PERGAMON

Progress in Neurobiology 64 (2001) 365–391

progress in Neurobiology

www.elsevier.com/locate/pneurobio

Arginine metabolism and the synthesis of nitric oxide in the nervous system

Heinrich Wiesinger *

Physiologisch-Chemisches Institut der Universität, Hoppe-Seyler-Straße 4, D-72076 Tübingen, Germany

Received 19 June 2000

Abstract

The biochemistry and physiology of L-arginine have to be reconsidered in the light of the recent discovery that the amino acid is the only substrate of all isoforms of nitric oxide synthase (NOS). Generation of nitric oxide, NO, a versatile molecule in signaling processes and unspecific immune defense, is intertwined with synthesis, catabolism and transport of arginine which thus ultimately participates in the regulation of a fine-tuned balance between normal and pathophysiological consequences of NO production. The complex composition of the brain at the cellular level is reflected in a complex differential distribution of the enzymes of arginine metabolism. Argininosuccinate synthetase (ASS) and argininosuccinate lyase which together can recycle the NOS coproduct L-citrulline to L-arginine are expressed constitutively in neurons, but hardly colocalize with each other or with NOS in the same neuron. Therefore, trafficking of citrulline and arginine between neurons necessitates transport capacities in these cells which are fulfilled by well-described carriers for cationic and neutral amino acids. The mechanism of intercellular exchange of argininosuccinate, a prerequisite also for its proposed function as a neuromodulator, remains to be elucidated. In cultured astrocytes transcription and protein expression of arginine transport system y^+ and of ASS are upregulated concomittantly with immunostimulant-mediated induction of NOS-2. In vivo ASS-immunoreactivity was found in microglial cells in a rat model of brain inflammation and in neurons and glial cells in the brains of Alzheimer patients. Any attempt to estimate the contributions of arginine transport and synthesis to substrate supply for NOS has to consider competition for arginine between NOS and arginase, the latter enzyme being expressed as mitochondrial isoform II in nervous tissue. Generation of NOS inhibitors agmatine and methylarginines is documented for the nervous system. Suboptimal supply of NOS with arginine leads to production of detrimental peroxynitrite which may result in neuronal cell death. Data have been gathered recently which point to a particular role of astrocytes in neural arginine metabolism. Arginine appears to be accumulated in astroglial cells and can be released after stimulation with a variety of signals. It is proposed that an intercellular citrulline–NO cycle is operating in brain with astrocytes storing arginine for the benefit of neighbouring cells in need of the amino acid for a proper synthesis of NO.

Contents

Abbreviations: ADC, arginine decarboxylase; ADMA, asymmetric N^G,N^G-dimethyl-L-arginine; AS, argininosuccinate; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CAT, cationic amino acid transporter; CNS, central nervous system; CPS, carbamoylphosphate synthetase; CSF, cerebrospinal fluid; DDAH, N^G,N^G-dimethyl-L-arginine dimethylaminohydrolase; HE, hepatic encephalopathy; IFN, interferon; LPS, lipopolysaccharide; NMDA, *N*-methyl-D-aspartate; NMMA, N^G-monomethyl-L-arginine; NOS, nitric oxide synthase; OCT, ornithine carbamoyltransferase.

* Tel.: +49-7071-2973338; fax: +49-7071-295360.

E-mail address: heinrich.wiesinger@uni-tuebingen.de (H. Wiesinger).

0301-0082/01/$ - see front matter
PII: S0301-0082(00)00056-3

1. Introduction and scope of the review

The biochemistry and physiology of the amino acid L-arginine (also 'arginine' in the following) has been a field of active research for many years and a plethora of data on the metabolism and function of this amino acid has been gathered in the basic and clinical sciences. However, the recent discovery of the many diverse biological functions of nitric oxide, NO, shed new light on the role of arginine, which is the only physiologically significant substrate for NO synthesis. In the nervous system NO participates in a variety of signaling processes and in the tissue's defense against pathogens, but is also a noxious agent when produced in excess or at the wrong place. Thus, the regulation of supply of arginine for NO generation in neural cells may be of pivotal importance for a delicate balance between normal and pathophysiological events in the peripheral and central nervous systems. The present review will give an overview of mammalian arginine metabolism before turning to the particular findings obtained for nervous tissue in vitro and in vivo. The functions of NO in the nervous system will be summarized together with the pathological consequences of NO overproduction. A focus on the brain appears justified since the diversity of neuronal and glial cell populations complicates the interrelationship between arginine and NO metabolic pathways at the organ level. A compilation of very recent data on astroglial arginine will allow to put forward a hypothesis of astrocytes as central players in the regulation of NO production within brain parenchyma.

