrier endothelium. This same group (506) subsequently reported that the high-affinity system L1 was distributed symmetrically between luminal and abluminal membranes of the bovine brain endothelium and further established that L-alanine was transported by two Na$^+$-dependent systems located exclusively in the abluminal membrane. In addition to system A ($K_m \sim 600 \mu M$), another Na$^+$-dependent transport system B$^{0,+}$, originally described in mouse blastocysts (610), was found to mediate transport of L-alanine in abluminal vesicles derived from rat and bovine microvessels (340, 506). Sánchez del Pino et al. (506) excluded system ASC as a major pathway for Na$^+$-dependent L-alanine uptake in abluminal vesicles and suggested that the involvement of system ASC in L-alanine transport reported by Tayarani et al. (559) may in fact have been due to system B$^{0,+}$ activity. System B$^{0,+}$ in bovine brain endothelial vesicles has an extremely low affinity ($K_m \sim 2.1 \mu M$), and as L-alanine concentrations in the brain extracellular space may not reach 2 mM, transport capacity via system B$^{0,+}$ may be limited.

Recent studies in abluminal vesicles from the rat blood-brain barrier have shown that oxoproline, an intracellular product of γ-glutamyl amino acids transported across the luminal membrane of brain endothelium, stimulates Na$^+$-dependent transport systems A and B$^{0,+}$, albeit activation of system B$^{0,+}$ (−20%) was significantly less than reported for system A (340). This study and the conclusions reached Sánchez del Pino et al. (506) suggest that system B$^{0,+}$ plays a minor role in Na$^+$-dependent amino acid transport across the blood-brain barrier. As illustrated in Figure 5, luminal and abluminal membranes of the brain endothelium express the Na$^+$-independent, high-affinity system L1, whereas Na$^+$-dependent systems A and B$^{0,+}$ are expressed exclusively at the abluminal membrane. Lee et al. (340) proposed that the γ-glutamyl cycle may regulate the availability and content of brain amino acids, with GGT catalyzing the transfer of the γ-glutamyl moiety from extracellular GSH (micromolar concentrations, see Ref. 1) to acceptor amino acids at the

![Figure 5](https://www.prv.org/content/prv/vol83/i1/a202/Figure05.png)
luminal membrane of the brain endothelium (8, 446). γ-Glutamyl amino acids formed at the outer surface of the luminal membrane of the brain endothelium are then transported into the brain endothelial cell, where oxoproline is thought to stimulate Na\(^+\)-dependent amino acid transporters. As oxoproline increases amino acid transport within minutes, it may act as an allosteric activator of the classical amino acid transport systems (J. Víña, personal communication). Activation of amino acid transport across the abluminal membrane of the blood-brain barrier by oxoproline was proposed to facilitate the exit of amino acids from brain to blood, thereby protecting the brain against elevated amino acid levels. However, Lee et al. (340) queried whether oxoproline concentrations in vivo are high enough to stimulate Na\(^+\)-dependent transport. To our knowledge, this remains the only study implicating oxoproline as a modulator of blood-brain barrier amino acid transport. L-Glutamine transport into abulminal-enriched membrane vesicles isolated from bovine brain endothelium is mediated by the Na\(^+\)-dependent systems A and N (~80% total transport at 100 \(\mu\)M), with facilitative carriers mediating efflux of nitrogen-rich amino acids from the brain (341).

A full-length cDNA encoding the bovine blood-brain barrier large neutral amino acid transporter, with 89–92% identity to the LAT1 isoform, has recently been reported (63, 175). Injection of bovine brain LAT1 and 4P2hc mRNA into oocytes yields a 10-fold increase in Na\(^+\)-independent l-tryptophan transport, which was inhibited specifically by other LNAA. The striking observation in this study was that the measured \(K_m\) (35 \(\mu\)M) for l-tryptophan transport was similar to values reported for the blood-brain barrier in vivo (see Table 2). Moreover, mRNA levels for bovine brain LAT1 were ~100-fold higher in isolated brain capillaries than a C6 rat glioma (63). LAT1 has recently also been identified in mouse and rat brain capillary endothelial cells, where it mediates high-affinity uptake of L-leucine and L-DOPA (see Refs. 296, 312, 389).

The consensus from studies in a variety of species and preparations is that many LNAA are transported into the brain endothelium via the Na\(^+\)-independent system L (and the high-affinity system L1). Finally, it is worth noting that \(K_m\) estimates for LNAA transport by human brain capillary endothelium are 10- to 100-fold lower than values reported for rat, dog, or bovine brain endothelial preparations (see Table 2).

2. Cationic amino acid transport

Studies of cationic amino acid uptake by the blood-brain barrier in vivo yielded conflicting results in the early 1970s, with Yudilevich and Rose (660) failing to identify a cationic transport system in the dog brain, while Oldendorf et al. (441) reported low BUI uptakes (16–22%) for L-arginine, L-lysine, and L-ornithine in the rat brain. In rat brain, L-arginine uptake was inhibited by histidine at low extracellular pH, suggesting that at low pH the protonated form of histidine reduced L-arginine entry without itself being transported (442). Subsequently, application of the in situ brain perfusion technique provided the first kinetic constants for L-arginine, L-lysine, and L-ornithine transport across the rat blood-brain barrier endothelium (567). Cationic amino acid influx was stereospecific, Na\(^+\) independent, and inhibitable by L-homoarginine, L-arginine, L-ornithine, and diaminobutyric acid but not BCH, MeAIB, or L-glutamate. The calculated \(K_m\) values compared favorably with those reported for transport of cationic amino acids by system y\(^+\) (CAT-1) expressed in Xenopus oocytes (318, 632), and interestingly CAT-1 mRNA was enriched 38-fold in brain capillaries compared with total brain tissue (567). Saturable L-arginine transport has also been measured in neuronal and astroglial cells, the choroidal epithelium, and the blood-choroid plexus barrier in vivo (475, 512, 566, 568, 638).

3. Anionic amino acid transport

Blood-brain barrier transport of the acidic amino acids L-aspartate and L-glutamate across the luminal membrane is limited (441, 661), and only one study has described a high-affinity Na\(^+\)-dependent uptake for L-glutamate in rat brain microvessels (279). Net efflux of L-glutamate from the brain is up to 20-fold higher than influx, suggesting that the low-capacity acidic transport system in the blood-brain barrier may be involved in mediating the efflux of excitatory neurotransmitters from the brain (reviewed in Ref. 455). Measurements of unidirectional uptake of L-aspartate and L-glutamate at the blood side of the isolated perfused choroid plexus have established that transport was saturable, with net uptakes reduced due to rapid efflux from the choroid plexus (475). Although recent evidence in vivo suggests that pretreatment of rats for 12 h with the electrophilic agent DEM increases uptake of L-cystine (but not 3-O-methylglucose) across blood-brain and blood-retinal barriers (271), this study could only assay transport of L-cystine against the background of plasma amino acids. Transport was inhibited by extracellular L-glutamate but unaffected by L-aspartate (infused into the internal carotid artery), suggesting involvement of the anionic transport system xCT, induced by DEM in human umbilical vein endothelial cells and artery smooth muscle cells (407, 539). More recently, this same group (595) reported the first kinetic constants for L-cystine transport in rat cultured retinal endothelial cells (see Table 2). RT-PCR analyses established that DEM (100 \(\mu\)M) induced a time-dependent expression of xCT and 4F2hc mRNA, with levels of xCT increasing within 6–12 h and 4F2hc after 24–48 h of exposure to DEM (595).
4. Sulfonic amino acid transport

There is evidence that dietary taurine is transported across the blood-brain barrier, and the high intracellular concentration of taurine in the brain (and retina) suggests that this sulfur-containing amino acid plays a role in volume regulation during osmotic stress (280). Experiments, using the in situ brain perfusion technique in anesthetized rats, provided direct evidence that taurine transport across the luminal surface of the blood-brain barrier was saturable and dependent on Na$^+$ and Cl$^-$ gradients (48). An absolute requirement for extracellular Na$^+$ was demonstrated since an inwardly directed Cl$^-$ gradient alone could not energize taurine uptake. Because taurine is primarily zwitterionic at pH 7.4, transport was inhibited mostly by hyptaurine and β-alanine but not GABA, which has an additional methylene group. Studies in cultured cerebral endothelial cells have confirmed that taurine and β-alanine transport is saturable; reduced by low temperature, dinitrophenol, and sodium azide; and inhibited selectively by structural analogs of taurine (328, 587). A Hill plot analysis revealed that transport of one β-alanine molecule was coupled to the transport of two Na$^+$ and one Cl$^-$. When β-alanine was transported at the luminal and abluminal membranes of bovine brain endothelial cells cultured on Transwell filters, it was apparent that, although transport at both membranes was dependent on the Na$^+$ and Cl$^-$ gradients, influx was more efficient across the luminal membrane (328). Even though these studies concluded that a specific transport system for β-like amino acids is expressed in the brain endothelium, none actually determined whether brain endothelial cells express the taurine/β-alanine transporter recently cloned from mouse brain and retinal cDNA libraries (353, 620) and human placental cDNA library (484). The protein sequence of the mouse retinal transporter (designated mTauT) reveals >93% sequence identity to the canine kidney, rat brain, mouse brain, and human placental taurine transporter. $K_m$ values estimated for taurine and β-alanine in Xenopus oocytes range between 4.5 and 60 μM (353) and are very similar to values reported for brain capillary endothelial cells (see Table 2).

B. Human Fetal Endothelium

1. Neutral amino acid transport

Amino acid transport in fetal endothelial cells cultured from human umbilical vein was initially characterized using a rapid indicator dilution technique (378). Endothelial cells were cultured on microcarriers and superfused in small heated columns with a Krebs-Henseleit buffer. Significant unidirectional tracer uptakes were measured for LNAAs, small neutral, and cationic amino acids, whereas uptakes for the system A analog MeAIB, glucose, serotonin, and dopamine were below 10% and at the limit of resolution of this technique. Figure 4 compares amino acid uptake by the blood-brain barrier in vivo and cultured umbilical vein endothelial cells measured using the rapid indicator dilution technique. As in the brain endothelium, transport of L-leucine in fetal endothelial cells was saturable (see Tables 2 and 3), Na$^+$ independent, and inhibited markedly by L-methionine, L-phenylalanine, BCH, and L-DOPA, but not MeAIB, L-cysteine, L-proline, L-hydroxyproline, β-alanine, L-aspartate, or glycine (inset in Fig. 4, top panel). These characteristics and the observed sensitivity of L-leucine transport to transstimulation are consistent with influx being mediated via system L, as confirmed in a subsequent study in human umbilical vein endothelial cells (97).

Neutral amino acid transport has also been studied in human umbilical vein endothelial cell monolayers in microtiter plates (97, 189, 339, 372). As accumulation of homocyst(e)ine in homocystinuria has been linked with thrombotic vascular occlusions (174, 206), Ewadh et al. (189) investigated the transport of homocyst(e)ine in confluent, nonstarved umbilical vein endothelial cells. Transport was pH insensitive and mediated by both Na$^+$-dependent and Na$^+$-independent saturable carriers, resembling systems ASC and L, respectively (see Table 3). There was no evidence in either amino acid-replete or amino acid-deprived (15–20 h) cells that system A contributed to the Na$^+$-dependent uptake of L-homocyst(e)ine in umbilical vein endothelium, suggesting uptake was mediated via system ASC (189). Bussolati et al. (97) further discriminated amino acid transport systems in umbilical vein endothelial cells by screening amino acid uptake (20–100 μM) in the absence or presence of Na$^+$ and after increasing periods of incubation in Earle’s balanced salt solution containing dialyzed serum, a procedure known to deplete the intracellular amino acid pool (see sect. viA). Amino acid deprivation was associated with time- (1.5–6 h) and protein synthesis-dependent increases in Na$^+$-dependent MeAIB and L-proline transport. The lack of MeAIB transport activity reported in an earlier study in umbilical endothelial cells (378) most likely reflects repression of system A under amino acid-replete conditions. Glutamine transport in umbilical vein endothelial cells appears to be mediated by Na$^+$-dependent (MeAIB- and threonine-inhibitable) and Na$^+$-independent (BCH-inhibitable) systems (97), and this study and others in pulmonary artery endothelial cells (257, 258) concluded that system N plays a negligible role in these endothelial cells (see Table 3). In contrast, system N transport activity appears to be expressed in rat lung microvascular endothelial cell (502). Sakurai et al. (502) did not determine whether uptake of L-histidine was mediated via the SN1, SN2, or SN3 transporter isoform, and the question remains as to whether species differences and/or the origin of endothelial cells

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macro- vs. microvascular) account for the lack of system N activity in pulmonary artery endothelium.

### 2. Cationic amino acid transport

The initial report that L-arginine is the physiological precursor of endothelium-derived NO (451) precipitated studies of L-arginine transport in endothelial cells. Rapid indicator dilution studies first demonstrated that radiolabeled L-arginine and L-ornithine are taken up by human umbilical vein endothelial cells (378). Although radiolabeled L-arginine uptake was initially found to be partly Na⁺ dependent, subsequent kinetic studies of L-arginine transport over a wide range of substrate concentrations (0.05–1 mM) failed to confirm Na⁺-dependent uptake for L-arginine in umbilical vein endothelial cells (97, 547). The kinetics of L-arginine transport in umbilical vein endothelial cells were best fitted by a Michaelis-Menten equation.
with a nonsaturable component rather than by two saturable components (see Table 3). Transport (100 μM) was pH insensitive and components (inhibited by 10-fold excess L-lysine and L-ornithine, but not L-serine or L-NNA or its methyl ester L-NAME. We and others (97, 547) have confirmed that influx of L-arginine transport was stereospecific and inhibited by 10-fold excess L-lysine and L-ornithine, but not L-serine or L-NNA or its methyl ester L-NAME. We and others (97, 547) have confirmed that influx of L-arginine and tetraphenylphosphonium (a membrane potential-sensitive probe) is significantly reduced in umbilical vein endothelial cells depolarized with K+. In addition to system y+, recent studies have identified system y-L-like activity and y+LAT1 and y+LAT2 mRNA in human umbilical vein endothelial cells (13, 503).

Other kinetic studies of L-arginine transport over a concentration range from 0.001–10 mM reported the existence of two different Na+-independent (75% of total transport) and one Na+-dependent (25% of total transport) systems in umbilical vein endothelial cells (452). The Na+-independent components of L-arginine transport were attributed to systems y+ and bH+, with system bH+ accounting for only 15–20% of uptake. Because kinetic data for L-arginine transport via system bH+ and the Na+-dependent system were not presented, it is difficult to reconcile these findings with the numerous other reports in umbilical vein endothelial cells attributing entry of L-arginine principally to systems y+ and y+LAT1 (97, 503, 547, 548, 537). Interestingly, we have found that maximal rates of saturable L-arginine transport are ~10-fold higher in endothelial cells derived from human placental microvasculature (L. Sobrevia and G. E. Mann, unpublished data) and saphenous vein (A. W. Wyatt, D. Chambers, and G. E. Mann, unpublished data) than other peripheral endothelial cell types (see Table 3). The low Km values and specificity of L-arginine transport in human placental and saphenous vein endothelial cells are consistent with influx mediated via system y+.

Studies in a human umbilical vein endothelial cell line SGHEC-17 have reported that transport of L-NMMA is saturable (Table 3) and inhibited markedly (at tracer concentrations) by L-arginine, canavanine, and asymmetric and symmetric dimethyl-L-arginine (ADMA, SDMA), and to a lesser extent by L-citrulline, aminoguanidine, or L-NNA or its methyl ester L-NAME (69). Although kinetic inhibition experiments were limited, it seems likely that entry of L-NMMA, ADMA, and SDMA was mediated via system y+, based on previous reports in J774 murine macrophages and endothelial cells (45, 66, 395, 513). In this context, ADMA and SDMA significantly inhibit L-arginine transport and iNOS activity in LPS-activated J774 macrophages (44), and in human dermal microvascular endothelial cells only very high concentrations of ADMA (100 μM) inhibited L-arginine influx (657).

In summary, human umbilical vein endothelial cells express transporters for CAT-1 and CAT-2B (107, 132, 380, 503) and y+LAT1 and y+LAT2 (503). A related human endothelial cell line EA.hy926 apparently only expresses CAT-1 (132), with activity of this transporter inhibited following activation of protein kinase C (PKC) with phorbol 12-myristate 13-acetate (PMA) (230). Entry of L-arginine via the y+LAT1 and y+LAT2 transporter isoforms has characteristics resembling system y+L described originally in human erythrocytes (164) and more recently in human platelets (400). Based on our own extensive characterization of L-arginine transport in human umbilical vein endothelial cells (see Figs. 2 and 3 in Ref. 547) and studies reported by Gazzola and colleagues (97, 503), we conclude that L-arginine transport in this endothelial cell type is mediated predominantly via systems y+ and y+L.

Km values of L-arginine determined for systems y+ and y+L in umbilical endothelial cells differ threefold (Table 3), and because system y+ is less sensitive to inhibition by neutral amino acids than system y+L (162, 450), it seems likely that under physiological conditions influx of L-arginine via system y+ prevails (503).

3. Anionic amino acid transport

Studies of anionic amino acid transport in fetal endothelial cells are limited. Aspartate and glutamate have been used to probe the Na+ dependence of anionic amino acid transport in human umbilical vein endothelial cells (97, 407, 545). Influx of L-aspartate is predominantly Na+-dependent and mediated via system XAg (97), and there are reports of Na+-dependent uptake of L-glutamate (50 μM) via two systems, one shared by L-aspartate and the other shared by neutral amino acids (407). In the latter study, a significant fraction of L-glutamate and L-cystine transport was Na+-independent and inhibited by homocysteate, α-amino adipate, and α-amino adipimelate, preferred substrates for the anionic Na+-independent system x−c. H2O2 generated by glucose/glucose oxidase stimulates metabolism of L-arginine to L-citrulline and results a time-dependent (4–24 h) and protein synthesis-dependent increase in Na+-independent L-cystine transport (407, 530). The kinetics of saturable, Na+-independent L-cystine transport (see Table 3), sensitivity to inhibition by L-glutamate and the induction of transport activity (and adaptive increases in intracellular GSH) by oxidative stress are consistent with uptake mediated via system x−c (25). Although Pan et al. (454) attributed Na+-independent L-glutamate transport to system XAg, their limited kinetic studies could not exclude the involvement of system x−c in L-glutamate transport. The consensus is that transport of anionic amino acids in human umbilical vein endothelial cells is mediated predominantly via the Na+-independent system x−c.
4. Involvement of GGT in cystine transport

In addition to the system $x_{\gamma}^C$, GGT has been implicated as an alternative pathway for L-cystine uptake into human umbilical vein endothelial cells (146). Inhibition of GGT activity with antithiogalactoside (inhibitor of GGT, see Ref. 405) dose-dependently inhibited initial rates of L-cystine uptake and depleted cellular GSH levels. Thus system $x_{\gamma}^C$ and the $\gamma$-glutamyl cycle could mediate L-cystine uptake into endothelial cells derived from human umbilical vein and the rat blood-brain barrier (see Fig. 5 and Ref. 340). $\gamma$-Glutamyl amino acids, formed on or near the cell membrane by the action of membrane-bound GGT on extracellular glutathione and amino acids, are translocated into cells with the subsequent release of free amino acids within the cell (446). Evidence for a direct role for the $\gamma$-glutamyl cycle in amino acid translocation remains controversial, insofar as it has received both support (101, 146, 340, 546, 576, 616) and criticism (275, 519, 646).