2. Arginine in mammals: an overview

Arginine has been classified as a 'semi-essential' or 'conditionally essential' amino acid. This characteriza-

tion alludes to the fact that arginine has to be extracted from the diet (i.e. is an 'essential' amino acid) as a supplement to the endogenous synthesis in growing mammals and in adult animals or humans during disease or trauma; however, arginine can be synthezised in sufficient quantity in the healthy adult (Rose, 1937; Barbul, 1986). Nevertheless, even in the adult mammal not every tissue expresses all enzymes necessary for the de novo synthesis of arginine and also catabolism of the amino acid is highly compartmented. Therefore, a complex inter-organ trafficking of the amino acids arginine and citrulline has to secure the balance between arginine production and arginine consumption, and intracellular metabolism as well as membrane transport are important determinants of the roles that arginine plays in the normal and pathophysiology of the body.

2.1. Metabolism of arginine

The prominent feature of arginine metabolism is a complex differential expression of relevant enzymes when one looks at the major mammalian organs. A simplified scheme of pathways in arginine metabolism is depicted in Fig. 1. However, there is not a single organ or cell that expresses all the enzymes shown in the Figure. Subcellular compartmentalization and expression of various isoenzymes complicates the picture further. Therefore, a slightly varied diagram may be drawn for every organ or cell type under consideration. The central pivot in arginine metabolism is the urea cycle. This first biochemical cycle to be described (Krebs and Henseleit, 1932) is the body's route of disposal of surplus nitrogen and thus, outside the nervous system, provides the means of detoxification of neurotoxic ammonia (Withers, 1998). It is the input of ornithine, the ability to convert ornithine into citrulline, and the catabolism of arginine mainly by arginase and NO synthase that determine the role of an organ or cell as arginine producer or consumer.

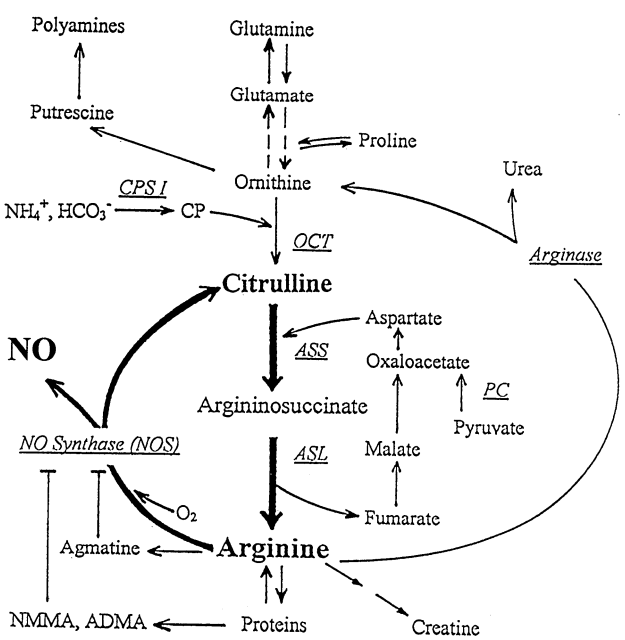

Fig. 1. Arginine metabolism in mammalian cells with emphasis on its interrelationship with the generation of NO and a citrulline–NO cycle. For differential tissue and cellular expression of the pathways, for mitochondrial localization of some of the enzymes and for further details of intermediary steps, see text as well as the review by Wu and Morris (1998). CP, carbamoylphosphate; PC, pyruvate carboxylase; other abbreviations as listed.

De novo synthesis of arginine (Fig. 1) depends on the presence of ornithine carbamoyltransferase (OCT; EC 2.1.3.3) which, together with carbamoylphosphate synthetase I (CPS I; EC 6.3.4.16), is located in the mitochondrial matrix. Expression of both enzymes in animals is restricted to the periportal hepatocytes in liver, the epithelial cells in the mucosa of the small intestine and, to a minor extent, the colonocytes of the large intestine (Raijman, 1974; Knecht et al., 1979; Morris, 1992), and is notably lacking in cells of the nervous system (Section 5.1.1). Consequently, only the former tissues are able to utilize ornithine for the generation of L-citrulline. Due to a further cellular restriction of biosynthetic enzymes in the adult, substantial production of ornithine from diet- and blood-derived glutamine or enteral proline (Fig. 1) occurs exclusively in the gut which releases citrulline into the circulation (Windmueller, 1982). Most of this citrulline is taken up into the kidney and utilized by the cells of the proximal tubulus for the synthesis of arginine which, in turn, is released into the blood for the benefit of other organs (Windmueller and Spaeth, 1981; Dhanakoti et al., 1992; Rabier and Kamoun, 1995). However, a role of the small intestine in arginine biosynthesis before weaning has to be considered (De Jonge et al., 1998). In contrast to the mitochondrial enzymes of the urea cycle, cytosolic argininosuccinate synthetase (ASS; EC 6.3.4.5) and argininosuccinate lyase (ASL; EC 4.3.2.1) appear to be expressed in every cell examined (Ratner, 1973; Yu et al., 1995). This may reflect the role of these enzymes in an intracellular citrulline–NO cycle (Fig. 1) since so far no cell has been found which does not express at least one isoform of NOS. The role of arginine as a precursor of NO will be dwelt upon extensively (see Section 3). It shall be emphasized that the rate-limiting step in the utilization of citrulline is the reaction catalyzed by ASS which needs aspartate as a cosubstrate. Cytosolic concentration of aspartate, and consequently synthesis of argininosuccinate (AS), may be regulated by the activity of mitochondrial pyruvate carboxylase which generates oxaloacetate, substrate of aspartate aminotransferase for the synthesis of aspartic acid (Rabier and Kamoun, 1995).

Hydrolytic cleavage of arginine to urea and ornithine (Fig. 1) is catalyzed by the two isoforms of arginase (EC 3.5.3.1) the expression of which is a further example of compartmentalization at the organ and subcellular level in arginine metabolism. Substantial activities of cytosolic arginase I are present in periportal hepatocytes and enable the liver to dispose of all of arginine nitrogen in the form of urea despite the fact that the highest rates of arginine synthesis in the body are found in this organ (Yu et al., 1995; Gotoh et al., 1997; Miyanaka et al., 1998). Catabolism of arginine by arginase I and ornithine aminotransferase was also reported for perivenous hepatocytes (O'Sullivan et al., 1998). Mitochondrial arginase II which is encoded by a separate nuclear gene, is expressed in extrahepatic tissues such as kidney, small intestine, and brain (Gotoh et al., 1997). Restriction of arginase to a segment in the nephron lacking arginine biosynthetic capacity is in accordance with kidney's role as arginine supplier for the body (Guder and Morel, 1992; Miyanaka et al., 1998). Macrophages express both arginase isozymes (Louis et al., 1999). Roles of arginase I in the cytosolic synthesis of polyamines and of arginase II in the generation of ornithine for synthesis of proline and glutamate within the mitochondria have to be considered (Wu and Morris, 1998; Louis et al., 1999; Ozaki et al., 1999).

Arginine is also catabolized in the biosynthetic pathway leading to creatine. Expression of mitochondrial arginine:glycine amidinotransferase mainly in kidney and pancreas and of cytosolic guanidinoacetate *N*-methyltransferase predominantly in liver and to a lesser extent in pancreas and kidney points to an interorgan cooperation in creatine biosynthesis (for discussion, see Wu and Morris, 1998).

Two minor pathways of arginine metabolism have been focussed upon over the last few years. For a long time, decarboxylation of arginine was believed to take place in microorganisms and plants exclusively; however, mitochondrial arginine decarboxylase (ADC; EC 4.1.1.19) and its product 4-(aminobutyl)guanidine (= agmatine) have been identified recently in several mammalian tissues including brain (Li et al., 1994; Regunathan and Reis, 2000). Roles for agmatine as a signaling molecule at imidazoline and α_2-adrenergic receptors (Li et al., 1994; Molderings et al., 1999), as a modulator of *N*-methyl-D-aspartate (NMDA) type glutamate receptor (Olmos et al., 1999; Yang and Reis, 1999), and in the regulation of cellular polyamine content and, consequently, cell proliferation (Satriano et al., 1999; Vargiu et al., 1999) have been described. The identification of N^G-methylated arginine analogues, in particular N^G-monomethyl-L-arginine (NMMA) and asymmetric N^G,N^G-dimethyl-L-arginine (ADMA), and of N^G,N^G-dimethyl-L-arginine dimethylaminohydrolase (DDAH; EC 3.5.3.18) in mammalian tissues, again including brain (Ueno et al., 1992; Kimoto et al., 1993, 1995; Tojo et al., 1997; Leiper et al., 1999), provokes speculation about the physiological role(s) of these arginine metabolites and their catabolizing enzyme. The possible integration of endogenous agmatine and methylated arginine analogues into a network regulating NO synthesis will be discussed (Sections 3.2 and 5.3.2).