C. Aortic Endothelium

Transport of neutral and cationic amino acids has been investigated in cultured aortic endothelial cells superfused in microcarrier columns (66, 68, 70, 379) or in static monolayers (70, 513). Rapid indicator dilution techniques revealed significant uptake (16–42% relative to d-mannitol) for LNAA, small neutral, cationic, and acidic amino acids in bovine and porcine aortic endothelial cells superfused in microcarrier columns (66, 379). As in fetal endothelial cells (see sect. IVB), kinetics of L-arginine transport in aortic endothelial cells were best fitted by a Michaelis-Menten equation plus a nonsaturable linear component. Saturable L-arginine transport (see Table 3) in porcine aortic endothelial cells was sensitive to trans-stimulation and alterations in membrane potential; unaffected by changes in extracellular pH, Na$^+$, or Ca$^{2+}$; and inhibited by L-lysine, L-ornithine, but not MeAIB, BCH, 6-diazo-5-oxo-norleucine, L-glutamine, L-phenylalanine, L-cysteine, or L-glutamate (66, 70). This same group reported the first evidence that cationic NO synthase inhibitors L-NMMA and Nω-nitro-imidoethylnornithine (L-NIO) interact with the endothelial cell L-arginine transporter, whereas the neutral NO synthase inhibitors L-NNA and L-NAME were ineffective inhibitors, suggesting to these authors that L-arginine analogs entered endothelial cells via a neutral amino acid transport system(s) (70) similar to findings in J774 macrophages (45). Kinetics studies of L-NMMA and L-NNA transport in porcine aortic endothelial cells have confirmed these findings (513), although this latter study reported both high- and low-affinity transport systems for L-NMMA, L-NNA, and L-arginine (see Table 3). Interestingly, NOS inhibitors have also been shown to be transported by system $y^+$L in human erythrocytes (204) and the Na$^+/Cl^-$-coupled transport system B$\text{0}^+$ in human retinal pigment epithelial cells (252).

More recent evidence in bovine aortic endothelial cells has confirmed that L-arginine transport is mediated predominantly by system $y^+$, with the Na$^+$-dependent system B$\text{0}^+$ and passive diffusion contributing only minimally to L-arginine entry (440). Interestingly, this same group concluded that system B$\text{0}^+$ and $y^+$L were not operative in bovine aortic endothelial cells, further highlighting potential differences in amino acid transporter expression in arterial, venous, and pulmonary vascular beds.

Molecular studies have identified a taurine transporter in bovine aortic endothelial cells (477), which shares a high degree of sequence homology with the taurine transporters previously cloned from mammalian brain, retina, and placenta (353, 484, 620). Influx of taurine was saturable ($K_m \sim 4.9 \mu M$) and inhibited by $\beta$-alanine and homotaurine, requiring at least two Na$^+$ and one Cl$^-$ to transport taurine. Pretreatment of bovine aortic endothelial cells with PMA, but not its less active analog 4α-phorbol 12,13-didecanoate (4α-PDD), reduced taurine uptake by $\sim 55\%$, while growth of cells for 24 h in media supplemented with either 100 mM NaCl or 200 mM raffinose increased the $V_{\text{max}}$ for taurine transport (see Table 3). Based on their preliminary observations, Qian et al. (477) postulated that prolonged exposure of endothelial cells to elevated glucose may lead to depletion of intracellular taurine and apoptosis as a result of a downregulation of the taurine transporter by glucose, similar to findings in retinal pigment epithelial cells (565).

D. Pulmonary Endothelium

1. Neutral and acidic amino acid transport

Amino acid uptake by the pulmonary endothelium was originally investigated in the isolated perfused rat lung in situ using the rapid indicator dilution technique (577). In this study, initial rapid tracer uptakes for L-alanine, glycine, L-methionine, L-phenylalanine, L-lysine, taurine, and L-glutamate (relative to $^{131}$I-albumin) ranged between 2 and 25% and were insensitive to inhibition by competitor amino acids, suggesting to these authors that endothelial cells in the intact lung did not rapidly transport amino acids. However, amino acid transport has been characterized in cultured endothelial cells derived from bovine and porcine pulmonary arteries. Steiger et al. (563) were one of the first groups to report that L-glutamate uptake by bovine pulmonary artery endothelial cell monolayers was poorly saturable at concentrations up to 6 mM, partially Na$^+$ dependent, and inhibited by L-aspartate and cysteate but not MeAIB. Similar studies in porcine pulmonary artery endothelial cells revealed that L-glutamate, MeAIB, and BCH have no effect on Na$^+$-dependent L-glutamine transport, whereas influx was
reduced significantly by system ASC substrates (cysteine, threonine, and alanine) and system N substrates (histidine and asparagine) (257, 258). The inhibition profile of L-glutamine transport and its insensitivity to pH changes and insulin, glucagon, or dexamethasone led these authors to conclude that L-glutamine influx in pulmonary artery endothelial cells was mediated by the Na\(^+\)-dependent system ASC. In contrast, endothelial cells cultured from the rat lung microvasculature express system N-like transport activity (502), raising the question whether amino acid transporters are expressed differentially in macro- and microvascular endothelium.

2. Cationic amino acid transport

The specificity and kinetics of L-arginine transport in porcine pulmonary artery endothelial cells suggest that ~70% of L-arginine transport is mediated by the Na\(^+\)-independent systems y\(^+\) and b\(^0\)-L (232). This group also described a high-affinity Na\(^+\)-dependent transport system resembling system B\(^0\)-L, and a subsequent study attributed ~20–40% of total L-arginine uptake to system B\(^0\)+ (60). As in aortic and umbilical vein endothelial cells (70, 513, 547), cationic L-arginine analogs are potent inhibitors of NOS and system y\(^+\) in porcine pulmonary artery endothelial cells (395). This latter study is of particular interest to the field of NO research, since it provided the first evidence that L-NMMA, N\(^0\)-amino-L-homoarginine, and N\(^0\)-iminooethyl-L-lysine inhibited system y\(^+\) in endothelial cells, with \(K_i\) values ranging between 140 and 780 \(\mu\)M. Although there are conflicting reports concerning the number and contribution of different cationic amino acid transporters mediating L-arginine influx in pulmonary artery endothelial cells (see Table 3), detailed kinetic inhibition studies in porcine endothelial cells concluded that Na\(^+\)-independent L-arginine transport is mediated predominantly by the CAT-1 transporter (system y\(^+\)).

3. Cystine and glutamate transport via system \(x_c^-\)

Denke et al. (159) noted that transport of L-cystine and L-glutamate, but not L-aspartate, L-leucine, or AIB, was stimulated in bovine pulmonary artery endothelial cells exposed to DEM (an electrophilic agent known to conjugate enzymatically with GSH) or hyperoxia. The increase in system \(x_c^-\) transport activity was associated with an elevation in intracellular GSH, and both responses were dependent on de novo protein and RNA synthesis. It is worth noting that the induction of L-cystine transport by DEM or hyperoxia was not triggered by an initial depletion of GSH. Subsequent studies documented similar findings in pulmonary artery endothelial cells exposed to arsenite, disulfiram, and the heavy metal cadmium (573), confirming the stimulation of system \(x_c^-\) activity in human umbilical vein endothelial cells (407, see sect. nB4). Inhibition of glutathione reductase with N,N-bis(2-chloroethyl)-N-nitrosourea (24 h) results in concentration-dependent increases in L-cystine transport and GSH levels in pulmonary artery endothelial cells, an effect blocked by excess L-glutamate (160, 573).

E. Myocardial Endothelium

In coronary endothelial cells isolated from spontaneously diabetic BB rats, impaired NO synthesis was not attributed to reduced L-arginine uptake but rather to a depletion of cofactors required for NO production (653). Diabetes-induced alterations in endothelial cell metabolism of L-arginine were inferred from a reduced activity of arginase and formation of L-ornithine and urea from L-arginine. Indicator dilution studies in the isolated perfused guinea pig heart attributed the initial uptake of radiolabeled L-arginine (relative to d-mannitol) to coronary endothelial and smooth muscle cells (331), although the lack of a rigorous compartmental analysis of coronary sinus effluent profiles raises the question whether uptake can be attributed to endothelial cells exclusively.

V. GLUCOSE TRANSPORTERS IN ENDOThelial CELLS

The blood-brain barrier is the major endothelial tissue expressing GLUT isoforms; however, glucose transporters have also been detected in other endothelia, including umbilical vein (239, 459), adrenal capillaries (211), aorta (211), retina (218, 431), heart (593), placenta (239, 254), iris and cornea (334), and testis (190). GLUT1 is the most abundant isoform in proliferating endothelial cells and the blood-brain barrier, and GLUT1 mRNA levels have been detected in the brain, retina, and rat heart (see Table 4).

A. Blood-brain, Blood-retinal, and Corneal Endothelium

1. Blood-brain barrier endothelium

The mechanisms mediating glucose uptake across the blood-brain barrier have been reviewed (see Refs. 76, 333, 366, 415, 463), and numerous studies have evaluated cerebral glucose metabolism in vivo by positron emission tomography (see Ref. 147). As originally described by Crane (149), rapid indicator dilution, BUI, and in situ perfusion techniques attributed glucose uptake across the luminal membrane of the blood-brain barrier to a facilitative, stereospecific, energy-independent, and saturable transporter with characteristics of GLUT1 (Table 4).

A high-affinity, Na\(^+\)-independent transport system for 2-deoxyglucose (\(K_m\) ~93 \(\mu\)M) has been described in isolated capillaries from rat brain (228) and endothelial cells...
## Table 4. Glucose transporter isoforms detected in blood-brain, blood-retinal, and peripheral vascular endothelium

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Detection</th>
<th>Endothelial Cell Type</th>
<th>Main Findings</th>
<th>Reference Nos.</th>
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<td>Cerebral endothelium</td>
<td>436</td>
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<td>Protein</td>
<td>Human cerebellum microvessels</td>
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<td>Levels increased in hemangioblastoma</td>
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<td>More GLUT3 than GLUT1</td>
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<td>GLUT1 in 21-week endothelium</td>
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<td>GLUT1</td>
<td>mRNA</td>
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<td>VEGF (50 ng/ml, 24 h) † 3-O-methylglucose uptake via PKCβ</td>
<td>553</td>
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<td>GLUT1</td>
<td>Protein/mRNA</td>
<td>Rat brain</td>
<td>Asymmetric distribution: −4-fold higher on abluminal membranes</td>
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<td>GLUT1</td>
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<td>Regulated postnatal</td>
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<td>Expressed at both luminal and abluminal endothelial cell membranes</td>
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<td>Similar in fed and fasted animals</td>
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<td>GLUT1</td>
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<tr>
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<td>Protein</td>
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<td>Asymmetric distribution: −3-fold higher on abluminal membrane, similar density cortex, hippocampus, and cerebellum</td>
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<td>GLUT1</td>
<td>Protein/mRNA</td>
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</table>

PKC, protein kinase C; PKA, protein kinase A; VEGF, vascular epidermal growth factor; 2-DG, 2-deoxyglucose.
isolated from bovine brain large arteries (434). In rat brain capillaries, 2-deoxyglucose transport was temperature dependent and inhibited by glucose ($K_r \sim 100 \mu M$), phloretin, and other hexoses. In the same preparation, transport of 3-O-methylglucose (nonmetabolized glucose analog) was inhibited by cytochalasin B and sensitive to accelerative exchange diffusion (54). In isolated brain capillaries, Na$^+$-independent transport of 2-deoxyglucose is more sensitive to inhibition by phloretin (inhibitor of facilitative glucose transport) than phlorizin and insensitive to 2,4-dinitrophenol and ouabain (228). These authors concluded that transport was not rate-limiting for brain metabolism, and because $\sim 90\%$ of 2-deoxyglucose retained within isolated brain capillaries was not phosphorylated, glucose concentrations in brain-blood barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial metabolites, glucose concentrations in blood-brain barrier endothelial cells would lie between plasma and interstitial inflamed that initial rates ($<2$ min) of 2-deoxyglucose and 3-O-methylglucose uptake were Na$^+$ independent, unaffected by 2,4-dinitrophenol and ouabain, but significantly inhibited by phloretin and cytochalasin B (621). In bovine brain microvascular endothelial cells, transport of 3-O-methylglucose under normal glucose concentrations is equilibrative, temperature dependent, Na$^+$ independent, inhibited by cytochalasin B and phloretin, and sensitive to trans-stimulation by D-glucose, D-mannose, D-xylose, D-galactose, and D-ribose (581, 582). Recent studies have shown that the brain endothelial cell line RBE4, derived from primary cultures of rat brain capillary endothelium, also transports 3-O-methylglucose via a facilitative glucose transporter (apparently GLUT1) sensitive to glucose deprivation (487). As reported by Maxwell et al. (392), this cell line may provide a useful model to explore modulatory actions of astrocytes on brain endothelial cell glucose transport.

There is general agreement that GLUT3 is localized predominantly in neural cells in the brain (see Ref. 333). Gerhart et al. (216), using an antiserum directed against the COOH terminus of human GLUT3, claimed to have detected GLUT3 in the canine blood-brain barrier; however, Maher et al. (373) queried the specificity of their antiserum. More recent ultrastructural studies of the rat brain have identified GLUT1 in the endothelium, with GLUT3 localized primarily in pre- and postsynaptic nerve endings (345). Thus it seems unlikely that GLUT3 plays a physiological role in the glucose transport across the blood-brain barrier.

Is there Na$^+$-dependent transport of glucose in the brain? Glucose transport was reported to be stereospecific and energy independent in luminal and abluminal endothelial membrane vesicles isolated from bovine cerebral microvessels, with very similar kinetic constants ($K_m \sim 10 \text{ mM}, V_{\text{max}} \sim 9 \text{ nmol} \cdot \text{mg protein}^{-1} \cdot \text{min}^{-1}$) determined using both membrane preparations (342). This group, however, also described the presence of Na$^+$-dependent transport at the abluminal membrane of the blood-brain barrier, with a higher affinity ($K_m \sim 0.13 \text{ mM}$) and a lower capacity ($V_{\text{max}} \sim 1.6 \text{ nmol} \cdot \text{mg protein}^{-1} \cdot \text{min}^{-1}$) than GLUT1. This same group subsequently confirmed that functional GLUT1 proteins are distributed asymmetrically at luminal and abluminal membranes (1:4) of bovine cerebral microvessels (538) but unfortunately failed to discuss these findings in relation to their previous description of Na$^+$-dependent glucose uptake (342). Although a Na$^+$-dependent glucose transport system, capable of transporting methylazoxymethanol beta-D-glucoside (inhbitable by specific transport analog alpha-D-glucoside), has been described in cultured bovine brain endothelial cells (390), this study could not discriminate transport at the luminal and abluminal surfaces of the cell monolayer. Na$^+$-dependent glucose transport has predominantly been detected in large cortical vessels and endothelial cells from large cerebral arteries (434, 435). The editorial comment to the study by Nishizaki and Matsuoka (435), however, concluded that the main pathway for glucose uptake in brain endothelium under normal glucose conditions is via GLUT1-facilitated diffusion. The general consensus is that GLUT1 is distributed asymmetrically between the luminal and abluminal membranes of the blood-brain barrier (ratio 1:4, see Ref. 190), with the luminal membrane representing the key interface for glucose entry into the brain.

2. Blood-retinal and corneal endothelium

Mechanisms regulating blood-retinal barrier integrity, permeability, and nutrient transport have been reviewed (see Ref. 153). Glucose transport across the endothelium of the inner blood-retinal barrier and from the choroidal vessels across the retinal pigment epithelium of the outer blood-retinal barrier nourishes the neuroretina (584), with rates of glucose transport exceeding metabolic rates of the retinal endothelium (333).

Early studies in primary cultures of bovine retinal endothelial cells revealed that facilitated 3-O-methylglucose transport was rapid, sensitive to trans-stimulation, and inhibited by 2-deoxyglucose, D-mannose, D-galactose, and D-xylose but not by L-glucose, D-ribose, and D-fructose (58). Transport was also inhibited by phloretin and cytochalasin B but unaffected by depletion of intracellular ATP or dissipation of the electrochemical gradient for Na$^+$ (53). A comparison of initial rates of glucose transport in porcine retinal and aortic endothelial cells indicates that the aortic endothelial cell membrane acts as a barrier to glucose uptake, while glucose transport into the retinal endothelium is not rate limiting for glucose utilization (444). Recent kinetic studies of 3-O-methylglucose

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transport in primary cultures of human retinal endothelial cells revealed that elevated glucose (22 mM) increases the $V_{\text{max}}$ 2.5-fold with no changes in GLUT1 mRNA or protein levels (96). The precise mechanism(s) mediating activation of glucose transport remains to be defined. GLUT1 mRNA and protein have been detected in human and monkey (96, 325, 334, 431), bovine (376), and chicken (218) retinal endothelial cells (see Table 4). As in the brain endothelium, GLUT1 is expressed on luminal and abluminal membranes of human and rat blood-retinal barriers, suggesting that GLUT1 mediates transcellular transport of glucose to the neuroretina.

Bovine cultured retinal pericytes express a Na$^+$-dependent SGLT2-like glucose transport system, which under conditions of hyperglycemia mediates increased uptake of glucose and α-methyl-D-glucoside (629). Moreover, inhibition of SGLT2-like glucose transport in bovine retinal pericytes by phlorizin and captopril significantly ameliorates glucose-induced swelling and pericyte loss (628). In contrast, glucose uptake by bovine retinal endothelial cells in culture is Na$^+$ independent (629), consistent with glucose transport mediated via GLUT1. In the human retinal endothelial cells, Knott et al. (325) reported that GLUT1 increases after exposure of cells for 8 h to 15 mM glucose, although it is worth noting that mRNA levels were similar in cells cultured in 5 or 25 mM glucose. GLUT3 has not been detected in the endothelium of the inner blood-retinal barrier in vivo (see Ref. 333). Immunohistochemical studies have also failed to detect expression of the insulin-sensitive glucose transporter GLUT4 or GLUT5 in retinal endothelial cells.

Transport of radiolabeled 3-O-methylglucose, ascorbic acid, and L-glucose from blood into the rat and guinea pig corneal endothelium, aqueous humor, and stromal compartments has been measured in pulse-chase experiments in vivo (165). Rate constants for L-glucose and 3-O-methylglucose transfer into the corneal endothelium were high, indicating a rapid equilibrium with the aqueous humor and possibly passive diffusion of glucose into corneal endothelial cells (165). As GLUT1 has been detected in the human corneal endothelium (334), this may provide a basis for characterizing glucose transport in corneal vascular beds.

In summary, upregulation of glucose transport may have important implications for microvascular dysfunction in diabetic retinopathy (see Ref. 333). Increased translocation of GLUT1 transporters to the plasma membrane of retinal endothelial cells in response to hypoxia and growth factors would lead to increased glucose transport, resulting ultimately in metabolic and structural changes in the blood-retinal barrier. In view of the sensitivity of the retinal vasculature to hyperglycemia-mediated injury, it is surprising how little is known about the cellular mechanisms regulating glucose transport and metabolism.