Further details of peripheral arginine metabolism are depicted in excellent recent reviews. In particular, Wu and Morris (1998) elaborate the concept of arginine being 'one of the most versatile amino acids in animal cells' and discuss extensively quantitative aspects of compartmentalization and regulation of arginine metabolism. The molecular biology and genetics of urea cycle enzymes have been reviewed expertly by Takiguchi and Mori (1995).

2.2. Transport of citrulline and arginine

Inter-organ compartmented metabolism of arginine necessitates transport of citrulline and arginine across the plasma membranes of a variety of cells. Physiological characterization of amino acid transport has led to the concept that groups of amino acids are accepted by carrier proteins according to the biochemical properties of the substrates. Thus, neutral amino acids share transport systems different from the ones responsible for cellular uptake of charged amino acids (Christensen, 1990). Many of these transporters operate according to the laws of facilitated diffusion. In addition, specific sodium-dependent carriers mediate secondary active transport of metabolites. Only scarce data exist on the transport of citrulline in mammalian tissues. A sodium-dependent transporter catalyzes citrulline uptake into the small intestine (Vadgama and Evered, 1992) which reflects the obligatory accumulation of the amino acid in this organ. No accumulating transport of citrulline from blood into the lumen of the kidney has been reported. Uptake studies in peripheral macrophages (Baydoun et al., 1994) and cultured neural cells (Section 5.2) pointed to the system L for large neutral amino acids as mediator of citrulline transport.

In contrast, transport of arginine as a paradigmatic cationic amino acid has been characterized extensively already in the pioneering work of Christensen's group (White et al., 1982). Transport is mediated mainly by a system denoted y^+ with minor roles being played by systems $b^{0,+}$, $B^{0,+}$, and y^+L (Deves and Boyd, 1998). High affinity for amino acids with a positively charged side chain, independence on the concentration of extracellular Na^+ and *trans*-stimulation of arginine transport by the other cationic amino acids L-lysine and L-ornithine are the physiological hallmarks of system y^+ which has been detected in many peripheral cells, among them macrophages, endothelial cells, platelets,

or vascular smooth muscle cells (Bogle et al., 1992; Schmidt et al., 1993; Vasta et al., 1995; Zharikov et al., 1997; Baydoun et al., 1999), and in a variety of neural cell populations (Section 5.2). Recently several transmembrane proteins named CATs (cationic amino acid transporters) have been cloned which upon expression in Xenopus oocytes or transfected cell lines exhibit the properties of system y^+. CAT-1 and CAT-2B are expressed in a wide variety of rodent and human tissues (Kakuda et al., 1998; Hattori et al., 1999), whereas CAT-3 was detected in (rodent) brain exclusively (Hosokawa et al., 1997; Ito and Groudine, 1997). A recently reported human CAT-4 gene is transcribed in brain, testis and placenta (Sperandeo et al., 1998). System y^+ is also responsible for cellular uptake of NOS inhibitors based on modification of arginine structure as long as a positive charge is retained in the side group. This holds for the N^G-methylated arginine analogues whereas the molecules containing a nitro group (e.g. N^G-nitro-L-arginine and its methylester) have a neutral charge distribution and appear to be transported by system L (Baydoun and Mann, 1994; Schmidlin and Wiesinger, 1994; Schmidt et al., 1994; Bogle et al., 1995; Closs et al., 1997). For further information on basic features of arginine transport the reader is referred to recent reviews (Malandro and Kilberg, 1996; Deves and Boyd, 1998; Closs and Mann, 1999). Up-regulation of arginine transport by proinflammatory mediators and other stress factors will be discussed below (Section 3.4).

2.3. Physiology and pathophysiology of arginine

Physiological roles of arginine are largely determined by the metabolic fates of the amino acid as described above. As a precursor of urea arginine has a central function in the detoxification of ammonia derived from amino acid and nucleotide catabolism. Inborn errors in any of the five urea cycle enzymes, therefore, lead to hyperammonemia resulting in severe clinical symptoms including mental retardation (Shih, 1978). Ammonia intoxication is also a factor contributing to hepatic encephalopathy (HE; Hazell and Butterworth, 1999; see Section 5.2). Generation of ornithine from arginine may be the main function of induced arginase II, particularly in macrophages (Wu and Morris, 1998; Ozaki et al., 1999). Stimulation of biosynthesis of polyamines and proline promotes wound healing by enhancing cell proliferation and collagen synthesis (Albina et al., 1988). It should not be neglected in this context that arginine itself is a proteinogenic amino acid and, once incorporated into proteins, can be posttranslationally N^G-methylated to NMMA and ADMA (Clarke, 1993, see Section 2.1) or deiminated to citrulline (Rothnagel and Rogers, 1984).

Polyamines have been described recently as being modulators of ion channels and neurotransmitter receptors (Williams, 1997). Therefore, the idea is quite intriguing that arginine can be considered as precursor of several signaling molecules including polyamines and NO (Wu and Morris, 1998). Creatine biosynthesis from arginine should not be underestimated, and arginine may indirectly be of importance in polarized cells which rely on creatine phosphate as means not only of energy storage, but also of energy transport (Wallimann et al., 1992). Roles of arginine in the immune system and as a secretagogue as well as nutritional effects of arginine have been reviewed previously (Barbul, 1986; Redmond and Daly, 1993). For a proper appreciation of the (patho-) physiological roles of arginine in peripheral organs, the reader is also referred to a recently published book (Eremin, 1997). The mechanism of action of arginine on the secretion of hormones (Palmer et al., 1975) may involve the generation of nitric oxide (Schmidt et al., 1992); this is just one example that it is worthwhile to reconsider literature on arginine written before 1987 with today's knowledge of the interrelationship of arginine and NO metabolism which will be elaborated in the next paragraph.

3. Arginine and NO

Arginine is substrate of all isoforms of NO synthase which generates from L-arginine and molecular oxygen NO and L-citrulline in a five-electron transfer reaction (Fig. 2). Moreover, it seems that arginine is the only physiological substrate for the generation of NO in eucaryotic cells, and arginine analogues are potent inhibitors of NOS (Grant et al., 1998). Thus the (patho-)physiologicol roles of arginine (Section 2.3) are intertwined with the biological effects of NO (Morris, 1999), and many functions of NO have been delineated from the attenuation or enhancement of the respective effect in the presence of arginine-based NOS inhibitors. Any specification of the numerous biological roles attributed to NO, notably in the cardiovascular and immune systems, is beyond the scope of this review, and the reader is referred to published summaries (e.g. Gross and Wolin, 1995; Kröncke et al., 1995; Mayer and Hemmens, 1997; Ignarro et al., 1999). It should be emphasized that, due to the diffusible nature of the radical NO, generation of the molecule is already the major control point for its action and is regulated at the transcriptional level and by a variety of epigenetic factors (Nathan and Xie, 1994). In this context several aspects of the modulation of NO synthesis by the availability of arginine deserve mentioning.

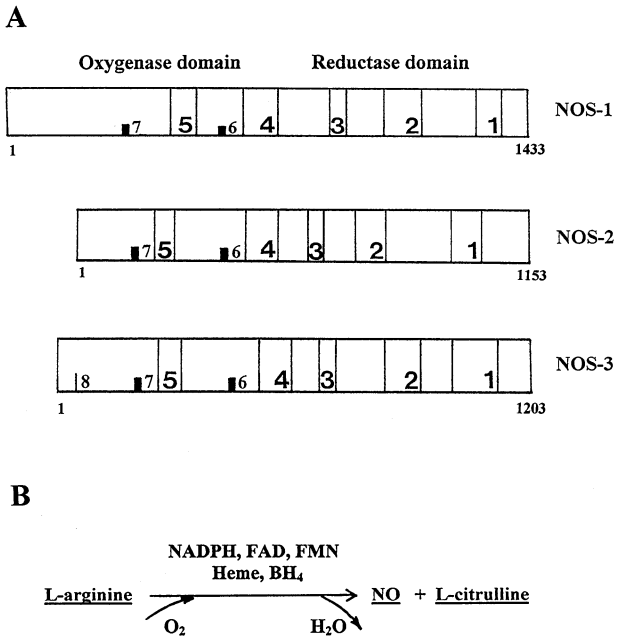

Fig. 2. Schematic representation of the three NOS isoforms (A), and the reaction catalyzed by them (B). Note that the enzymes are active as homodimers only. A nomenclature was adopted which acknowledges the order of cloning of the isoforms. NOS-1 and NOS-3 are generally considered as constitutive enzymes ('cNOS') and were first decribed in brain neurons (NOS-1 = 'bNOS' or 'nNOS') and in vascular endothelial cells (NOS-3 = 'eNOS'), respectively. Both enzymes are activated by binding the Ca^{2+}/calmodulin complex (another explanation for 'cNOS') after the concentration of intracellular Ca^{2+} has been raised transiently. NOS-2 was first described in macrophages ('mNOS') treated with LPS and IFN-γ. The enzyme is induced ('iNOS') by a wide variety of agents, prominently various combinations of cytokines. Due to the high affinity for Ca^{2+}/calmodulin NOS-2 is not regulated by changes in the concentration of intracellular Ca^{2+}. In many cell types including brain cells all isoforms can be detected under appropriate conditions, and transcriptional regulation of all isoforms to some extent was reported. Therefore, any nomenclature which implies a specific localization or mode of expression should be avoided. Schematically depicted are the binding sites for NADPH (1), FAD (2), FMN (3), calmodulin (4), heme (5), arginine (6) and tetrahydrobiopterin (BH_4,7). NOS-3 contains a myristoylation site at its N-terminus (8). Numbers of amino acids per polypeptide chain are given for the rat enzymes.