B. Human Fetal Endothelium

In the early 1980s, studies in simian virus 40-transformed human umbilical vein endothelial cells demonstrated that transport of 2-deoxyglucose and 3-O-methylglucose was saturable ($K_m$ ~3–5 mM, $V_{\text{max}}$ ~0.56 pmol·μl$^{-1}$·min$^{-1}$), inhibited by phloretin and phlorizin, but unaffected by KCN, dinitrophenol, or ouabain (137). Facilitative diffusion of glucose was also shown to be unaffected by serum deprivation (18 h) or treatment with high concentrations of insulin (10$^{-5}$ M). More recent studies in human umbilical vein endothelial monolayers and superfused microcarrier cultures could only detect relatively low rates of glucose transport (378, 459). In the human placenta, GLUT1 is abundant at both maternal and fetal surfaces of the syncytiotrophoblast and yet apparently undetectable in fetal umbilical vein endothelium (38). Because GLUT1 has been localized in the fetal endothelium of human, marmoset, and rat placenta (see Table 4), further studies are necessary to resolve whether the human umbilical vein endothelium expresses functional GLUT1 transporters.

C. Aortic, Pulmonary, Myocardial, and Adrenal Endothelium

Transport of glucose analogs has been characterized in cultured endothelial cells isolated from bovine aorta (211, 298, 358, 376, 507) and a bovine aortic endothelial cell line GM7373 (221). In freshly isolated aortic endothelial cells, transport of 2-deoxyglucose was inhibitable by cytochalasin B, and as in capillaries of the adrenal medulla, uptake was increased reversibly by glucose deprivation (211). Similar to reports for brain capillaries, increased transporter activity correlated with an increased expression of GLUT1. Thus glucose deprivation may be a signal for upregulation of glucose flux by increasing the number of transporters rather than the intrinsic properties of existing transporters. In bovine aortic endothelial cells, antidiabetic drugs such as metformin, used to restore insulin sensitivity in diabetic patients (507), elevate the rate of 2-deoxyglucose and 3-O-methylglucose uptake approximately two- to threefold, suggesting that regulation of glucose transport in aortic endothelial cells may not necessarily be dependent on glucose phosphorylation.

Studies of glucose transport and expression of GLUT isoforms in pulmonary artery endothelial cells are limited. Rapid indicator dilution experiments in the isolated perfused rat lung concluded that the pulmonary endothelium does not readily take up glucose (577), and more recent evidence indicates that GLUT1–5 and SGLT1 are not expressed in the human lung (161). Although glucose transport has been studied in explant cultures from rat lung (533), interpretation of this study is confounded by the
labeled L-arginine in bradykinin (100 nM) or ATP (100 μM) transiently stimulates L-arginine influx and NO release (68). In endothelial cells, constitutive activity of Kir-type K+ channels and the activation of other K+ channels (BKCa, IKCa, SKCa, KATP) by vasoactive agonists such as bradykinin and ATP induces hyperpolarization (reviewed in Refs. 432, 586). We have reported that basal and bradykinin-stimulated L-arginine transport was reduced in cells depolarized with 70 mM K+, while agonist-induced NO and PGI2 release were still detectable (66). In the same study inhibition of NO production by L-NNA attenuated the increase in L-arginine uptake evoked by bradykinin (see Fig. 2 in Ref. 68), suggesting that NO (or a downstream mediator) modulates L-arginine transport (see sect. VI).

Changes in membrane potential modulate L-arginine transport in porcine aortic, porcine pulmonary artery, and human umbilical vein endothelial cells (66, 97, 547, 668) and Xenopus oocytes expressing CAT-1 or CAT-2 activity (307, 425). Interestingly, in umbilical vein endothelial cells, histamine (1–100 μM, 5 min) has no effect on L-arginine transport (547, 550), even though it rapidly elevates intracellular Ca2+ and release of NO and PGI2. The discrepancy between the actions of histamine and bradykinin on L-arginine transport may reflect differences in endothelial cell type. Although a definitive link between L-arginine transport and NO production has not been identified in endothelial cells, system y+ and eNOS are known to colocalize in plasma membrane caveolae in porcine pulmonary artery endothelial cells (396). Furthermore, endothelial cells may contain a localized L-arginine pool (132, 244), enabling preferential delivery of extracellular L-arginine to eNOS, with the rate of L-arginine utilization partly determining the rate of refilling of the localized L-arginine pool via CAT transporters (see sect. x).

In human umbilical vein endothelial cells, adenosine (10 μM, 2 min) and the select A2-purinoceptor agonist N-ethylcarboxamido-adenosine (CGS-21860, 100 nM) acutely stimulate L-arginine transport and NO synthesis via a Ca2+-insensitive mechanism involving membrane hyperpolarization (551). Activation of the L-arginine-NO pathway was abolished by selective A2a antagonists but unaffected by the A1 receptor agonist N0-cyclopentyladenosine (CPA, 100 nM). Adenosine increases protein tyrosine phosphorylation in umbilical vein endothelial cells (523), and we have recently shown that A2a purinoceptor agonists stimulate the endothelial cell L-arginine-NO pathway via the activation of p42/p44 mitogen-activated protein kinases with release of NO leading to a membrane hyperpolarization and increased L-arginine transport (656). Similarly, Ca2+-independent activation of eNOS by shear stress has been associated with tyrosine phosphorylation and is mimicked by tyrosine phosphatase inhibitors (19, 166, 202, 209). Although studies with bovine
aortic endothelial cells have reported that the dinucleotide $P^4$,$P^4$-diadenosine 5'-tetraphosphate stimulates L-arginine and L-citrulline uptake (262), the intracellular signaling mechanism(s) was not investigated.

$\beta_2$-Adrenoceptor stimulation of human umbilical vein endothelial cells also results in Ca$^{2+}$-insensitive activation of the L-arginine-NO signaling pathway (199), with isoprenaline acutely increasing L-arginine transport and NO production (L. Queen, A. W. Wyatt, G. E. Mann, and A. Ferro, unpublished data). The stimulatory effects of isoprenaline were mimicked by forskolin and dibutyryl cAMP, suggesting that an elevation in cAMP and activation of protein kinase A (PKA) may mediate these processes. In functional studies, $\beta_2$-adrenoceptor-mediated relaxation of human umbilical vein rings was abolished by removal of the endothelium or by inhibition of eNOS (199). The mechanism by which $\beta$-adrenoceptor stimulation or elevation of cAMP activates the L-arginine-NO transduction pathway in endothelial cells remains to be elucidated, although recent studies have shown that the endothelium-dependent component of cAMP-mediated relaxation of rat pulmonary arteries is critically dependent on availability of extracellular L-arginine (~1–7 $\mu$M) (see Ref. 276). Dependence on extracellular L-arginine was agonist and tissue specific, since ACh-induced relaxation of the aorta or isoprenaline-mediated relaxation of mesenteric arteries was unaffected by removal of exogenous L-arginine.

In cultured coronary microvascular endothelial cells, activation of H$_1$ receptors by histamine stimulates glucose transport (~10–50%), reaching a maximum after 5 min of histamine application (593). GLUT1 mRNA and protein was detected in these microvascular cells, suggesting that acute stimulation of glucose transport by histamine may involve modulation of GLUT1 expression and/or activity. Transport of 3-O-methylglucose in umbilical vein endothelial cells is unaffected by ATP, adenosine, or histamine (459), reflecting potential differences between micro- and macrovascular endothelium (see Ref. 298). However, because histamine-mediated release of PGI$_2$ from human macrovascular and microvascular endothelial cells is impaired in hyperglycemia (see Ref. 549), further studies of the effects of elevated glucose in coronary microvascular endothelial cells are warranted.

In summary, accumulating evidence from studies with cultured endothelial cells suggests that a distinct intracellular pool of L-arginine could limit availability of substrate for agonist-stimulated NO synthesis (132, 244, 396). This hypothesis is supported by functional studies with rat pulmonary arterial rings in which stimulation of endothelium-derived NO production by stretch, isometric tension, or cAMP is critically dependent on the uptake of L-arginine (276). Studies from our laboratory and others (18, 67, 132, 429) have reported that transport of L-arginine is rate-limiting for NO production via iNOS in LPS-

and cytokine-activated J744 macrophages. Superfusion of activated J774 macrophages with an L-arginine-free medium abolishes nitrite release, while restoration of extracellular L-arginine after 20–50 h of substrate depletion leads to a rapid and sustained production of nitrite (18).

Although these experiments did not directly assay L-arginine transport, they emphasize the importance of L-arginine delivery in sustaining NO production in vascular cells expressing iNOS. In this context, NO production by cytokine-activated macrophages is virtually abolished in CAT2$^{−/−}$ knock-out mice (see Ref. 429).

Recent studies in platelets from chronic renal failure patients have shown that both basal and ADP-stimulated NO production is dependent on enhanced L-arginine transport via the high-affinity amino acid transport system y$^+$L (89). Clearly, further studies are required to elucidate the roles of systems y$^+$ and y$^+$L in regulating the delivery of L-arginine for NO production in different blood cells and vascular endothelial and smooth muscle cells.

B. Modulation of Amino Acid and Glucose Transport by NO

In view of the importance that NO plays in modulating vascular tone, it is surprising that only limited information is available on the effects of NO on amino acid transport in endothelial cells. We have previously suggested that agonist-induced modulation of system y$^+$ in porcine aortic endothelial cells might in part be regulated by NO generation. Although 2-min exposure to sodium nitroprusside (SNP), which generates NO in aqueous solution and elevates endothelial cell cGMP levels, did not affect L-arginine transport (66), we could not exclude the possibility that longer exposure to NO would have modulated system y$^+$. Convincing experiments in bovine aortic endothelial cells (440) have documented that the NO donors S-nitroso-N-acetylpenicillamine (SNAP; 200 $\mu$M, equivalent to 0.4 $\mu$M NO) or dipropylendiamine NONOate (DPTA; 1 $\mu$M, equivalent to 2 $\mu$M NO) acutely (~15 min) stimulate L-arginine uptake via system y$^+$, whereas longer exposure (1–4 h) to these NO donors inhibited transport (see Fig. 1). The initial stimulation of L-arginine transport by SNAP was not associated with a membrane hyperpolarization. The inhibitory effects of NO on system y$^+$ activity were attributed to the oxidation of sulfhydryl moieties within the transporter protein, as originally proposed by Patel et al. (461), who reported that prolonged exposure (4–24 h) of porcine pulmonary artery endothelial cells to exogenous NO results in a reversible inhibition of L-arginine transport (50 $\mu$M, 30 s) via both Na$^+$-dependent (system B$^{\text{H}^+}$) and Na$^+$-independent (system y$^+$) systems. The influence of NO on different endothelial cationic amino acid transporters may be tissue specific, since NO donors were found to have no effect on system...
B<sup>0</sup>+ (~10% of total L-arginine transport) in bovine aortic endothelial cells (440).

Our recent studies have confirmed that acute exposure (2–5 min) of human umbilical vein endothelial cells to SNP and SNAP (100 μM) stimulates L-arginine transport and phosphorylation of p42/p44MAPK, with an ensuing membrane hyperpolarization leading to activation of transport (413, 656). Although there are no immediate explanations for the discrepancy in the effects of NO donors on membrane potential in bovine aortic versus human endothelial cells, application of the whole cell patch-clamp technique to monitor outward K<sup>+</sup> currents elicited by adenosine in umbilical vein endothelial cells has provided a sensitive measure of NO-mediated changes in membrane currents (see Fig. 8 in Ref. 656).

Treatment of bovine aortic endothelial cell monolayers with SNAP (>1 mM, 1 h) depletes cellular GSH levels, whereas longer exposure (4–10 h) to lower concentrations of SNAP (≤1 mM) results in adaptive increases in L-cystine transport and intracellular GSH levels (349). Activation of L-cystine transport by SNAP (0.01–1 mM) was dependent on both RNA and protein synthesis, and, as illustrated in Figure 6, was unaffected by acivicin (an inhibitor of GGT, see Ref. 405), suggesting that Meister’s γ-glutamyl cycle (see sect. III B4) was not responsible for the L-glutamate-inhibitable induction of L-cystine influx. This same study, using a Transwell coculture system, established that NO released from cytokine-activated RAW.264.7 macrophages induced adaptive increases in L-cystine transport and intracellular GSH levels in bovine aortic endothelial cells (349).

Peroxynitrite (ONOO<sup>-</sup>) generated from the reaction of NO with superoxide anion O<sub>2</sub><sup>-</sup> leads to alterations in cell signaling and function via the modification of cellular lipids and protein thiols (47, 480). In bovine aortic endothelial cells 3-morpholinosydnonimine (SIN-1; 0.05–2.5 mM), a donor of NO and ONOO<sup>-</sup>, caused a transient depletion of GSH followed by a prolonged increase in L-cystine transport and intracellular GSH levels after 6–9 h after exposure (93). Induction of L-cystine transport in both aortic endothelial and smooth muscle cells in response to SNAP or SIN-1 was abolished by inhibitors of protein and RNA synthesis. These findings, and the fact that L-glutamate (5–10 mM) inhibited SIN-1-stimulated increases in L-cystine transport and GSH levels, implicated system x<sub>c</sub> in the adaptive responses to oxidative and/or nitrosative stress. Buckley and Whorton (93) could not exclude nitrosoperoxygenate (ONOOCO<sub>2</sub>, formed from the reaction of ONOO<sup>-</sup> with CO<sub>2</sub>), as a potential mediator of these adaptive responses. Interestingly, basal rates of L-cystine uptake were fourfold higher in aortic smooth muscle cells than endothelial cells. It seems likely that under conditions of overproduction of NO or ONOO<sup>-</sup> in inflammation (see sect. VIII A), endothelial cells can adapt by increasing system x<sub>c</sub> transport activity and their GSH levels to counteract NO-mediated changes in cellular thiols (349).

Molecular identification of a taurine transporter in...
bovine aortic endothelial cells (477) and the report that prolonged exposure of human retinal pigment epithelial cells to NO (released via SIN-1, 18–24 h) stimulates taurine transport and steady-state levels of mRNA for the TAUT transporter (see Ref. 79), provides a basis for exploring regulation of taurine transport by NO in vascular endothelial and smooth muscle cells. In retinal pigment epithelial cells, NO may stimulate taurine transporter activity and expression via mechanisms involving cGMP (79).

To our knowledge, there are no reported studies on the acute or chronic effects of NO on glucose transport in cultured vascular endothelial cells. Injection of LPS into 3-h fasted rats has been reported to increase GLUT1 protein levels fourfold in hepatic endothelial cells (557). Although this study did not directly determine whether GLUT1 activity or expression was modulated by NO released in response to LPS, it provided evidence that hepatic endothelial cells have an increased capacity to eliminate H2O2 during endotoxemia (559). The importance of LPS-induced stimulation of glucose uptake and activity of the hexose monophosphate shunt in the elimination of H2O2 from hepatic endothelial cells is highlighted by the fact that restriction of glucose delivery increased intracellular H2O2 levels. A role for NO as a modulator of glucose transport in skeletal muscle has been documented, with GLUT4 implicated in the four- to fivefold increase in basal glucose transport mediated by SNP (187). In contrast, myocardial glucose uptake is increased in the presence of the NOS inhibitor L-NAME and in preparations isolated from eNOS knock-out mice, suggesting that NO may in some vascular beds downregulate glucose uptake via a cGMP-dependent mechanism (579). Further studies are warranted, since glucose metabolism and transporter expression are likely to be modulated by NO-dependent mechanism(s) at sites of inflammation.

VII. ADAPTIVE AND DEVELOPMENTAL REGULATION OF AMINO ACID AND GLUCOSE TRANSPORT IN ENDOTHELIAL CELLS

A. Amino Acid Deprivation

Amino acid deprivation induces adaptive responses in amino acid transport and intracellular amino acid levels in a variety of cultured cell types (reviewed in Refs. 205, 214, 236, 397). Prolonged incubation of cells in an amino acid-free medium results in a time- and protein synthesis-dependent stimulation of system A transport activity, which is decreased upon restoration of extracellular amino acid availability. The recent cloning of transporter isoforms with properties resembling the Na+/H+ antiporter system A (251 570, 571, 631, 658) enabled Alferi et al. (4) to identify ATA2 as the primary isoform of system A in porcine pulmonary artery endothelial cells, with activation of MeAIB influx in response to hypertonic stress dependent on increased ATA2 mRNA levels.

The term adaptive regulation is generally defined as substrate-induced changes in system A activity, and the underlying molecular mechanisms have only recently been elucidated in human cultured fibroblasts (215). Incubation of fibroblasts (4–16 h) in Earle’s balanced salt solution supplemented with 10% dialyzed fetal bovine serum led to a time-dependent increase in L-proline transport via system A, which was prevented by 2 mM L-proline or MeAIB and reversed following restoration of an amino acid-rich medium. Gazzola et al. (215) further demonstrated that mRNA levels for the system A transporter ATA2 closely paralleled changes in L-proline transport, with amino acid starvation increasing ATA2 mRNA levels nearly 10-fold and amino acid resupplementation down-regulating both ATA2 mRNA and transport activity. Thus adaptive regulation involves a derepression phase, in which amino acid deprivation stimulates transport activity via an increased synthesis of new transporters and a repression phase, in which system A substrates decrease transport activity due to a degradation of system A transporters and/or an inhibition of new transporter synthesis (see Ref. 215). Similar studies in rat C6 glioma cells provided further novel evidence that total amino acid deprivation initially results in a recruitment of preformed ATA2 transporters to the plasma membrane, whereas prolonged starvation is associated with an increase in ATA2 mRNA levels (351). Importantly, only substrates of system A specifically reversed the adaptive increases in ATA2 mRNA levels and transport activity in fibroblasts and C6 glioma cells.

Our studies in human umbilical vein endothelial cells have established that depletion of intracellular L-glutamine (12.7 vs. 1 mM) in response to total amino acid deprivation (0.5–1 h) is associated with an increase in the Vmax for Na+/H+ antiporter system A transport activity (372). In view of the fact that extracellular L-glutamine prevents amino acid starvation-induced increases in ATA2 expression/activity in fibroblasts and C6 glioma cells (215, 351), it would be of interest to examine the effects of amino acid deprivation on system A transport activity and ATA2 expression in human umbilical endothelial cells. Preliminary evidence in human umbilical endothelial cells also suggests that amino acid deprivation (6 h) stimulates system x− activity via a protein synthesis-dependent mechanism (97). Because murine and human cDNAs encoding system x− were only recently isolated (39, 319, 509, 510), future studies will need to determine whether mRNA and protein levels are upregulated in response to amino acid starvation.

Although amino acid starvation apparently did not stimulate system y− activity in C6 glioma cells (351), Hyatt et al. (283) reported an approximately threefold...
increase in CAT-1 mRNA levels in Fao cells, which remained elevated for 24 h and decreased after amino acid resupplementation. These authors concluded that the increased CAT-1 mRNA levels supported synthesis of CAT-1 protein during starvation (without altering the plasma membrane distribution of CAT-1 transporters) and increased cationic amino acid transport rate. In endothelial cells deprived of L-arginine for 1–24 h, total intracellular L-arginine levels decrease within 1 h in bovine aortic (0.84 vs. 0.2 mM) and porcine aortic (0.9 vs. 0.4 mM) but not in human umbilical vein (0.88 vs. 0.9 mM) endothelial cells (see Fig. 2 and Refs. 42, 43, 66). The maintained intracellular L-arginine pool in umbilical vein endothelial cells in the absence of extracellular L-arginine is surprising and may reflect protein degradation in this cell type. Decreases in intracellular L-arginine levels (0.25 vs. 0.097 mM, albeit lower basal L-arginine concentrations) have also been detected in bovine aortic endothelial cells deprived of extracellular L-arginine for 2 h (474). Recent studies with the human umbilical vein endothelial cell line EA.hy296 established that incubation of cells with a competitive inhibitor of system y+ (2 mM L-lysine) decreased free intracellular L-arginine levels (3.5 vs. 0.6 mM), albeit a significant residual fraction of L-arginine was not depletable (132). As the activity of eNOS was unaffected in EA.hy296 cells in the presence of 2 mM L-lysine, these authors postulated that endothelial cells have a membrane-associated pool capable of supplying L-arginine directly to eNOS. Unlike endothelial cells, the nonfreely exchangeable L-arginine pool in macrophages is not accessible to iNOS (132). Hardy and May (244) have recently reported a high correlation between L-arginine uptake and L-arginine concentration in bovine aortic endothelial cells (see Ref. 132) and found that a fraction of intracellular L-arginine may reside in a compartment limited by transport of L-arginine.

Omission of L-arginine from culture media closely mimics the experimental conditions employed originally by Palmer et al. (451) to monitor basal and agonist-stimulated NO production from superfused microcarrier cultures of porcine aortic endothelial cells. When Bogle et al. (66) assayed unidirectional transport of L-arginine, L-lysine, and L-ornithine in porcine aortic endothelial cells under similar experimental conditions, deprivation of L-arginine enhanced cationic amino acid transport activity. This increase in transport activity may reflect adaptive responses in system y+ and potentially system y+L activity, although the involvement of system y+L was not investigated. Further studies are required to establish whether amino acid starvation alters mRNA levels for CAT-1, CAT-2B, y+LAT1, and y+LAT2 in different endothelial cell types and whether potential changes in cationic transporter expression influence endothelium-derived NO production.

### B. Effects of Hypoxia

Exposure of pulmonary artery endothelial cells to 0% oxygen for 4 h reduces L-arginine transport via system y+ by ~32% (60). Longer term hypoxia (5% O2, 3–5 wk) results in an irreversible loss of intracellular L-arginine and reduced Vmax for L-arginine transport via systems y+ and B0+, and CAT-1 the key transporter mediating L-arginine uptake in porcine pulmonary artery endothelial cells (668). A comparison of L-arginine transport in porcine pulmonary artery endothelial cells and membrane vesicles initially attributed the hypoxia-induced inhibition of system y+ activity to a membrane depolarization (670). Subsequent studies with the same cell type revealed that the CAT-1 transporter interacts with the actin-binding protein fodrin, which has high-affinity binding sites for integral membrane proteins (669). Increased fodrin proteolysis in long-term hypoxia was thought to disrupt the functional association between CAT-1 and actin microfilaments, resulting in an inhibition of L-arginine transport (669). Because CAT-1 and eNOS can colocalize in endothelial caveolae (396), Zharikov et al. (671) speculated that modifications of the actin cytoskeleton would alter not only L-arginine transport via system y+ but also potentially NO production. When these authors treated porcine pulmonary artery endothelial cells with agents known to target the actin cytoskeleton (swinholide A/Swish, a microfilament-disrupting marine toxin, and jasplakinolide/Jasp, a microfilament-stabilizing agent), Swinh (50 nM, 2 h) decreased L-arginine transport and NO production, whereas Jasp (100 nM, 2 h) reversibly increased activity of the L-arginine-NO pathway. Because changes in L-arginine transport were not paralleled by changes in CAT-1 transporter expression (or eNOS protein content), these authors concluded that alterations in the cytoskeleton in response to Swinh or Jasp either weakened or strengthened the interaction of CAT-1 with proteins involved in regulating transporter activity.

An overview of the effects of hypoxia on gene expression and activity of eNOS is beyond the scope of this review, and we refer readers to a recent study which compared the effects of hypoxia (1–10% O2, 6–24 h) and metabolic inhibitors (DNP, rotenone, 2-deoxyglucose) on eNOS mRNA and protein levels and NO synthesis in porcine aortic endothelial cells (267). Exposure of endothelial cells to 3% O2 for 12 h increased basal NO production approximately twofold, with increases in eNOS mRNA expression inversely proportional to Po2. Hypoxia-mediated increases in eNOS mRNA expression were mimicked by inhibitors of cellular metabolism and paralleled by increases in e-jun expression, leading these authors to conclude that hypoxia-induced increases in the cellular NAD(P)H/NAD(P) ratio enhances eNOS expression via redox-sensitive AP-1-mediated transcription. In summary,
the L-arginine/NO signaling pathway can be modulated by redox-sensitive mechanisms and agents known to affect the actin cytoskeleton. Further studies with endothelial cells from the pulmonary circulation and other peripheral vascular beds are necessary to determine whether hypoxia affects L-arginine transport and NO production similarly in different cell types.

Prolonged hypoxia causes an inhibition of oxidative phosphorylation leading to important adaptive increases in glucose transport (667). Hypoxia increases brain capillary density and blood-brain barrier transport of glucose and binding of cytochalasin B to GLUT1 in cerebral microvessels (247). Exposure of immature rats to hypoxia (2.5 h) has also been shown to enhance GLUT1 mRNA and protein levels in cerebral microvessels (606, see Table 4). Retinal hypoxia often precedes proliferative diabetic retinopathy, and an increase in intracellular glucose in retinal vascular cells is thought to be an important factor in the development of diabetic retinopathy (336). As retinal ischemia increases intracellular adenosine concentrations, which in turn activate hypoxia-inducible genes, Takagi et al. (580) investigated the effects of hypoxia on GLUT1 mRNA expression in bovine cultured retinal capillary endothelial cells. Exposure of endothelial cells to hypoxia caused time-dependent changes in GLUT1 mRNA levels, with an 8.9-fold increase detected after 12 h paralleled by a 2- to 3-fold increase in 2-deoxyglucose transport and immunoactive GLUT1. The hypoxia-mediated increase in GLUT1 transport activity was mediated in part via adenosine A2 receptors and the cAMP-PKA pathway, since antagonists of A2 purinoceptors and PKA suppressed hypoxia-induced GLUT1 expression.

Hypoxia also modulates glucose transport in human fetal and bovine aortic endothelial cells (358). Endothelial cells cultured under low oxygen conditions (14 mmHg) for up to 96 h exhibited increased rates of glucose transport and generated more lactic acid than normoxic cells. Moreover, activation of glucose transport by hypoxia required several hours and was associated with an increased expression of GLUT1 protein and mRNA. As inhibitors of oxidative phosphorylation mimicked the effects of hypoxia, these authors proposed that oxidative metabolism might serve as an important signal for adaptive responses in endothelial cells to hypoxia (358). In the absence of glucose, coronary microvascular endothelial cells become markedly sensitive to hypoxia (402).

C. Effects of Hyper- and Hypoglycemia

1. Amino acid transport

Adaptive responses in system y+ activity and NO synthesis have been identified in human umbilical vein endothelial cells exposed to elevated D-glucose (549, 550). In these experiments, hyperglycemia induced a time-dependent increase in system y+ activity (549, 550). Hypoxia increased the expression of L-arginine and L-lysine (data not shown) transporters and eNOS. Modulation of MAPK by glucose appears to affect L-arginine transport (1.3 vs. 2.6 pmol · min−1 · mg−1 protein) in human umbilical vein endothelial cells exposed to elevated glucose, suggesting a selective activation of cationic amino acid transport by hyperglycemia (550). Stimulation of L-arginine and L-lysine transport following exposure of endothelial cells to 25 mM glucose for 24 h was not paralleled by detectable changes in mRNA levels for CAT-1 or CAT-2B (380) or membrane potential (550). However, our recent experiments in human umbilical vein endothelial cells using real-time RTPCR have established that CAT-2B mRNA levels increase twofold after only 4-h exposure to 25 mM glucose, returning to basal levels within 8–24 h. These changes in CAT-2B mRNA were paralleled by increases in activity, protein, and mRNA levels for eNOS (203). Although CAT-2B protein levels have not been determined in human umbilical vein endothelial cells, it seems likely that increased cationic amino acid transport in response to elevated glucose was the consequence of increased transporter expression.

Elevated basal levels of intracellular Ca2+ (65 vs. 136 nM) in human umbilical vein endothelial cells exposed to high glucose are consistent with the time- and concentration-dependent increase in NO production (Fig. 7C) and eNOS protein levels (380). A similar finding has been reported in human aortic endothelial cells treated with 22 mM glucose for 5 days (145). Our preliminary studies in human foreskin microvascular endothelial cells confirm that hyperglycemia (25 mM, 24 h) also increases the Vmax for L-arginine transport (1.3 vs. 2.6 pmol · min−1 · μg protein−1 · mg−1) (L. Sobrevia and G. E. Mann, unpublished data). Interestingly, in rat islet capillary endothelial cells, production of nitrite and L-citrulline was increased significantly by elevated glucose (574).

We hypothesize that increased delivery of arginine via CAT-2B, and potentially y+L, sustains the increased activity of eNOS in human endothelial cells exposed to hyperglycemia. Whether increased delivery of L-arginine is mediated via cationic transporters associated with membrane caveolae and/or the plasma membrane remains to be elucidated. As members of the p42/p44 MAPK cascade may be sequestered within cholesterol-sensitive microdomains in the plasma membrane (see Refs. 10, 182, 458), our recent findings that elevated glucose activates p42/p44MAPK in human umbilical vein (413) and bovine retinal (S. Vine, R. Chibber, and G. E. Mann, unpublished data) endothelial cells provide a basis for investigating the role of MAPK in the regulation of endothelial CAT transporters and eNOS. Modulation of MAPK by glucose ap-
pears to vary in endothelial cells from different vascular beds, since in bovine pulmonary artery endothelial cells elevated glucose (25 mM) increased JNK1 and ERK5 activity without affecting the activity of either p42/p44 or p38 MAPKs (355). Moreover, activation of ERK5 by elevated glucose was unaffected by inhibition of aldose reductase with 0.4 mM sorbinil but inhibited significantly by pretreatment of cells with the antioxidant N-acetylcysteine, while glucose flux through the hexosamine pathway generating glucosamine-6-phosphate (see sect. II A) contributed to JNK1 activation.

2. Glucose transport

Hyperglycemia has been implicated in the pathogenesis of micro- and macrovascular complications in diabetes (reviewed in Refs. 133, 471, 549), yet little is known concerning the regulation of glucose transporters in endothelial cells (see Table 5). Glucose transport via GLUT1 in the blood-brain barrier of spontaneously hyperglycemic diabetic mice is downregulated compared with normoglycemic CD-1 mice (139), suggesting that prolonged hyperglycemia in vivo decreases blood-brain barrier glucose transport. Streptozotocin-induced diabetes, but not dietary-induced hyperglycemia, is also associated with a reduction in GLUT1 expression in rat brain capillaries (472). As palmitoylation of GLUT1 was increased in hyperglycemic and diabetic rats (472), this implies that palmitoylation may be involved in the modulation of glucose transporters in hyperglycemia. The Oldendorf intracarotid injection method (BU1 uptake index) has recently been employed to document an increase in 18F-2-deoxy-D-glucose uptake within a few minutes of initiating seizures in anesthetized rats (142). Thus seizure activity capable of depleting brain glucose levels within distinct regions of the brain may well elevate glucose transport across the blood-brain barrier endothelium. High glucose downregulates glucose transport and GLUT1 protein in retinal pericytes, but not in endothelial cells, perhaps reflecting a selective action of hyperglycemia on retinal pericytes in vivo (376).

Exposure of bovine aortic endothelial cells to elevated glucose (1.2–22 mM, 24 h) has negligible effects on

![FIG. 7. Hyperglycemia-induced activation of the L-arginine-nitric oxide signaling pathway in HUVEC. A: basal rates of l-arginine transport (100 μM, 1 min) via system y\(^{-}\) were measured after incubating HUVEC for specified times in culture medium 199 containing serum and either 5 mM (○) or 25 mM D-glucose (●) for 0–48 h. Cells were then cultured for a further 48–96 h in either 5 or 25 mM D-glucose. When cells were cultured in the presence of 5 mM D-glucose + 20 mM D-mannitol (as an osmotic control), transport of l-arginine was unaffected [data not shown; see Fig. 1 in Sobrevia et al. (550)]. L-Arginine influx was measured over 1 min during incubation of cells with a HEPES-buffered Ringer solution. B: treatment of cells with elevated D-glucose for 24 h induces a concentration-dependent stimulation of L-arginine transport (100 μM, 1 min) with half-maximal stimulation occurring at 11.5 ± 3.3 mM glucose concentration. C: cGMP levels were determined in cell extracts after incubating cells for 24 h in medium 199 containing serum and either 5 mM (control) or 25 mM D-glucose. Basal (data not shown) and 25 mM D-glucose-stimulated cGMP production was abolished during coincubation of cells with Nω-nitro-L-arginine methyl ester (L-NAME, 100 μM), a selective NO synthase inhibitor that does not inhibit endothelial cell arginine transport (70, 513). [Data replotted from Sobrevia et al. (550) and Mann et al. (380).]
2-deoxyglucose or 3-O-methylglucose transport or GLUT1 mRNA or protein levels, whereas high glucose decreased the $V_{\text{max}}$ for transport in bovine aortic and human smooth muscle cells (208). This differential regulation of glucose transport in primary cultures of smooth muscle and endothelial cells may contribute to the endothelial dysfunction associated with chronic hyperglycemia. In another study, incubation of bovine aortic endothelial cells with 22 mM glucose for 24–48 h has no effect on GLUT1 mRNA or protein levels or 3-O-methylglucose transport, whereas the rate of 2-deoxyglucose phosphorylation was significantly reduced (619). These findings contrast with the autoregulation of glucose uptake in fibroblasts, muscle, and glial cells. The increase in glucose transport in a bovine endothelial cell line GM7373, with no changes GLUT1 expression (221), raises concern whether this particular endothelial cell line reflects transport processes in primary bovine endothelial cell cultures. The insensitivity of glucose transport in endothelial cells to hyperglycemia remains puzzling.

Glucose starvation increases the $V_{\text{max}}$ for 3-O-methylglucose transport (increased 40–70%) and GLUT1 protein expression in bovine brain microvascular endothelial cells, with glucose restoring transport rates to control values within 48 h (581). In contrast, longer term exposure of cells to 25 mM glucose had no effect on transport (Table 5). Glucose starvation (11 vs. 5 mM, 48 h) also enhances cytochalasin B-sensitive 2-deoxyglucose uptake in cultured brain, adrenal capillary, and aortic endothelial cells (211). Although increases in transport activity in all three cell types correlated with increased GLUT1 expression, it is worth noting that adaptation of aortic and adrenal endothelial cells to glucose starvation and refeeding occurred much earlier than in brain capillary endothelial cells.

Rapid indicator dilution studies in humans have recorded an increase in glucose uptake (~55%) from blood to brain following a few days of starvation (463), although this study could not exclude the possibility that the increase was due to changes in permeability-surface area rather than the activity of facilitated glucose transporters. Similarly, Blomqvist et al. (61), using positron emission tomography in humans, demonstrated that moderate hypoglycemia (with no change in plasma insulin concentration or cerebral blood flow) increased facilitated transport of D-[U-13C]glucose. Because chronic hypoglycemia increases the expression of GLUT1 mRNA and protein in brain endothelium (see Table 5), this may account for the compensatory increase in glucose transport activity that occurs during low circulating glucose levels (333, 335). An increase in the number of glucose transporters would maintain cerebral glucose utilization at normal levels, with cerebral blood flow increasing more moderately than during acute hypoglycaemia (see Ref. 426).

D. Effects of Diabetes Mellitus

1. Amino acid transport

Detailed studies of the effects of diabetes on amino acid transport in endothelial cells are limited. In streptozotocin-diabetic rats, increased competition by elevated plasma amino acids was found to reduce influx of LNAA and L-lysine across the luminal membrane of the blood-brain barrier in vivo (81, 381). Human umbilical vein endothelial cells, derived from diet-controlled gestational diabetic pregnancies, exhibit decreased rates of L-leucine and thymidine incorporation during their log phase of growth in serum-containing medium (547). Gestational diabetes was associated with an increase in the $V_{\text{max}}$ for L-arginine transport and a ~2.5-fold increase in intracellular cGMP levels, which were abolished by the NO synthase inhibitor L-NAME (see Fig. 8C). Because fetal endothelial cells sustained a small membrane hyperpolarization (nondiabetic: ~70 mV vs. diabetic: ~78 mV), it is plausible that rates of L-arginine and L-lysine (data not shown) transport were elevated in part due to the sustained membrane hyperpolarization, increasing the driving force for cationic amino acid entry (66, 97, 307, 425, 547, 668). An intriguing finding is that fetal umbilical vein endothelial cells isolated from gestational diabetic pregnancies maintain their phenotypic alterations during culture in vitro for up to five cell passages (547), suggesting that fetal vascular endothelial cells may become “imprinted” in vivo (reviewed in Ref. 32). We have observed a similar preservation of “diabetes-induced” characteristics in fetal umbilical vein endothelial cells isolated from non-insulin- and insulin-dependent diabetic pregnancies, insofar as basal rates of L-arginine transport and NO production are increased compared with endothelial cells isolated from nondiabetic pregnancies. In view of the accumulating evidence of in utero programming of chronic disease in the offspring, it seems likely that altered cell signaling and transport properties observed in the fetal umbilical vasculature may provide valuable indicators of subsequent cardiovascular dysfunction in adults (107, 547, 564).

Insulin resistance is a common feature of non-insulin-dependent diabetes mellitus, and insulin-mediated vasodilation is impaired in patients with insulin resistance (reviewed in Refs. 33, 133, 471, 549). Hyperglycemia impairs the actions of insulin in umbilical vein endothelial cells isolated from gestational diabetic pregnancies (552). Basal rates of L-arginine transport and NO production are paradoxically increased in gestational diabetic cells cultured in medium containing 20% serum and 5.5 or 25 mM glucose (see Fig. 8, A and C). However, in endothelial cells from diabetic pregnancies, hyperglycemia abolished
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<td>Uregulation in long-standing diabetes without associated retinopathy</td>
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<td>↔ Glucose</td>
<td>↑ Glucose</td>
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<td>GLUT1 ↑ early SIV infection</td>
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<td>Bovine retinal endothelial cells</td>
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<td>Hypoglycemia</td>
<td>↑ Hypoglycemia</td>
<td></td>
<td>Hyperglycemia ↓ GLUT1 mRNA only in pericytes</td>
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<td>Bovine retinal endothelial cells</td>
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<td>↔ IGF-I</td>
<td>↑ IGF-I</td>
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<td>Bovine brain endothelial cells</td>
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<td>Bovine brain endothelial cells</td>
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<td>GLUT1 expression in brain endothelial cells ↑ by astrocyte or gloma cell conditioned medium</td>
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<td>$V_{max}$ and $K_m$ similar at luminal and abluminal membranes; Na/glucose transport detected</td>
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<td>Bovine aorta</td>
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<td>↑ Metformin</td>
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<td>Increased uptake of 2-deoxyglucose (ED$_{50}$ ~ 0.8–1 mM)</td>
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<td>Bovine pulmonary artery</td>
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<td>↑ 17β-Estradiol</td>
<td>↑ 17β-Estradiol</td>
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<td>↑ Hyperglycemia</td>
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<td>Rat placenta (fetal side)</td>
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Data are retabulated from reviews in Refs. 106, 324, 415, 438, 445, 516, 594. IGF-I, insulin-like growth factor I; PI, phosphatidylinositol; EGP, epidermal growth factor.

the dose-dependent inhibition of elevated l-arginine transport and cGMP accumulation induced by insulin (see Fig. 8, B and C). Thus changes in insulin sensitivity and/or its signaling cascade induced by hyperglycemia in gestational diabetes may result in an “insulin resistance” in endothelial cells derived from the fetal vasculature. The limited availability of endothelial cells from maternal resistance arteries prevents a direct comparison with established diabetes, known to lead to impaired endothelium-dependent relaxation (reviewed in Ref. 493).