3.1. Generation of superoxide by NO synthase

It may sound trivial that in the absence of substrate arginine NOS is not generating NO. However, it is not trivial at all that at low concentrations of arginine a new catalytic function is exhibited by NOS, i.e. generation of superoxide radical, O_2^- (Pou et al., 1992; Xia et al., 1998). Because the reaction of superoxide dismutase with O_2^- is less efficient than combination of this radical with even traces of NO to peroxynitrite, $ONOO^-$ (Beckman and Koppenol, 1996), NOS activity gives rise to a highly toxic molecule. $ONOO^-$-mediated cellular injury was demonstrated in a NOS-transfected human kidney cell line after arginine depletion (Xia et al., 1996). Similarly, at low concentrations of intracellular arginine cytotoxicity of macrophages against *Escherichia coli* was enhanced after induction of NOS-2 (Xia and Zweier, 1997).

3.2. Arginine analogues and inhibition of NO synthase

A complex pattern of regulation of all isoforms of NOS may be imposed by the endogenous arginine analogues, agmatine, NMMA and ADMA (Section 2.1). Agmatine inhibits NOS activity with K_i values between 0.2 and 7.5 mM depending on the isoform (Galea et al., 1996). NMMA and ADMA inhibit NOS activity (Hibbs et al., 1987; Fukuto and Chaudhuri, 1995; Jin and D'Alecy, 1996) as well as arginine transport (Section 2.2); IC_{50} values for NOS inhibition by ADMA are 2 and 200 μM in vitro and in intact immune cells, respectively (MacAllister and Vallance, 1998). If present in sufficiently high concentrations, these compounds may pose additional constraints on the intracellular availability of arginine and cause NO synthesis to become arginine-dependent. Colocalization of DDAH and NOS has been reported (Kimoto et al., 1993; Tojo et al., 1997). It should be noted that hydrolysis of NMMA or ADMA by DDAH has two effects with the potential of promoting NO synthesis: inhibition of NOS is relieved and citrulline is generated which may enter the NO-citrulline cycle (Section 3.4). Although generation of citrulline by this pathway under normal conditions does not seem to be of quantitative significance, alterations in the levels of NOS inhibitors NMMA and ADMA may have pathophysiological consequences. Indeed, in isolated rat aorta rings inhibition of DDAH increased tone considerably, an effect which was abolished by L-arginine (MacAllister et al., 1996). DDAH activity was reduced in hypercholesterolemia in rabbits which in part may explain the endothelial dysfunction leading to atherosclerosis (Ito et al., 1999).

3.3. Arginine and constitutive NO synthase

Intracellular availability of arginine may not only modulate product specificity of NOS (Section 3.1), but may be of general importance for the synthesis of NO in amounts adequate for the physiological function of the respective NOS isoform (see also Section 3.4). At first consideration, regulation of the activity of the constitutive isoforms of NOS by arginine appears improbable. K_M values of purified NOS for the substrate arginine are between 1 and 10 μM, and intracellular concentrations of arginine are at least 100 μM and amount to 0.8 mM in cultured endothelial cells (McDonald et al., 1997) thus being sufficient to meet the requirements of any constitutive NOS for functioning under substrate saturation. Nevertheless, dependence of the activity of NOS-3 on the extracellular concentration

of arginine has been reported and termed the 'arginine paradox' (Kurz and Harrison, 1997). A particular subcellular colocalization of arginine transporter CAT-1 and NOS-3 in plasma membrane caveolae may provide an explanation (McDonald et al., 1997). Intracellular arginine may be sequestered in a pool which is not available for NOS-3, whereas the close apposition of CAT-1 and NOS-3 enables the direct delivery of extracellular arginine to the enzyme.