2. Glucose transport

Glucose transport across the blood-brain barrier is downregulated in hyperglycemic rodents (139, 225). Application of the BUI technique has demonstrated that transport of glucose, 2-deoxyglucose, and 3-O-methylglucose is reduced in streptozotocin- and alloxan-diabetic rats, whereas transport of LNAA and cationic amino acids was unaffected (393). More recent studies of 3-O-methylglucose transport and [3H]cytochalasin B binding in the rat blood-brain barrier have confirmed that diabetes reduces the BUI for 3-O-methylglucose uptake without altering the number of cytochalasin B binding sites (414). Interestingly, treatment of diabetic rats with insulin restored BUI measurements to values in nondiabetic animals. A reduction in the number of perfused brain capillaries and/or alterations in membrane properties of the blood-brain barrier were thought to account for the decrease in non-carrier-mediated diffusion of glucose in streptozotocin-diabetic rats (248). In the rat blood-retinal barrier (retinal uptake index RUI), streptozotocin-diabetess apparently increases $K_m$ values for high-affinity ($K_m \sim 0.24$ mM) and low-affinity ($K_m \sim 7.8$ mM) glucose transport pathways (183). Localized upregulation of GLUT1 expression at the luminal surface of blood-retinal barrier in long-standing diabetes (Table 5) not affected by retinopathy may be associated with the deleterious effects of chronic hyperglycemia on the retinal microvasculature (333, 336).

As micro- and macrovascular endothelial cells exhibit metabolic and structural differences (321), GLUT1 may also be modulated differentially by elevated glucose. Experimental diabetes mellitus is associated with a down-regulation of GLUT4 expression and 2-deoxyglucose uptake in renal vascular smooth muscle cells, perhaps contributing to glomerular hyperfiltration and hypertension in the early stages of diabetes (385). In bovine cultured aortic endothelial and smooth muscle cells, metformin (an antidiabetic agent used to restore insulin responsiveness) causes a dose- and time-dependent increase in 2-deoxyglucose and 3-O-methylglucose uptake, which in smooth muscle cells was correlated with increased GLUT1 protein content (507). It is noteworthy that in this study the action of metformin on hexose transport re-
quired an exposure of 4–8 h and high concentrations of the drug ~2 mM. These authors concluded that metformin increases translocation of GLUT1 to the plasma membrane rather than affecting its intrinsic activity, confirming similar findings in adipocytes and cardiac myocytes (200, 391).

E. Effects of Insulin, Insulin-like Growth Factors, Vascular Endothelial Growth Factor, and Transforming Growth Factor-β

Specific receptors for insulin and insulin-like growth factors I (IGF-I) and II (IGF-II) have been identified in endothelial cells from micro- and macrovessels (see Refs. 29, 321). Vascular endothelial growth factor (VEGF) plays a key role in angiogenesis (198), and there are only limited studies of the effects of VEGF or transforming growth factor-β (TGF-β) on endothelial cell amino acid and glucose transport. The insulin receptor in endothelial cells is structurally similar to that in other cell types and is activated by tyrosine autophosphorylation. Internalization of insulin receptors in endothelial cells is activated by PMA following serine phosphorylation (238). Human endothelial cells exhibit only small increases in nucleic acid synthesis in response to insulin, although potent effects are observed in the presence of endothelial cell growth factors (321). Other effects of insulin in cultured endothelial cells include stimulation of glucose and amino acid transport, glucose oxidation, and increased protein and DNA turnover (29).

1. Amino acid transport

As illustrated in Figure 8, treatment of human umbilical vein endothelial cells with insulin (for 8 h in the presence of 20% serum) induces a concentration-depen-

![Figure 8](https://example.com/figure8.png)

FIG. 8. Regulation of the L-arginine-nitric oxide signaling pathway by human insulin in umbilical vein endothelial cells derived from human normal or gestational diabetic pregnancies. A: basal rates of L-arginine transport (100 μM, 1 min) via system y* in fetal umbilical vein endothelial cells were measured after incubating cells for 24 h in medium 199 containing serum and either 5 mM (○) or 25 mM (●) D-glucose, in the absence or presence of human insulin added during the last 8 h of the 24-h incubation period. In cells exposed to 5 mM D-glucose, insulin caused a concentration-dependent (0.1–10 nM) increase in arginine transport and also increased the V_{\text{max}} for saturable transport (4.9 vs. 12.2 pmol · μg protein $^{-1} \cdot \text{min}^{-1}$) with negligible changes in K_{m} (83 vs. 88 μM). Activation of L-arginine transport induced by insulin was prevented by cycloheximide (17 μM, 24 h) or preexposure of cells to 25 mM D-glucose (data not shown, see Fig. 2 in Ref. 552). B: L-arginine transport was elevated in umbilical vein endothelial cells derived from gestational diabetic pregnancies [compare basal transport rates (○) in A and B]. In the absence of insulin, elevated rates of L-arginine transport in cells cultured in 5 mM (○) or 25 mM (●) D-glucose were not inhibited by cycloheximide [data not shown, see Fig. 3 in Sobrevia et al. (552)]. Hyperglycemia (24 h) abolishes the inhibitory effects of insulin on elevated rates of arginine transport in cells cultured in 5 mM (○) or 25 mM (●) D-glucose and then preincubated in HEPES-buffered Ringer for 15 min with 0.5 mM 3-isobutyl-1-methylxanthine and 100 μM L-arginine. cGMP levels were then determined in endothelial extracts in the absence (open bars) or presence (solid columns) of human insulin (1 nM, 8 h) and/or L-NAME (100 μM, hatched bars). [Data replotted from Sobrevia et al. (549, 550, 552).]
high concentrations of insulin (1 nM) increase in basal rates of L-arginine and L-lysine transport, with negligible changes in transport for L-leucine, L-serine, and L-cystine (550). Activation of system y\textsuperscript{+} was paralleled by a ~2.5-fold increase in the production of endothelium-derived NO (Fig. 8C) and PGl\textsubscript{2} (data not shown), and it is likely that an intracellular mediator(s) generated after activation of the insulin receptor stimulated NO production (550, 552). The 2.5-fold increase in \(V_{\text{max}}\) for L-arginine transport via system y\textsuperscript{+} induced by insulin was abolished in cells adapted to elevated glucose (see Fig. 8B). The resting membrane potential, determined using the whole cell patch-clamp technique, was not altered by elevated glucose. Insulin receptors may be sequestered within plasmalemmal caveolae (237), raising the question whether insulin can acutely modulate the CAT-1, eNOS, and p42/p44\textsuperscript{MAPK} activity in endothelial cell caveolae. It is unfortunate that the only other study examining the actions of insulin on endothelial cell cationic amino transport pretreated porcine pulmonary artery endothelial cells with relatively high concentrations of insulin (1 \(\mu\text{M}\)). To our knowledge, there are no other reports correlating the acute actions of physiological concentrations of insulin on L-arginine transport and NO synthesis in endothelial cells. Thus comparative studies with human pulmonary artery, coronary artery, as well as aortic and venous endothelial cells are required to determine whether insulin directly modulates NO production in endothelial cells isolated from different vascular beds.

In this context, insulin activates Na\textsuperscript{+}-independent L-lysine transport in rat pancreas (416) and L-arginine transport in gastric mucosa (135) via a y\textsuperscript{+}-like system, CAT-1 expression in rat liver cells (655), and steady-state levels of CAT-1 mRNA (but not CAT-2A or CAT-2B) in rat coronary myocytes (534). Stimulation of the pentose cycle by insulin, as noted in bovine microvascular endothelial cells, will increase the supply of NADPH required for NO synthesis (651). These cellular actions of insulin are consistent with accumulating evidence that insulin activates the L-arginine-NO vasodilator pathway in skeletal muscle vasculature in humans, where this islet hormone may act both on endothelial and smooth muscle cells (reviewed in Ref. 33). Although targeted gene disruption of endothelial and neuronal NOS in mice renders these animals insulin resistant (526), the question remains as to whether reduced NO bioavailability attenuates insulin-mediated changes in skeletal muscle blood flow and glucose uptake.

In bovine pulmonary artery endothelial cells, TGF-\(\beta\)1 (2 ng/ml) causes a depletion of the key intracellular antioxidant GSH and an inhibition of L-cystine and L-glutamate, but not L-leucine, uptake (74). Although this study concluded that TGF-\(\beta\)1 decreased cellular GSH levels via a downregulation of L-cystine influx, prolonged pro-oxidant effects of this cytokine may have potentially downregulated expression of \(\gamma\)-glutamylcysteine synthetase, the enzyme responsible for the formation of GSH.

2. Glucose transport

Early studies of the effects of insulin on glucose transfer across the blood-brain barrier of fasting patients only noted a small increase in glucose flux (0.46 vs. 0.66 \(\mu\text{mol} \cdot \text{g brain}^{-1} \cdot \text{min}^{-1}\)) during infusion of insulin (259). Because net fluxes of glucose were unaltered, these authors concluded that insulin increased the efflux of glucose from the brain. Studies in conscious rats, examining rate constants for radiolabeled 3-O-methylglucose entry and exit from the brain, also detected only small changes in glucose fluxes (424). Short-term incubation of bovine brain microvascular endothelial cells with insulin (10 \(\mu\text{g/ml}, 20 \text{ min}\)) has no effect on uptake of either 3-O-methylglucose or 2-deoxyglucose, confirming similar studies in bovine retinal and brain microvascular endothelial cells (53, 56, 394), bovine aortic endothelial cell clone JVO17A (465), and human umbilical vein endothelial cells (137, 459). Only one study in bovine retinal endothelial cells (5) has documented a time- and protein synthesis-dependent increase in glucose transport in response to insulin (1 ng/ml). Because activation of glucose transport by insulin in rabbit coronary microvascular endothelial cells requires serum and glucose deprivation (220), this may explain the insulin insensitivity of glucose transport observed in the isolated dog brain in vivo and the majority of studies in cultured endothelial cells.

IGF-I (25 ng/ml) stimulates glucose transport in primary cultures of retinal endothelial cells via PI 3-kinase and PKC-dependent mechanisms, involving rapid (15-min) phosphorylation of mitogen-activated protein kinases (ERK1/2, see Ref. 157). Selective inhibition of PI 3-kinase or ERK phosphorylation abolished IGF-I-induced activation of glucose transport. IGF-I did not alter GLUT1 protein or mRNA levels, reminiscent of findings reported by this same group for VEGF-stimulated glucose transport in retinal endothelial cells (553). DeBosch et al. (157) suggested that IGF-I-induced stimulation of glucose transport in retinal endothelial cells involves the translocation of cytosolic GLUT1 to the plasma membrane. Activation of PI 3-kinase is an essential step in the pathway by which insulin stimulates glucose transport in a variety of tissues (see Ref. 479), and in this context, epidermal growth factor (EGF) has been reported to stimulate GLUT1 mRNA expression in cultured bovine corneal endothelial cells (287).

GLUT4 is expressed at high levels in fat and skeletal muscle (reviewed in Ref. 415). Increased expression of insulin-sensitive glucose transporter was originally reported in microvascular endothelial cells after administration of insulin to animals in vivo (615), although a subsequent study,
employing a number of different and specific antibodies, was unable to reproduce these results (541). Immunofluorescence and RT-PCR have confirmed that insulin-regulatable GLUT4 is highly expressed in smooth muscle cells of the rat renal microvasculature (80).

VEGF enhances microvascular permeability and modulates Ca\(^{2+}\) signaling in endothelial cells (reviewed in Ref. 403). In bovine retinal endothelial cells (553), VEGF stimulates 3-O-methylglucose transport via a PKC-\(\beta\)-mediated translocation of preexisting cytosolic GLUT1 transporters to the plasma membrane (see Fig. 9). The ability of LY379196, a selective inhibitor of PKC-\(\beta\), to abolish VEGF-stimulated uptake of 3-O-methylglucose implicates PKC-\(\beta\) as the isoform in glucose-mediated changes in retinal blood flow and permeability in diabetes (553). This action of VEGF on GLUT1 is reminiscent of the actions of insulin in GLUT4-sensitive tissues. Previous studies in a bovine aortic endothelial cell clone JVO17A reported that VEGF at comparable concentrations caused an approximately threefold increase in 2-deoxyglucose uptake and a fivefold increase in GLUT1 transcript (465). Sone et al. (553) suggested that the discrepancy in the action of VEGF on GLUT1 mRNA levels may be due to the fact that bovine retinal endothelial cells possess a threefold higher density of VEGF receptors compared with bovine aortic endothelial cells (592). Alternatively, inherent differences in retinal microvascular and peripheral microvascular endothelial cells may account for the differential effect of VEGF.

F. Effects of Thyroid Hormones and 17\(\beta\)-Estradiol

Impaired brain development and function have been associated with iodine deficiency, yet only limited studies have examined the role of thyroid hormones on glucose transport across the blood-brain barrier. Chronic maternal dietary iodine deficiency results in significant decreases in the brain uptake index for 2-deoxyglucose measured in brain of rat pups (572), implying that impaired glucose transport in the brain of offspring may be secondary to an altered thyroid status. An imbalance in cerebral metabolism and thyroid hormone levels has also been implicated in the susceptibility of the genetic epilepsy-prone rat to seizures (500).

Treatment of ovariectomized rats with 17\(\beta\)-estradiol for 12–14 days causes a dose- and time-dependent increase in 2-deoxyglucose uptake and GLUT1 protein expression in brain microvessels (528). GLUT1 mRNA increased transiently after 15 min and then returned to basal levels within 2 h, leading these authors to conclude that 17\(\beta\)-estradiol modulated both GLUT1 protein and mRNA levels. To our knowledge there are no reports discriminating the genomic and nongenomic actions of sex steroids on endothelial cell amino acid transport. Genomic activation of eNOS by 17\(\beta\)-estradiol is well documented (reviewed in Refs. 497, 498). Accumulating evidence indicates that estrogens can elicit acute vascular responses that do not require transcription or protein synthesis: 1) stimulation of NO production via Ca\(^{2+}\)-independent or -dependent mechanisms involving activation of protein tyrosine kinases (108, 226, 317) and 2) inhibition of L-type Ca\(^{2+}\) and activation of maxi K\(^{+}\) channels in vascular smooth muscle cells (499, 605). In view of the potential cardioprotective action of estrogen (and phytostrogens) in premenopausal women, it would be of interest to investigate potential mechanisms by which sex steroids modulate glucose and amino acid transport and NO synthesis in endothelial cells, and whether eNOS activation is limited by L-arginine supply via membrane caveolae coexpressing estrogen receptor \(\alpha\) (112, 317).

G. Developmental Changes

Structural changes in brain cerebral capillaries during development have implications for amino acid and glucose transport rates across the luminal membrane of the blood-brain barrier, and we refer readers to an out-
AMINO ACID AND GLUTOSE TRANSPORTERS IN VASCULAR CELLS

standing handbook on the physiology and pharmacology of the blood-brain barrier (75). In the context of this review, we have selected only a few studies that highlight changes in amino acid and glucose transport during development. Our searches of available databases have not found data on developmental modulation of amino acid or glucose transport in endothelial cells or microvessels isolated from peripheral vascular beds.

Positron emission tomography, using L-[11C]methionine as model system L analog, has revealed that transport decreases approximately sevenfold in patients aged 4.5 years exceeding values in adults (447). Between 2 and 71 years, with blood to brain transfer of methionine at 4.5 years exceeding values in adults (447). These findings are paralleled by similar developmental changes in animals. Experiments in neonatal and adult rats have reported an age-dependent decrease in amino acid transport across the blood-brain barrier (see Refs. 7, 28) and in isolated brain microvessels from 9-, 12-, and 21-day-old chick embryos, and 30-day-old chicken activities of neutral amino acid transport and alkaline phosphatase are also much higher in early embryonic microvessels (430). Although initial rates of L-valine and L-tyrosine uptake across the blood-brain barrier of C57BL/6 mice in vivo are unaffected by age, their incorporation into protein exhibits a marked decline between 3 and 8 mo of age (504). An increased influx of amino acids early in life, as documented in the majority of studies, seems essential to sustain an enhanced supply of precursor amino for cerebral protein synthesis during rapid brain growth.

The developing brain undergoes a series of functional and anatomic changes, which affects its rate of cerebral glucose utilization. Transport of glucose across the rabbit brain capillary endothelium in vivo is also modulated in the postnatal period (72, 181). In rabbits, 14, 28, and 70 days postpartum, immunoreactive GLUT1 protein was initially downregulated between birth and upregulated after 14 days, whereas GLUT1 mRNA levels remained unchanged from 14 to 70 days (181). Moreover, application of the BUI technique in newborn, 14-day-old suckling, 28-day-old weanling, and adult rabbits confirmed that glucose transport in the brain endothelium increased with age and was followed by increases in cerebral blood flow (140). Similar studies in rats have established that GLUT1 expression is upregulated in the developing blood-brain barrier (72, 607), with increases in glucose transport correlated with GLUT1 expression (607). In the rat, maturational increases in cerebral glucose utilization were more closely related to the pattern of nonvascular GLUT1 and GLUT3 expression, implying that during early postnatal development nonvascular expression of the glucose transporter proteins is rate-limiting for glucose utilization. In postmortem human brain samples, similar levels of GLUT1 were detected in the endothelium from preterm and newborn neonates and adults, suggesting that increased GLUT1 expression occurs earlier during development (384).

VIII. MODULATION OF AMINO ACID AND GLUTOSE TRANSPORT BY ENDOTOXIN, CYTOKINES, AND OXIDIZED LIPOPROTEINS IN ENDOTHELIAL CELLS

Proinflammatory cytokines, such as TNF-α, interleukin (IL)-1β, and interferon-γ (IFN-γ), are mediators of host responses to infection in inflammation (21, 326, 501). Reactive oxygen species generated in endothelial and smooth muscle cells in response to cytokines and LPS can function as intracellular signaling molecules, modulating permeability, leukocyte adhesion, actin filament organization, and redox-sensitive transducers such as Ras, Rac, PKC, and the transcription factors activator protein-1 and nuclear factor-κB (for overview, see Fig. 5 in Ref. 365). Numerous studies have implicated NO as a cytokine-induced vasodilator in bacterial sepsis, and increased plasma and urinary nitrite levels in humans are consistent with an overproduction of NO in inflammation (460). Activity of a Ca2+-independent isoform of NO synthase (iNOS), similar to that in first described in macrophages (260), has been identified in porcine aortic and mouse brain endothelial cells exposed to TNF-α, IFN-γ, and/or LPS (233, 481 and see Refs. 326, 521 for review). Generation of NO by iNOS occurs with a lag period of 2 h, reaching a maximum between 6 and 12 h, is inhibited by glucocorticoids and dependent on supply of extracellular L-arginine (18, 41, 67, 260).

Although it has been more difficult to demonstrate expression and activity of iNOS in human vascular endothelium, a Ca2+-dependent isoform has been reported in human umbilical vein endothelial cells, where enzyme activity is apparently increased due to an augmented supply of the limiting cofactor BH4 (496). Human postmortem retinal endothelial cells constitutively express eNOS, which is activated by Ca2+, while iNOS activity has been detected in retinal pericytes after exposure to LPS (111). As discussed in section VIII, A and B, cytokines modulate amino acid and glucose transport activity in endothelial cells.

A. Effects of Endotoxin and Cytokines on Amino Acid Transport

1. Neutral and anionic amino acid transport

Treatment of porcine pulmonary artery endothelial cells with either TNF-α or IL-1β induces a time- (~12 h) and concentration-dependent increase in the maximal transport capacity for l-glutamine entry via the Na+-dependent system ASC (554, see Table 6), confirming similar
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Endothelial Cell Type</th>
<th>TNF-α</th>
<th>IL-1β</th>
<th>Endotoxin</th>
<th>Summary of Findings</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>Human umbilical vein</td>
<td>↑ V&lt;sub&gt;max&lt;/sub&gt;</td>
<td></td>
<td></td>
<td>TNF-α (0.1–2 ng/ml, 8 h) ↑ V&lt;sub&gt;max&lt;/sub&gt; ~2-fold with no change in K&lt;sub&gt;m&lt;/sub&gt;, phorbol ester (TPA, 10–1,000 nM, 8 h) stimulated V&lt;sub&gt;max&lt;/sub&gt; ~4-fold and blocked by PKC inhibitor chelerythrine chloride (6.5 μM)</td>
<td>452</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>Human umbilical vein</td>
<td></td>
<td>↑ CAT-2B</td>
<td></td>
<td>TNF-α (3–30 ng/ml) ↑ CAT-2B, but not CAT-1, expression within 1 h and maintained over 24 h; nitrite levels only 1.7-fold in cells treated with TNF-α for 24 h</td>
<td>286</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>Human umbilical vein</td>
<td>↑ V&lt;sub&gt;max&lt;/sub&gt;</td>
<td>↑ V&lt;sub&gt;max&lt;/sub&gt;</td>
<td></td>
<td>TNF-α transport within 9–10 h via NEM-sensitive system y′ with no change detected for system y′ L; ↑ CAT-2B expression precedes stimulation of transport but not associated with nitrite production or iNOS expression</td>
<td>503</td>
</tr>
<tr>
<td>Porcine pulmonary artery</td>
<td>↑ Influx</td>
<td>↑ Influx</td>
<td></td>
<td></td>
<td>LPS (0.01–1 μg/ml, 12 h), TNF-α (500–1,000 U/ml, 8–12 h), or IL-1 (500–1,000 U/ml, 8–12 h) increased Na&lt;sup&gt;+&lt;/sup&gt;-independent (~2- to 4-fold) and Na&lt;sup&gt;+&lt;/sup&gt;-dependent (~2-fold) influx, LPS effect ↓ by anti-TNF-α antibody or IL-1 receptor antagonist</td>
<td>100</td>
</tr>
<tr>
<td>Bovine pulmonary artery</td>
<td>↑ Uptake</td>
<td></td>
<td></td>
<td>TNF-α (0.5 ng/ml) and LPS (0.5 μg/ml) for 24 h ↑ L-[&lt;sup&gt;14&lt;/sup&gt;C]arginine uptake 25%, ↑ CAT-2 mRNA, eNOS, and iNOS protein expression, nitrite and urea</td>
<td>427</td>
<td></td>
</tr>
<tr>
<td>Bovine aorta</td>
<td>↑ Influx</td>
<td>No change</td>
<td>↑ Influx</td>
<td></td>
<td>TNF-α (1–10 ng/ml) and LPS (0.3–3 μg/ml) increased Na&lt;sup&gt;+&lt;/sup&gt;-independent influx, no effects of IL-1β (1–10 ng/ml) or IFN-γ (1–10 U/ml). TNF-α (100 ng/ml, 24 h) ↑ V&lt;sub&gt;max&lt;/sub&gt; of low- and high-affinity transporters (see Table 7). Nitrite accumulation or ↑&lt;sup&gt;[14]&lt;/sup&gt;C]citrulline production not detectable</td>
<td>177</td>
</tr>
<tr>
<td>Rat aorta</td>
<td>↑ Influx</td>
<td>↑ Influx</td>
<td></td>
<td></td>
<td>TNF-α (100 ng/ml, 24 h) and/or IL-1β (10 ng/ml, 24 h) ↑ influx and nitrite accumulation, requires de novo protein synthesis, angiotensin (100 nM) slightly ↑ arginine influx and ↓ nitrite accumulation. CAT-2 but not CAT-1 mRNA ↑ in response to cytokines.</td>
<td>222</td>
</tr>
<tr>
<td>Rat cardiac microvascular</td>
<td>↑ Influx</td>
<td></td>
<td></td>
<td>IL-1β (4 ng/ml) + IFN-γ (500 U/ml) for 24 h ↑ L-arginine uptake 2-fold, paralleled by significant L-citrulline and nitrite production, both inhibited by dexamethasone (3 μM)</td>
<td>535</td>
<td></td>
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<tr>
<td>L-NMMA</td>
<td>Human umbilical vein SGHEC-7 cell line</td>
<td>↑ Influx</td>
<td></td>
<td>LPS (1 μg/ml, 6 h) stimulated influx inhibited by cycloheximide (1 μM) but not dexamethasone (1 μM)</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>Porcine pulmonary artery</td>
<td>↑ V&lt;sub&gt;max&lt;/sub&gt;</td>
<td></td>
<td>LPS (1 μg/ml, 8–12 h) induced ~2-fold ↑ V&lt;sub&gt;max&lt;/sub&gt;, required de novo protein synthesis and mRNA transcription, basal influx unaffected by cycloheximide</td>
<td>257, 258</td>
<td></td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>Human umbilical vein</td>
<td>↑ V&lt;sub&gt;max&lt;/sub&gt;</td>
<td></td>
<td>TNF-α (1,000 U/ml, 12 h) and IL-1 (1,000 U/ml, 12 h) ↑ V&lt;sub&gt;max&lt;/sub&gt; only 1.4- to 1.7-fold with no change in K&lt;sub&gt;m&lt;/sub&gt;, requires de novo protein synthesis and mRNA transcription, insensitive to PKC inhibition</td>
<td>554</td>
<td></td>
</tr>
<tr>
<td>L-Cystine</td>
<td>Human umbilical vein</td>
<td>↑ Influx</td>
<td></td>
<td>TNF-α (100 U/ml, 24 h) increased cystine transport (50 μM) 1.8-fold</td>
<td>M. Jay, R. C. M. Siow, H. Sato, and G. E. Mann, unpublished data</td>
<td>454</td>
</tr>
</tbody>
</table>

L-NMMA, N<sup>ω</sup>-monomethyl-L-arginine; TNF-α, tumor necrosis factor-α; IL-1, interleukin-1; LPS, lipopolysaccharide; NEM, N-ethylmaleimide; IFN-γ, interferon-γ.
findings from this group on LPS-stimulated L-glutamine transport (257, 258). Activation of L-glutamine transport was dependent on de novo protein and RNA synthesis, and as L-glutamine supplementation protected pulmonary artery endothelial cells from H$_2$O$_2$ injury (264), these adaptive changes in transport may serve to protect the lung microvasculature from endotoxin damage. In human umbilical vein endothelial cells, TNF-α (10 ng/ml) induces a time-dependent (4–24 h) increase in Na$^+$-independent (but not Na$^+$-dependent) L-glutamate transport activity, which is blocked by pretreatment of cells with actinomycin D and cycloheximide and unaffected by activators of PKC (454). It is unfortunate that this study did not further explore the involvement of anionic amino acid transport system x$c^-$ (407) in TNF-α-induced increases in L-glutamate transport, since system x$c^-$ would be activated by intracellular reactive oxygen radicals generated in response to TNF-α (21, 26). In this context, our preliminary experiments with human umbilical vein endothelial cells indicate that L-cystine transport via system x$c^-$ is stimulated approximately twofold after treatment of cells with TNF-α for 24 h (Table 6).

2. Cationic amino acid transport

Experiments using a human umbilical vein endothelial cell line SGHEC-7 were the first to correlate the effects of LPS (1–10 μg/ml) on NO synthesis and cationic amino acid transport, using radiolabeled L-NMMA as a substrate (69). Incubation of SGHEC-7 cells for 6 h with LPS caused an 80% increase in L-NMMA transport and a time-dependent accumulation of nitrite in the culture medium (see Table 6). LPS-induced activation of L-arginine, similar to reports in J774 macrophages and rat aortic smooth muscle cells (see sect. IxA), was blocked by cycloheximide and unaffected by dexamethasone. In contrast, studies in rat pulmonary artery endothelial cells reported that dexamethasone inhibited Na$^+$-independent L-arginine transport (453). Studies with rat cardiac microvascular endothelial cells have shown that IL-1β (4 ng/ml) in combination with IFN-γ (500 U/ml) stimulates L-arginine uptake; CAT-1, CAT-2B, and CAT-2A expression; and nitrite production (535). Cytokine-mediated increases in cellular BH$_4$ levels were reduced 12-fold in cardiac endothelial cells coincubated with dexamethasone (3 μM), suggesting that dexamethasone inhibits BH$_4$ synthesis by preventing induction of GTP cyclohydrolase I (496, 535, 637). Unpublished findings from Simmons et al. (535) suggest that L-arginine concentrations in cardiac endothelial cells are significantly depleted (50–90%) by cytokines and dexamethasone, reemphasizing the possibility that intracellular L-arginine could become rate limiting for iNOS. Glucocorticoids may thus limit L-arginine availability by inhibiting cytokine-activated CAT transporters (e.g., CAT-2B promoter contains a putative AP-1 site that may be repressed by glucocorticoids) and/or the induction of argininosuccinate synthase, necessary for the de novo synthesis of L-arginine (535). The differential effect of dexamethasone on cytokine-activated CAT transporters highlights potential differences in metabolism and/or signaling cascades in different vascular cell types.

In porcine pulmonary artery endothelial cells, LPS may stimulate system γ$^+$ activity through an autocrine release of TNF-α and IL-1, since pretreatment of cells with TNF-α antibodies or an IL-1 receptor antagonist attenuated the actions of LPS (109). Stimulation L-arginine uptake by TNF-α and IL-1 required de novo RNA and protein synthesis and was attributed primarily to entry via the Na$^+$-independent system γ$^+$ (Table 6). Unfortunately, this study did not specify whether IL-1α or -1β was used, nor did it correlate alterations in L-arginine transport with NO production. As summarized in Table 6, studies with human umbilical vein endothelial cells confirmed that TNF-α induces a time-dependent activation of L-arginine transport, with inhibition of PKC by chelerythrine chloride attenuating the effects of TNF-α (452). Subsequent experiments with the same cell type revealed that TNF-α-mediated increases in CAT-2B (but not CAT-1) mRNA are paralleled by increased nitrite accumulation (286). This latter study speculated that the 1.7-fold increase in nitrite production in TNF-α-treated endothelial cells was due to diminished L-arginine availability as a result of coinduction of arginase, known to be expressed in endothelial cells (94, 201, 348, 666).

Cytokines have been implicated in the induction of CAT-2B and iNOS in the aorta of rats with heart failure (562) and the activation of system γ$^+$ in erythrocytes and system γ$^+$L in platelets obtained from patients with chronic renal or heart failure (see Refs. 89, 241, 399–401). Molecular approaches have recently identified RT-PCR products for CAT-1, CAT-2B, γ$^+$LAT1, and γ$^+$LAT2 in TNF-α-stimulated human umbilical vein endothelial cells, with cytokine treatment only increasing CAT-2B expression after 3 h (503). TNF-α and LPS had no effect on the expression or activity of system γ$^+$L and actually decreased CAT-1 expression after 6 h. Although a sensitive fluorometric assay was used to determine nitrite levels, this study could not detect changes in NO production. The discrepancy in TNF-α-stimulated NO production in umbilical vein endothelial cells may reflect differences in the culture and assay conditions, but perhaps more importantly differences in the availability of the cofactor BH$_4$ (see Refs. 496, 535, 637).

In summary, stimulation of L-arginine transport in endothelial cells in response to LPS and/or cytokines seems to be restricted to the cationic transport system γ$^+$, with negligible changes in L-arginine transport via system B$^{0,+}$ or system y$^-$L (109, 177, 503). Advances in our understanding of the L-arginine-NO signaling pathway and availability of different cDNAs provide a basis for corre-
lating cytokine-mediated changes in \( \text{L-arginine} \) transport, NO production, and expression of CAT transporters, NOS isoforms, and GTP cyclohydrolase I in endothelial cells from cerebral, retinal, and peripheral vascular beds.

**B. Effects of Endotoxin and Cytokines on Glucose Transport**

There are only limited studies on the effects of LPS or proinflammatory cytokines on glucose transport in endothelial cells. Treatment of bovine aortic endothelial cells with TNF-\( \alpha \) (40 pM) and/or VEGF (100 pM) increases hexose transport and GLUT1 mRNA levels (454). Exposure of rat hepatic endothelial cells to LPS for 7 or 22 h causes a 10- to 25-fold increase in GLUT1 mRNA levels, while GLUT1 mRNA expression in parenchymal cells is low and insensitive to LPS (559). The reported upregulation of other antioxidant enzymes in these hepatic endothelial cells in response to LPS provides an adaptive mechanism for removing reactive oxygen radicals released by activated sinusoidal phagocytes.

**C. Effects of Oxidatively Modified Low-Density Lipoproteins and Lysophosphatidylcholine**

Pretreatment of human umbilical vein endothelial cells with oxidatively modified low-density lipoproteins (LDL) has no effect on \( \text{L-arginine} \) transport (100 \( \mu \)M, 1 min) assayed subsequently in Krebs-Henseleit buffer in the absence of LDL (293). However, in this study, pretreatment of cells with highly oxidized LDL (1–10 \( \mu \)g protein/ml for 1–24 h), but not native or mildly oxidized LDL, significantly inhibited histamine-stimulated NO and PG\( \text{I}_2 \) production. In this same study, butylated hydroxytoluene-treated native LDL (300 \( \mu \)g protein/ml), which has been reported to uncouple \( \text{L-arginine} \) metabolism from NO formation resulting in an increased formation of superoxide anions via eNOS (476), also had no effect on basal rates of \( \text{L-arginine} \) transport or NO synthesis (293). In contrast, pretreatment of bovine aortic endothelial cells deprived of \( \text{L-arginine} \) (16 h) with much higher concentrations of either oxidized LDL (100 mg protein/ml) or lysophosphatidylcholine (50–100 \( \mu \)M) inhibited \( \text{L-arginine} \) transport via CAT-1 without affecting ADP-induced Ca\(^{2+} \) transients assayed in fluo 3-loaded cells (314). The inhibition of NO production caused by lysophosphatidylcholine was partially reversed upon refeeding cells with 10 mM \( \text{L-arginine} \) but not \( \text{L-lysine} \), and these authors implicated the recruitment of a low-affinity, but high-capacity, transport pathway for \( \text{L-arginine} \) as a potential explanation for the partial restoration of NO synthesis (Fig. 10).

Although \( \text{L-arginine} \) transport was also inhibited in the intima of intact bovine aorta, it is worth noting that the LDL concentrations employed were ~1,000-fold higher than the physiological concentrations used in studies with umbilical vein endothelial cells (293). Apart from species differences, experiments in \( \text{L-arginine-deprived} \) bovine aortic endothelial cells (314) may have limited substrate supply to sustain NO production.

Nonetheless, studies in vivo have shown that \( \text{L-arginine} \) supplementation partially restores endothelial-derived NO release in hypercholesterolemic animals and humans (148, 224), suggesting that at significantly elevated plasma concentrations (>1–2 mM) \( \text{L-arginine} \) delivery may be increased via a low-affinity transport system(s). Figure 10 illustrates the possibility that at significantly elevated plasma \( \text{L-arginine} \) concentrations, entry of \( \text{L-arginine} \) may be mediated predominantly via a high-capacity cationic transport system providing substrate for eNOS (314). An alternative explanation for the inhibitory effects of oxidized LDL on eNOS activity is the finding that oxidized LDL, but not native or high-density lipoprotein (HDL), serves as an acceptor for cholesterol (59). Depletion of cholesterol from plasmalemmal caveolae leads to a translocation of eNOS to an intracellular compartment and impaired agonist-induced activation of the enzyme. The scavenger receptor CD36 was shown to mediate the effects of oxidized LDL on the cholesterol composition of caveolae and eNOS activation, with concentrations of HDL as low as 10 \( \mu \)g protein/ml replacing the sterol lost from caveolae upon exposure of cells to oxidized LDL (600). As suggested by these authors, the ability of HDL to protect against oxidized LDL-induced eNOS redistribution is most likely due to its direct action on endothelial signaling cascades localized to plasmalemmal caveolae.

**IX. REGULATION OF AMINO ACID AND GLUCOSE TRANSPORT IN VASCULAR SMOOTH MUSCLE CELLS**

**A. Modulation of Amino Acid Transport**

**1. Effects of starvation and glucose on amino acid transport**

Systems L and \( \text{y}^+ \) were originally described in vascular smooth muscle cells isolated by enzymatic digestion from rat aorta (363). \( \text{Na}^- \)-independent influx of \( \text{L-leucine} \) and \( \text{L-lysine} \) in quiescent smooth muscle cells deprived of serum was saturable and \( \text{trans-stimulated} \) in cells preloaded for 2 h with unlabeled amino acids (see Table 7).

Because \( \text{L-leucine} \) and other neutral amino acids partially
inhibited L-lysine influx, whereas L-lysine did not inhibit L-leucine uptake, these investigators examined whether additional transport pathways mediated uptake of these amino acids. The involvement of system b\textsuperscript{0,\textdagger} (610) and the high-affinity system y\textsuperscript{+}L, described originally in human erythrocytes (164), was excluded largely on the basis that inhibition of L-lysine uptake by L-leucine was non-competitive, with a \(~30\) fold difference in $K_i$ and $K_m$ values for L-leucine transport (363). This group further reported that L-leucine influx (but not efflux) via system L was enhanced in quiescent rat aortic smooth muscle cells deprived of glucose for 12–48 h, an effect reversed within 48 h of refeeding cells with glucose (364). System L transporter protein was thought to be regulated by an inhibitor peptide undergoing rapid turnover, with inhibition of its synthesis resulting in a stimulation of leucine transport activity. The delay in decreasing system L transporter activity after refeeding with glucose suggests that turnover of the transporter occurs more slowly.