3.4. Arginine and induced synthesis of NO

The long-lasting, Ca^{2+}-independent production of NO by NOS-2 after induction of the enzyme poses two interconnected questions: how is NOS-2 supplied in sufficient quantity with its substrate arginine, and may NOS-2 activity be regulated posttranslationally by the availability of arginine? An important step in answering these questions was the discovery that a variety of immunostimulants, i.e. bacterial lipopolysaccharide (LPS) or interferon-γ (IFN-γ) and other cytokines, not only induce NOS-2, but also stimulate the expression of pathways accessory to NO generation, e.g. synthesis of cofactor tetrahydrobiopterin (Sakai et al., 1995; Nussler et al., 1996; Frank et al., 1998) or regeneration of coenzyme NADPH (Garcia-Nogales et al., 1999), and synthesis as well as transport of substrate arginine. Up-regulation of arginine transport under conditions which induce NOS-2 was demonstrated for many peripheral cell types, notably for cytokine-stimulated macrophages (Bogle et al., 1992; Kakuda et al., 1999) or vascular smooth muscle cells (Baydoun et al., 1999; Hattori et al., 1999), but also for endothelial cells after shear stress-induced formation of NO (Posch et al., 1999). Up-regulation of arginine transport appears to be mainly due to activation of transcription of CAT-2B and incorporation of newly synthesized transporter protein into the plasma membrane (Closs et al., 1993; Stevens et al., 1996; Caivano, 1998), although up-regulation of other members of the CAT family has been reported (Simmons et al., 1996; Kakuda et al., 1998; Baydoun et al., 1999; Hattori et al., 1999).

The rate-limiting step in the recycling of NOS coproduct citrulline to NOS substrate arginine, i.e. the synthesis of argininosuccinate catalyzed by ASS (Section 2.1), is also sensitive to transcriptional activation. Up-regulation of ASS by immunostimulants as detected either on the mRNA or protein level has been reported for all non-hepatic mammalian cells examined (Hattori et al., 1994; Nussler et al., 1994; Flodström et al., 1995; Nagasaki et al., 1996; Nussler et al., 1996; Hammermann et al., 1998). In contrast, ASL appears to be a constitutive enzyme under proinflammatory conditions, although an up-regulation of ASL mRNA without a detectable increase in protein expression in rat tissues after intraperitoneal application of LPS has been reported (Nagasaki et al., 1996). ASS is co-stimulated with induction of NOS-2 in many cells and tissues as demonstrated in culture and in vivo studies (Hattori et al., 1995; Nussler et al., 1994; Nagasaki et al., 1996; Nussler et al., 1996). The terms arginine–citrulline cycle (Hecker et al., 1990) and citrulline–NO cycle (Nussler et al., 1994) have been coined, and the latter will be used throughout this review (Fig. 1).

It was attempted in several cases to estimate the contributions of recycling and uptake to arginine-dependent generation of NO (see Section 5.3.3). Such an estimate may be further complicated by the fact that also arginase is transcriptionally induced by LPS as was shown for arginase II in a macrophage cell line (Gotoh et al., 1996) and in endothelial cells (Buga et al., 1996) and for arginase I in rodent peritoneal macrophages, but also rodent lung and spleen (Sonoki et al., 1997; Salimuddin et al., 1999), two organs which express markedly NOS-2 after treatment of the animals with LPS (Nagasaki et al., 1996). Although arginase expression appears to be regulated independently of NOS-2 expression (Morris et al., 1998), a role in down-regulation of NO synthesis has to be considered (Wang et al., 1995; Hey et al., 1997; Gotoh and Mori, 1999). Indeed, pathophysiological implications of this antagonism have been reported (Cook et al., 1994; Jude et al., 1999). It is an intriguing observation that arginase I mRNA can be detected not before several hours after the appearance of NOS-2 mRNA (Sonoki et al., 1997), and sequential temporal expression of two enzymes competing for the same substrate may be a novel regulatory mechanism of general significance. In reversal of the situation, arginase is inhibited by N^G-hydroxy-L-arginine, the intermediate in the NOS reaction, which is released in substantial amounts from the enzyme (Buga et al., 1996; Waddington et al., 1998; Boucher et al., 1999).

4. NO in the nervous system

In order to lay the grounds for a discussion of the role of arginine in the regulation of neural NO synthesis a concise overview of NO in the nervous system will be provided first. Several seminal papers will be referenced, however, in general the reader is referred to recent summaries. Since the present review is focussed on the CNS, it shall only be mentioned in passing that NOS appears to be wide-spread in the peripheral nervous system (e.g. Grozdanovic et al., 1992); at the functional level, NO is well established as a neurotransmitter in the periphery, e.g. as the long sought-for non-adrenergic, non-cholinergic transmitter released from the myenteric plexus neurons in the gastrointestinal tract (Bult et al., 1990).