Elevated glucose also increases the $V_{\text{max}}$ for Na\textsuperscript{+}-independent L-arginine transport in human umbilical artery smooth muscle cells cultured in 10% serum (548). The kinetics of influx and the selective inhibition of L-arginine transport (100 $\mu$M) by 10-fold excess L-homoarginine and L-lysine, but not L-alanine, suggested that influx was mediated predominantly by system y\textsuperscript{+} and not system b\textsuperscript{0,\textdagger}. Stimulatory effects of glucose were concentration dependent (5–25 mM) and time dependent (3–24 h), prevented by cycloheximide, and not mediated by changes in membrane potential.

**FIG. 10.** Modulation of endothelial cell L-arginine transport by oxidatively modified low-density lipoproteins (LDL) and lysophosphatidylcholine (LPC). At physiological plasma concentrations, entry of L-arginine is mediated preferentially via a high-affinity cationic amino acid transport system and can be metabolized via eNOS (NOS) to NO. Endothelium-derived NO synthesis and/or bioavailability is impaired in atherogenic vessels. Lysophosphatidylcholine and oxidized low-density lipoproteins (ox-LDL) were proposed by Kikuta et al. (314) to inhibit NO production in endothelial cells by inhibiting a high-affinity transport system for L-arginine. Increasing extracellular L-arginine concentrations to \(~10\) mM partially restores NO synthesis, with transport of L-arginine mediated preferentially via a low-affinity but high-capacity transport system(s). As L-arginine supplementation in vivo reverses impaired endothelium-dependent relaxation in hypercholesterolemic patients (see Ref. 148), this model proposes that impaired eNOS activity can be restored by increased transport of L-arginine via a low-affinity carrier. Alternative mechanisms for impaired NO production in hypercholesterolemia are discussed in section \textsection VIII. \[Redrawn from Fig. 10 in Kikuta et al. (314).\]
2. Effects of hypertonic stress and serum deprivation on neutral amino acid transport

Most mammalian cells are capable of adapting to changes in extracellular hypertonicity, undergoing regulatory volume increases (reviewed in Ref. 338). Neutral amino acid substrates of the Na\(^{+}\)-dependent system A have been implicated in the regulatory volume increase in human fibroblasts (98). Activation of system A transport activity by hyperosmotic stress involves either the synthesis of new carrier protein and/or regulatory proteins thought to enhance the transport activity of existing carriers (397) and activation of p42/p44MAPK (205). The following section provides a brief overview of the effects of hypertonic stress on endothelial and smooth muscle cell amino acid transport.

Recent evidence confirms that cultured endothelial cells respond to hypertonic stress by upregulating system

### TABLE 7. Kinetics of amino acid transport in cultured smooth muscle cells from peripheral vascular beds

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>System</th>
<th>Cell Type</th>
<th>(K_{m}) (\mu M)</th>
<th>(V_{\text{max}}) pmol (\cdot \mu g^{-1} \cdot \text{min}^{-1})</th>
<th>Condition</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>y(^{*})</td>
<td>Human umbilical artery</td>
<td>117 9.1</td>
<td>Basal</td>
<td></td>
<td>550</td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>155 15.5</td>
<td>+25 mM d-glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>75 8.3</td>
<td>+LPS and IFN-(\gamma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>106 2.89</td>
<td>+PDGF (2–50 ng/ml, 6 h)</td>
<td></td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>2,100 7.87</td>
<td>+PDGF (2–50 ng/ml, 6 h)</td>
<td></td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>2,000 12.85</td>
<td>+PDGF (2–50 ng/ml, 6 h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>45 0.7</td>
<td>Static conditions</td>
<td></td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>68 1.2</td>
<td>Cyclic stretch for 72 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>68 0.99</td>
<td>Basal</td>
<td></td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>148 2.61</td>
<td>+LPS and IFN-(\gamma)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>25 0.43</td>
<td>Basal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>30 0.55</td>
<td>+TNF-(\alpha)</td>
<td></td>
<td>177</td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>690 1.21</td>
<td>Basal</td>
<td></td>
<td>177</td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>666 1.27</td>
<td>+TNF-(\alpha)</td>
<td></td>
<td>177</td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>68 8.07</td>
<td>Basal</td>
<td></td>
<td>314</td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>60 1.42</td>
<td>+100 (\mu M) lysophosphatidylcholine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>847 21.71</td>
<td>Basal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>1,130 14.67</td>
<td>+100 (\mu M) lysophosphatidylcholine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Lysine</td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>50–90 0.5</td>
<td>Basal</td>
<td></td>
<td>363</td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>62 1.65</td>
<td>Basal</td>
<td></td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>107 2.89</td>
<td>+PDGF (2–50 ng/ml, 6 h)</td>
<td></td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>2,480 11.37</td>
<td>Basal</td>
<td></td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>2,400 10.0</td>
<td>+PDGF (2–50 ng/ml, 6 h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>128 1.03</td>
<td>Basal</td>
<td></td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>217 4.28</td>
<td>+100 (\mu M) lysophosphatidylcholine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Ornithine</td>
<td>y(^{*})</td>
<td>Rat aorta</td>
<td>50–90 0.5</td>
<td>Basal</td>
<td></td>
<td>363</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>A</td>
<td>Fetal rat aortic cell line A10</td>
<td>4.5</td>
<td>Basal (301 mosmol/kg(H_2O))</td>
<td></td>
<td>116</td>
</tr>
<tr>
<td>MeAIB</td>
<td>A</td>
<td>Fetal rat aortic cell line A10</td>
<td>7.5</td>
<td>Hypertonic stress (375 mosmol/kg(H_2O))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIB</td>
<td>A</td>
<td>Rat aorta</td>
<td>175 0.197</td>
<td>Basal</td>
<td></td>
<td>117</td>
</tr>
<tr>
<td>AIB</td>
<td>A</td>
<td>Rat aorta</td>
<td>136 0.423</td>
<td>+Colchicine (2 (\mu M))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Proline</td>
<td>A</td>
<td>Human renal mesangial cells</td>
<td>1,179 1.01</td>
<td>↑ By 10 (nM) insulin</td>
<td></td>
<td>437</td>
</tr>
<tr>
<td>L-Proline</td>
<td>A</td>
<td>Human renal mesangial cells</td>
<td>1,179 1.01</td>
<td>↑ By 10 (nM) insulin</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>L</td>
<td>Rat aorta</td>
<td>823 1.02</td>
<td>+PGE(<em>2) (1 (\mu M) for 48 h) altered (K</em>{m}) for Na(^{+})-dependent transport</td>
<td></td>
<td>185</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>L</td>
<td>Rat aorta</td>
<td>217 0.142</td>
<td>Basal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Leucine</td>
<td>L</td>
<td>Rat aorta</td>
<td>233 0.276</td>
<td>+TGF-(\beta)_1 (10 ng/ml for 24 h)</td>
<td></td>
<td>364</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>L</td>
<td>Rat aorta</td>
<td>150 3.2</td>
<td>Basal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Leucine</td>
<td>L</td>
<td>Rat aorta</td>
<td>150 5.2</td>
<td>+48-h glucose deprivation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Cystine</td>
<td>x(^{-})</td>
<td>Human umbilical artery</td>
<td>30 0.61</td>
<td>+Native LDL (100 (\mu g) protein/ml)</td>
<td></td>
<td>539</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>x(^{-})</td>
<td>Human umbilical artery</td>
<td>40 0.74</td>
<td>+Mildly oxidized LDL (100 (\mu g) protein/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Cystine</td>
<td>x(^{-})</td>
<td>Human umbilical artery</td>
<td>29 0.86</td>
<td>+Highly oxidized LDL (100 (\mu g) protein/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human aorta</td>
<td></td>
<td></td>
<td>30 0.61</td>
<td>Elevated glucose (11–27 mM) ↓ cystine uptake and GSH levels</td>
<td></td>
<td>578</td>
</tr>
</tbody>
</table>

AIB, 2-aminoisobutyric acid; LPS, lipopolysaccharide; IFN-\(\gamma\), interferon-\(\gamma\); PDGF, platelet-derived growth factor; TGF-\(\beta\), transforming growth factor-\(\beta\); TNF-\(\alpha\), tumor necrosis factor-\(\alpha\); LDL, low-density lipoprotein; GSH, glutathione.
A transport activity (155, 310, 466), with an increase in ATA2 mRNA levels preceding stimulation of MeAIB influx in porcine pulmonary artery endothelial cells (4). In human saphenous vein endothelial cells, cell volume recovery required several hours and was associated with a significant increase in the intracellular pool of amino acids (but not inorganic cations), in particular L-glutamine, L-proline, and L-glutamate (155). Because cell density can modulate amino acid transport activity and hypertonicity reduces porcine pulmonary artery endothelial cell density, MeAIB transport assayed at different densities could overestimate the effects of hypertonicity (466). Exposure of rat liver sinusoidal endothelial cells to hyperosmotic stress (405 mosM) increases mRNA levels for 

The effects of hypertonic stress on transport of 

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3. Effects of cytokines, endotoxin, and angiotensin II on cationic amino acid transport

Indirect evidence that L-arginine transport is rate limiting for NO synthesis in cytokine-activated vascular smooth muscle cells was initially based on the observation that cationic amino acids inhibited L-arginine-mediated relaxation of aortic rings preexposed to LPS (515). In this study, L-ornithine and L-lysine increased the contractile tone of endothelium-denuded aortic rings and attenuated the relaxation caused by extracellular L-arginine, suggesting that inhibition of L-arginine uptake reduced NO synthesis via iNOS.

Studies in cultured vascular smooth muscle cells have correlated the effects of cytokines and LPS on L-arginine transport and NO synthesis (see Table 7). In rat aortic smooth muscle cells cultured in 10% serum, LPS alone and in concert with IFN-γ, but not TNF-α or IL-1α, induced a time-dependent (8–24 h) and concentration-dependent increase in L-arginine transport and nitrite production, a stable breakdown product of NO metabolism (642). This study provided convincing evidence that induction of L-arginine transport (V_{max} increased 2-fold) and NO synthesis by LPS/IFN-γ were dependent on de novo protein synthesis and exogenous L-arginine (IC_{50} ~30 μM). Treatment of cells with the glucocorticoid dexamethasone abolished nitrite production in response to LPS/IFN-γ, without altering elevated rates of L-arginine transport (642), confirming earlier reports in cultured J774 macrophages, where IFN-γ only potentiated LPS-stimulated NO production (40, 67). Furthermore, elevated rates of L-arginine transport were unaffected after inhibition of iNOS, indicating that induction of system y+ and iNOS in rat aortic vascular smooth muscle cells are regulated differentially. In quiescent rat aortic smooth muscle cells deprived of serum for up to 48 h, TNF-α and IL-1β synergize to induce L-arginine transport, nitrite production, and iNOS and CAT-2 mRNA expression, all of which were abolished by pretreatment of cells with cycloheximide (177, 222).

Rat cultured aortic smooth muscle cells constitutively express transcripts for CAT-1, CAT-2A, and CAT-2B (46), consistent with earlier evidence for low- and high-affinity L-arginine transport activity in this cell type (642). Enhanced expression of these CAT transcripts in response to LPS and IFN-γ was paralleled by increased rates of L-arginine transport and NO synthesis via iNOS (46). Stimulated CAT expression and L-arginine transport occurred independently of PKC, p42/p44 MAPK, whereas activation of p38 MAPK was found to be involved intimately in the regulation of both L-arginine transport and induced
NO synthesis (46). Thus upstream signaling events associated with enhanced L-arginine transport in vascular smooth muscle may, in part, be distinct from those leading to induction of iNOS, and yet converge on the p38\textsuperscript{MAPK} signaling pathway. Hattori et al. (253) have also reported that CAT-1 and CAT-2B mRNA are expressed constitutively in rat aortic smooth muscle cells and that LPS and IFN-\(\gamma\) enhance L-arginine transport, NO production, and expression of both transcripts. Although Hattori et al. (253) failed to detect CAT-2A in unstimulated or stimulated smooth muscle cells, the increased expression of CAT-2A reported in rat aortic smooth muscle cells has been confirmed in coronary microvascular endothelial cells and cardiomyocytes activated with IL-1\(\beta\) and IFN-\(\gamma\) (534). Nevertheless, the physiological relevance of CAT-2A (\(K_m \geq 2 \text{mM}\)) in the vasculature is not immediately apparent, since blood vessels are exposed to a circulating cationic amino acid concentration of \(\sim 200 \text{ \mu M}\).

Angiotensin II (100 nM, 6–24 h) increases L-arginine and L-lysine transport and CAT-1 and CAT-2 mRNA levels in rat aortic smooth muscle cells (361). Activation of L-arginine transport by angiotensin II was mediated by system y\(^+\), since the stimulatory effects of angiotensin II were inhibited by excess L-lysine and L-ornithine, but not by MeAIB or BCH. Similar studies in rat aortic smooth muscle cells cultured in 10% serum demonstrated that L-arginine transport was mediated by Na\(^+\)-dependent (\(\sim 60\%\)) and Na\(^+\)-dependent (\(\sim 40\%\)) pathways, with inhibition profiles characteristic of systems y\(^+\) and Bo\(+\) (494).

Acute and chronic administration of angiotensin II (100 nM, 1 h and 4 days) inhibited Na\(^+\)-dependent arginine transport without affecting systems y\(^+\), A, or L (494). Furthermore, angiotensin II and activation of PKC partially inhibited IL-1\(\beta\)-stimulated nitrite production, although effects on L-arginine transport were not investigated. The presence of system Bo\(+\) in rat aortic smooth muscle cells and its inhibition by angiotensin II are at variance with the findings of Low and Grigor (361). Because neither of these studies performed detailed saturation and inhibition kinetics for L-arginine transport, it is difficult to resolve these discrepancies. However, in the case of glucose transport (298), differences in the degree of serum deprivation may have altered signaling cascades associated with the regulation of cationic amino acid transporters.

4. Effects of LDL and lysophosphatidylcholine on cystine and cationic amino acid transport

In rat aortic smooth muscle cells deprived of serum, lysophosphatidylcholine (100 \(\mu\)M) induces a biphasic change in L-arginine transport, with influx of L-arginine and L-lysine (50 \(\mu\)M) initially decreasing over the first 2 h and subsequently increasing over the next 6–24 h (178). Adaptive increases in \(K_m\) and \(V_{\text{max}}\) values for L-ornithine transport were only assessed over a limited concentration range (5–500 \(\mu\)M) and abolished by pretreatment with 2 \(\mu\)M actinomycin D (see Table 7). Stimulation of L-ornithine transport was attributed to a single carrier (unlike L-arginine, see Refs. 177, 180) yet associated with transient increases in CAT-1 (\(\sim 3\)-fold) and CAT-2 (\(\sim 20\)-fold) mRNA levels (178). Although mRNA levels decayed to near basal values within 4–8 h, transport of L-ornithine remained elevated, suggesting a delay required for translation, posttranslational changes, and eventual insertion of CAT protein into the cell membrane. These investigators concluded that inhibition of iNOS activity together with coinduction of CAT transporter activity, arginase, and ornithine decarboxylase would direct intracellular metabolism of L-arginine and L-ornithine to polyamine biosynthesis. Lysophosphatidylcholine was also shown to inhibit IL-1\(\beta\)-stimulated nitrite production and iNOS protein levels, yet changes in the activity and expression of CAT transporter isoforms in relation to NO production were not investigated.

Well-defined human Cu\(^{2+}\)-oxidized LDL preparations (reviewed in Ref. 492) have been used to investigate the effects of native, mildly, and highly oxidized LDL on L-arginine and L-cystine transport in smooth muscle cells cultured from medial explants of human umbilical artery (293, 539). Treatment of umbilical artery smooth muscle cells with either native, mildly, or highly oxidized LDL (1–100 \(\mu\)g protein/ml, 24 h), had no effect on basal transport rates for L-arginine, confirming our findings in human umbilical vein endothelial cells (293). Mildly and highly oxidized LDL, but not native LDL, increased the \(V_{\text{max}}\) for L-cystine transport via system x\(_c\)-dependent (human umbilical arterial smooth muscle cells (see Table 7 and Ref. 539). Pretreatment of umbilical artery smooth muscle cells with physiological concentrations of vitamin C (10–100 \(\mu\)M) abolished the adaptive increases in L-cystine transport and intracellular glutathione levels induced by oxidized LDL, providing the first evidence that vitamin C spares endogenous adaptive antioxidant responses in human vascular smooth muscle cells exposed to atherogenic lipids (539).

5. Effects of insulin, IGF-I, TGF-\(\beta\), and platelet-derived growth factor on amino acid transport

In many cell types, activation of system A transport activity by insulin is paralleled by cell proliferation (reviewed in Ref. 397). Studies in rat aortic smooth muscle cells have investigated the signaling cascades involved in the stimulation of AIB transport by insulin and IGF-I (437). Treatment of quiescent aortic smooth muscle cells with either insulin (\(\sim 10 \text{nM}, 3 \text{h}\)) or IGF-I stimulated AIB uptake (0.12 vs. 0.15 nmol \(\cdot\) mg protein\(^{-1}\) \(\cdot\) min\(^{-1}\)), which was prevented by cycloheximide but not actinomycin D, implying translational regulation. Although rat aortic
smooth muscle cells express both insulin and IGF-I receptors (insulin:IGF-I $\sim 1/100$), these authors concluded that insulin and IGF-I acted via their own receptors. Wortmannin inhibited both insulin-stimulated AIB uptake and PI 3-kinase with an $IC_{50}$ of $\sim 5-10$ nM; however, the lack of specificity of this inhibitor raises the question whether insulin-stimulated AIB transport is mediated only via the activation of PI 3-kinase. In the same smooth muscle cell type, IGF-I (10 ng/ml), platelet-derived growth factor (PDGF; 2 ng/ml), and TGF-$\beta$ (1 ng/ml) stimulated uptake of L-leucine, L-proline, and L-arginine via a mechanism involving PI 3-kinase (261).

Activation of rat aortic smooth muscle cells with TGF-$\beta$1 induces a time-dependent (4–24 h) increase in AT2A mRNA, which precedes the twofold increase in the $V_{\text{max}}$ for transport of L-proline (185), a key amino acid required for collagen synthesis (see Table 7). Because actinomycin D blocked the stimulatory actions of TGF-$\beta$1, these authors concluded that increased transporter expression involved transcriptional activation of the AT2A gene. A point worth noting is that a measurable fraction of L-arginine transport was mediated by an as yet unidentified Na$^+$-independent system. TGF-$\beta$1 also increases the activity and expression of CAT-1 and arginase I in rat aortic smooth muscle cells (180). In addition to increasing transport of L-arginine, TGF-$\beta$1 stimulated intracellular metabolism of L-arginine to L-ornithine for the synthesis of polyamines and L-proline. The inhibition of L-arginine transport observed over the first 4 h of exposure to TGF-$\beta$1 was attributed to a decrease in the affinity of the transporter. The lack of coinduction of CAT-2 by TGF-$\beta$1 contrasts with the reported actions of PDGF, lysophosphatidylcholine, and angiotensin II (176, 178, 361). PDGF treatment also stimulates proliferation of rat aortic smooth muscle cells and decreases the affinity and increasing transport capacity for L-ornithine (see Table 7). It remains to be established whether induction of ornithine decarboxylase activity by PDGF serves as a signal to increase the expression of CAT-2B transporters, thereby sustaining elevated rates of L-ornithine transport. Although this study demonstrated that inhibition of ornithine decarboxylase (ODC) with difluromethylornithine (DFMO) inhibited PDGF-induced polyamine synthesis, it remains unclear whether inhibition of ODC activity attenuated the PDGF-induced increases in CAT transporter activity and mRNA levels.

Attempts have recently been made to correlate CAT transporter mRNA levels with transport activity, yet there is a notable lack of information on alterations in CAT protein levels in response to cytokines and other mediators, perhaps reflecting the limited specificity of available antibodies for the different CAT transporter isoforms.

B. Modulation of Glucose Transport

1. Effects of elevated glucose

There are relatively few studies of glucose transport kinetics in vascular smooth muscle cells (Table 8). A facilitated transport system for 2-deoxyglucose and 3-O-methylglucose has been identified in bovine aortic and human arterial smooth muscle cells cultured in serum-containing medium (298). Transport activity was inhibited $\sim 90\%$ by D-glucose and cytochalasin B, unaffected by $\alpha$-methyl-$\beta$-glucoside (preferred substrate for the Na$^+$-glucose cotransporter), and only marginally reduced by phlorizin. Elevating glucose from 1.2 to 22 mM (24 h) decreased GLUT1 protein levels and the $V_{\text{max}}$ for 2-deoxyglucose transport. GLUT1 mRNA levels were paradoxically unaffected by changes in glucose concentrations, and GLUT2, -3, and -5 mRNAs could not be detected by Northern blot analyses. Similar findings have been reported in quiescent smooth muscle cells from rat aorta, where elevated glucose (20 mM, 24 h) decreased transport rates for 2-deoxyglucose and glucose (273). In the absence of serum, the decreased $V_{\text{max}}$ for glucose trans-

<table>
<thead>
<tr>
<th>Hexose</th>
<th>Transporter</th>
<th>Cell Type</th>
<th>$K_{\text{m}}$ $\mu$M</th>
<th>$V_{\text{max}}$, pmol $\cdot$ $\mu$g protein$^{-1}$$\cdot$ min$^{-1}$</th>
<th>Condition</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-DG</td>
<td>GLUT1</td>
<td>Human aorta</td>
<td>0.85–1.12</td>
<td>6–12*</td>
<td>1.2–22 mM D-glucose</td>
<td>298</td>
</tr>
<tr>
<td>2-DG</td>
<td>GLUT1</td>
<td>Bovine aorta</td>
<td>0.89–0.86</td>
<td>8–20*</td>
<td>1.2–22 mM D-glucose</td>
<td>298</td>
</tr>
<tr>
<td>2-DG</td>
<td>GLUT1</td>
<td>Rat aorta</td>
<td>1,140</td>
<td>0.03*</td>
<td>Basal, lean animals</td>
<td>561</td>
</tr>
<tr>
<td>2-DG</td>
<td>GLUT1</td>
<td>Rat aorta</td>
<td>960</td>
<td>0.06*</td>
<td>Basal, Zucker obese animals</td>
<td>507</td>
</tr>
<tr>
<td>2-DG</td>
<td>GLUT1</td>
<td>Bovine aorta</td>
<td>1,153</td>
<td>22.7*</td>
<td>Basal</td>
<td>507</td>
</tr>
<tr>
<td>2-DG</td>
<td>GLUT1</td>
<td>Bovine aorta</td>
<td>1,390</td>
<td>35.0*</td>
<td>+ Metformin (2 mM, 24 h)</td>
<td>478</td>
</tr>
<tr>
<td>2-DG</td>
<td>GLUT1</td>
<td>Bovine pericytes</td>
<td>840</td>
<td>0.128</td>
<td>Angiotensin II $\uparrow$ transport 2–6 h</td>
<td>629</td>
</tr>
<tr>
<td>$\alpha$-MG</td>
<td>SGLT2</td>
<td>Bovine pericytes</td>
<td>2,840</td>
<td>0.198</td>
<td>Basal</td>
<td>629</td>
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<tr>
<td>$\alpha$-MG</td>
<td>SGLT2</td>
<td>Bovine pericytes</td>
<td>1,980</td>
<td>0.492</td>
<td>Inhibited by phlorizin and captopril</td>
<td>629</td>
</tr>
<tr>
<td>$\alpha$-MG</td>
<td>SGLT2</td>
<td>Rat mesangial cells</td>
<td>1,980</td>
<td>0.492</td>
<td>By high glucose</td>
<td>627</td>
</tr>
<tr>
<td>$\alpha$-MG</td>
<td>SGLT2</td>
<td>Rat mesangial cells</td>
<td>1,390</td>
<td>0.30</td>
<td>By high glucose</td>
<td>627</td>
</tr>
</tbody>
</table>

* Note $V_{\text{max}}$ values recalculated assuming $10^6$ cells $\sim 200$ $\mu$g protein. $V_{\text{max}}$ values reported in Ref. 561 are actually in fmol $\cdot$ $\mu$g protein$^{-1}$$\cdot$ min$^{-1}$. 2-DG, 2-deoxyglucose; $\alpha$-MG, $\alpha$-methyl-$\beta$-glucoside.
port was associated with a decrease in GLUT1 transporter protein, even though intracellular glucose concentrations remained elevated in cells exposed to 20 mM glucose. Downregulation of GLUT1 transporter activity thus does not appear to normalize intracellular glucose levels in smooth muscle cells and may account for the toxicity of elevated glucose in diabetes mellitus (273). Another study in vascular smooth muscle cells has confirmed that elevated glucose downregulates GLUT1 transport activity and reported that stimulatory effects of angiotensin II on 2-deoxyglucose transport are attenuated at lower glucose concentrations (478). The responsiveness of smooth muscle cells to angiotensin II was independent of PKC but dependent on extracellular Ca$^{2+}$.

2. Effects of insulin, IGF-I, angiotensin II, and PDGF on glucose transport

Insulin stimulates glucose transport in rat aortic smooth muscle cells and A10 and A7r5 smooth muscle cell lines (560). Treatment of A7r5 cells with insulin (1 nM-1 μM) or IGF-I for 20 min increased glucose transport activity, which was inhabitable by cytochalasin B but independent of protein synthesis (560). In rat aortic smooth muscle cells deprived of serum, IGF-I was more potent than insulin in stimulating 2-deoxyglucose transport, and wortmannin, used as an inhibitor of PI 3-kinase, reduced IGF-I and insulin-stimulated glucose transport (207). Interestingly, as reported for L-arginine transport in human smooth muscle cells (548), the stimulatory actions of insulin on glucose transport in rat aortic smooth muscle cells were attenuated in cells preadapted to 25 mM α-glucose (207). Antidiabetic agents such as troglitazone and metformin, used to enhance insulin sensitivity in humans, have been reported to increase hexose transport and GLUT1 mRNA levels in human and bovine aortic smooth muscle cells, respectively (313, 507).

The effects of angiotensin II, EGF, thrombin, and arginine vasopressin on facilitated 2-deoxyglucose transport have been examined in rat aortic smooth muscle cells deprived of serum for up to 48 h (362). Angiotensin II and EGF caused a rapid (~30 min) and protein synthesis-independent activation of 2-deoxyglucose transport, while sustained (4 h) increases in transport required de novo protein synthesis, as evidenced by the two- to fourfold increases in GLUT1 mRNA levels. Physiological concentrations (~16 nM) of insulin failed to stimulate 2-deoxyglucose transport or potentiate the actions of angiotensin II or EGF. Because pretreatment of quiescent smooth muscle cells with actinomycin D prevented the rapid (~30 min) increase in GLUT1 mRNA in response to angiotensin II, these authors concluded that increased GLUT1 transcription resulted in increases in GLUT1 protein and glucose transport (362).

Treatment of rat aortic smooth muscle cells with PDGF (1–100 ng/ml) results in a biphasic stimulation of 2-deoxyglucose transport, with the delayed phase (6–8 h) of transport stimulation dependent on protein synthesis (369). Although GLUT1 is the predominant isoform in this cell type, this study did not establish whether stimulation of transport involved an intrinsic activation of existing carriers or a redistribution of GLUT1 transporters. Furthermore, cAMP analogs increased 2-deoxyglucose uptake but had no effect on PDGF-activated glucose transport, whereas 8-bromo-cGMP potently inhibited PDGF- and cAMP-stimulated transport. Thus cAMP and PDGF signaling cascades converge on a common effector molecule(s) sensitive to regulation by cGMP. Because protein kinase G levels in cultured smooth muscle cells were extremely low, it seemed unlikely that the inhibitory effects of cGMP were mediated via protein kinase G (369).

X. ROLE OF PLASMA MEMBRANE CAVEOLAE

Caveolae are specialized invaginations of the plasma membrane (50–100 nm diameter) also known as “lipid rafts” formed as a result of localized accumulation of cholesterol, glycosphinogolipids, and structural proteins (reviewed in Refs. 210, 536). Caveolin plays a key role in vesicular and cholesterol trafficking and has recently been implicated in regulating signal transduction at the plasma membrane. The discovery of highly organized signaling molecules localized to membrane caveolae, including system y ($\text{CAT-1}$), eNOS, argininosuccinate synthase, Ca$^{2+}$-ATPase, MAPKs, Src family of tyrosine kinases, Ras, several PKC isoforms, receptors for insulin, EGF, PDGF, VEGF, estrogen receptor $\alpha$, and heterodimeric G proteins (see Refs. 182, 195–197, 201, 212, 226, 229, 237, 289, 317, 347, 458) provides an opportunity for investigating the role of caveolin-1 in modulating the L-arginine-NO signaling pathway in vascular endothelium.

Govers and Rabelink (229) have cautioned that the molecular interactions between CAT-1 (system y$^+$), eNOS, G protein-coupled receptors, and other proteins in caveolae require further characterization, since many studies could only document the presence of these proteins rather than their functional interactions due to the limited solubility of caveolae. Nonetheless, cell culture experiments in vitro have confirmed molecular interactions between eNOS and caveolin-1, and recent studies in caveolin-1 gene knock-out mice revealed that Ach-mediated relaxation of endothelium intact aortic rings is significantly enhanced in caveolin-1$^{-/-}$ compared with wild-type mice (172, 485). As impaired vasconstrictor responses to phenylephrine were restored by treatment of aortic rings with L-NAME, this implies that basal NO production is enhanced in caveolin-1 null mice. Although eNOS activity appears to be hyperactivated in mice lacking membrane caveolae, neither of these studies deter-
determined whether CAT transporter activity was affected. Conversely, in vivo targeting of the caveolin-1 scaffolding domain mimics the inhibitory actions of a NOS inhibitor on vascular permeability and inflammation (92), confirming findings in vitro that a functional association between eNOS and caveolin-1 inhibits NOS activity.

The schematic model in Figure 11 illustrates a potential role for plasmalemmal caveolae as conduits for \( \text{L-arginine} \) supply to eNOS in endothelial cells in health and disease states such as hypercholesterolemia, hypertension, diabetes mellitus, and preeclampsia, all of which are characterized by oxidative stress and impaired endothelium-dependent relaxation (reviewed in Refs. 173, 365, 481). If systems \( \text{y}^{+} \) and \( \text{y}^{-} \text{L} \) (and potentially other amino acid transporters such as \( \text{xCT} \)) are associated with eNOS in membrane caveolae, this may help explain 1) why low micromolar concentrations of ADMA and \( \text{L-NMMA} \) accumulated in the plasma of patients with hypercholesterolemia or renal failure inhibit eNOS activity and endothelium-dependent relaxation (290, 368, 604); 2) why \( \text{L-arginine} \) supplementation reverses ADMA- and \( \text{L-NMMA} \)-mediated inhibition of NO production and endothelial dysfunction in hypercholesterolemic patients (136, 148), when intracellular and plasma \( \text{L-arginine} \) concentrations are well above the \( K_{m} \) for eNOS; and 3) why a patient with a rare autosomal defect in dibasic amino acid transport (mutations in the \( \text{SLC7A7} \) gene resulting in lysinuric protein intolerance) due to defective cationic amino acid transport in the proximal tubule and small intestine, see Ref. 598) presents with diminished plasma \( \text{L-arginine} \) (21 vs. 98 \( \mu \text{M} \)) and nitrite (10 vs. 34 \( \mu \text{M} \)) levels and impaired flow-mediated dilation, with all parameters largely normalized upon elevating plasma \( \text{L-arginine} \) concentrations to 250–300 \( \mu \text{M} \) (303). The accompanying commentary to this study (360) concurred that it provided novel insights into the role of the \( \text{L-arginine-NO} \) pathway in vivo, yet also raised two important questions, namely, by what mechanisms does \( \text{L-arginine} \) infusion augment NO production in vivo and is eNOS uncoupled to generate superoxide anions from molecular oxygen as a result of the reduced \( \text{L-arginine} \) availability? Interestingly, erythrocytes and fibroblasts from patients with lysinuric protein intolerance exhibit normal rates of \( \text{L-lysine} \) and \( \text{L-arginine} \) influx via system \( \text{y}^{+} \text{L} \) (73, 156).

**XI. CONCLUDING REMARKS**

Cerebral, retinal, and peripheral vascular endothelial cells express selective transport systems for hexoses and neutral, cationic, sulfonic, and anionic amino acids. Although high-affinity Na\(^{+}\)-dependent transport activity is generally restricted to the abluminal membrane of the endothelium of the blood-brain and blood-retinal barriers (see Table 2 and Fig. 5), endothelial cells in peripheral blood vessels express both Na\(^{+}\)-independent and Na\(^{+}\)-dependent transporters (see Table 3). To our knowledge, localization of these transporters to either luminal or abluminal membranes of the endothelium in peripheral blood vessels has not been studied employing the specific membrane fraction markers used to distinguish transport processes at either surface of the blood-brain or blood-retinal barriers. Endothelial cell amino acid transport is sensitive to hormonal and adaptive regulation, with insulin, gestational diabetes, amino acid deprivation, and TNF-\( \alpha \)-stimulating system \( \text{y}^{+} \text{L} \) transport activity in human fetal umbilical vein (66, 503, 547, 549, 550) and hypertonicity evoking a four- to sixfold increase in system A transport activity in human saphenous vein (155) and bovine/porcine pulmonary artery (310, 466). Glucose transport in brain, retinal, and peripheral vascular endothelial cells is principally insulin insensitive (see sect. \( \text{VI} \)) and mediated via GLUT1 Even though prolonged serum and glucose deprivation is required to detect insulin-induced transport.
stimulation of glucose transport in coronary endothelial cells (220), it is worth recalling that serum deprivation itself alters glucose transport activity in endothelial cells (298).

Proinflammatory cytokines, LPS, and growth factors coinduce the activity and expression of CAT transporter isoforms and iNOS in endothelial and smooth muscle cells (Tables 6 and 7), macrophages (302, 429), and peripheral blood mononuclear cells isolated from patients with sepsis (486); however, cross-talk between the multiple intracellular signaling cascades remains to be investigated. The finding that iNOS-mediated NO production is significantly reduced in peritoneal macrophages from CAT2−/− mice, strongly suggests a functional association between CAT2 and iNOS and a critical dependency of NO production on L-arginine delivery (see Ref. 429).

Our understanding of the mechanisms by which secreted mediators such as NO and cytokines modulate endothelial and smooth muscle cell amino acid and glucose transporters is limited, and further studies will need to establish whether vascular cells isolated from cerebral, retinal, and peripheral vascular beds respond similarly. Because the expression of eNOS and the density of caveolae may vary in endothelial cells from macro- and microvessels (11, 626), activation of eNOS and potential modulation of nutrient transport by NO may depend on the origin of the endothelial (and smooth muscle) cell type. Prolonged exposure of endothelial cells to NO downregulates l-arginine transport via system γ⁺, while acute exposure to NO stimulates l-arginine influx (440, 461). We have further demonstrated that endogenous NO generated in response to acute A₂₃₃-purinoceptor activation increases system γ⁺ activity in umbilical vein endothelial cells most likely due to a NO-mediated activation of outward K⁺ currents (656).

Questions remain concerning the signal transduction and transcription factors involved in the activation of oxidant stress response genes such as system xCT, known to be induced in vascular cells by reactive oxygen radicals and atherosgenic lipids (93, 158, 159, 271, 272, 349, 407, 539, 573, 595). The availability of human cDNAs for xCT and 4F2hc (39, 319, 508, 509, 529) provides an opportunity for investigating whether NO and reactive oxygen radical-mediated increases in xCT and 4F2hc mRNA are paralleled by changes in L-cystine transport in different endothelial and smooth muscle cell types. It is interesting that upregulation of system xCT activity in human retinal pigment epithelial cells in response to prolonged NO exposure is associated with increases in mRNA levels for xCT but not 4F2hc (78). Recent findings in mouse brain endothelial cells have shown that diethylmaleate (100 μM, 12 h) induces a 1.6-fold increase in xCT mRNA levels, with negligible changes in 4F2hc mRNA levels attributed to the 46-fold greater amount of 4F2hc mRNA compared with xCT mRNA (595). Although a disulfide-linked heterodimer of xCT and 4F2hc is required for L-cystine transport, transfection of HEK293 cells with xCT alone can mediate transport due to the high endogenous levels of 4F2hc (529). In view of the important role that oxidative stress plays in the pathogenesis of vascular disease (reviewed in Refs. 173, 355), there is a notable lack of information concerning the induction and regulation xCT and CAT transporters in human vascular tissue in vivo in atherosclerosis, diabetes, intrauterine growth retardation, and preeclampsia. Perhaps one of the most interesting observations in our studies is that pregnancy-associated diseases, such as gestational diabetes, intrauterine growth retardation, and preeclampsia, induce phenotypic changes in the fetal vasculature, including alterations in the L-arginine–NO signaling pathway (107, 547, 564) and regulation of intracellular Ca²⁺ (564). We hypothesize that these alterations may have significant implications for long-term programming of the fetal cardiovascular system (for review, see Ref. 32). The significant advances in the molecular biology of amino acid and glucose transporters and intracellular signaling pathways within the last decade provide the necessary tools for characterizing the molecular and functional regulation of nutrient transporters in endothelial and smooth muscle cells derived from the brain, retinal, and peripheral vasculature in health and disease.

We are indebted to our collaborators whose names appear in the cited references and particularly thank Prof. Jeremy D. Pearson, Prof. Jose Viña, Prof. Richard J. Naftalin, Dr. A. Claudio Mendes Ribeiro, and Dr. Richard C. M. Siow for helpful discussions.

We gratefully acknowledge research funding from the Medical Research Council (UK); Biotechnology and Biological Sciences Research Council (UK); Wellcome Trust (UK); British Heart Foundation; Ministry of Agriculture, Fisheries, and Food (UK); British Council; Ministry of Education, Science, and Culture (Japan); Tommy’s—The Baby Charity and Community Fund (UK); Fondo Nacional para el Desarrollo Científico y Tecnológico (Chile) Grants 1971321, 1000354, and 7000354; and Dirección de Investigación, Universidad de Concepción (Chile) Grants 9733871-D and 201084003–1.0.

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