Synthesis of NO occurs throughout the CNS including spinal cord and retina (Dun et al., 1992; Donati et al., 1995; Sheng and Ignarro, 1996), and in brain in particular in the endothelial cells lining the capillaries (Seidel et al., 1997) and in most cell types of the parenchyma investigated. The pattern of NOS isoform expression is complex and seems to reflect the roles proposed for NO in the proper functioning but also in the pathophysiology of the CNS. After the discovery that NOS-1 is identical with NADPH-diaphorase (Dawson et al., 1991; Hope et al., 1991) numerous studies on the localization of the enzyme in nervous tissue appeared. However, many of the early results and interpretations may be hampered by an inappropriate application of the histochemical technique since proper fixation procedures have to be followed in order to obtain unequivocal results (Blottner et al., 1995; Wolf, 1997).

NOS-1 is expressed in neurons in many parts of the brain, prominently in the cerebellum and hippocampus (Bredt et al., 1990; Egberongbe et al., 1994; Endoh et al., 1994; Braissant et al., 1999a). In other areas such as cerebral cortex or the striatum, NOS-1-positive neurons comprise about 1 to 2% of the total neuronal population (Bredt et al., 1991; Iwase et al., 1998); modulation of various physiological functions by NO has been proposed, among them pain perception, sleep, feeding behaviour or thermoregulation, and, in particular, regulation of microcirculation (Szabo, 1996). Alternatively spliced forms of the enzyme appear to dominate in discrete brain regions (Eliasson et al., 1997). NOS-1 is targeted to proteins of the postsynaptic density in close association with the NMDA receptor (Brenman et al., 1996; Sattler et al., 1999). Calcium-dependent stimulation of NOS-1 activity has been implicated in excitatory glutamatergic neurotransmission, long term potentiation, and long term depression (Garthwaite et al., 1988; Schuman and Madison, 1994; Garthwaite and Boulton, 1995). Thus, NOS-1 may be involved in phenomena based on synaptic plasticity such as learning and memory formation, tasks which are supported, and in NOS-1-deficient neurons may be taken over, by NOS-3 (Dinerman et al., 1994; Wilson et al., 1997). In turn, nitric oxide has been implicated in excitotoxicity after excessive stimulation of neurons by glutamate, as occurs in stroke, although the underlying mechanisms of neuronal cell death as well as of the resistance of NOS-1 containing neurons to NO are still a matter of debate (Koh and Choi, 1988; Dawson et al., 1993; Lipton et al., 1993; Schulz et al., 1995). Nevertheless, excessive generation of neuronal NO appears to be responsible for ischemia-reperfusion and traumatic injury of the brain (Huang et al., 1994; Chabrier et al., 1999) which can only partly be overcome by the beneficial effect of endothelial cell-derived NO on blood flow (Prado et al., 1993; Schulz et al., 1995; Iadecola, 1997). Evidence is increasing that neurons are able to express NOS-2 in response to proper stimulation (Minc-Golomb et al., 1996; Heneka et al., 1998; Moro et al., 1998) and that endogenously produced NO leads to cell death by necrotic or apoptotic mechanisms (Heneka et al., 1998).

NOS-1 activity as well as protein have been detected in astrocytes in situ and in culture (Arbones et al., 1996; Kugler and Drenckhahn, 1996; Caggiano and Kraig, 1998), whereas NOS-1 transcript was not found in glial cells in situ in a recent study (Braissant et al., 1999a). In cultured astrocytes NOS-1 is activated upon incubation of the cells with Ca^{2+}-rising agents such as endothelin (Saadoun and Garcia, 1999). Astroglial NOS-1-derived NO may support the regulation of local cerebral blood flow in response to neuronal activity. Transcriptional activation of the NOS-2 gene in astrocytes occurs upon incubation with LPS or several proinflammatory cytokines, either alone or in various combinations (Murphy et al., 1993; Murphy and Grzybicki, 1996). Other activators include HIV coat protein (Hori et al., 1999) and β-amyloid peptide (Rossi and Bianchini, 1996; Hu et al., 1998) or physical stimuli such as intraocular pressure (Liu and Neufeld, 2000). Recently, NOS-3 was also detected in astrocytes and possibly mediates an interdependence between cortical neuronal activity and local blood flow (Colosanti et al., 1998; Wiencken and Casagrande, 1999). Thus this prominent population of macroglial cells can express all three isoforms of NOS.

Published data on the expression of NOS isoforms in oligodendrocytes are more ambiguous or plain contradictory (Merrill et al., 1997; Keilhoff et al., 1998; Hewett et al., 1999). Ependymal cells as a source of nitric oxide apparently have not been investigated. Evidence for NOS-2 expression in microglial cells has been gathered for rodent brain from in situ and cell culture experiments, whereas generation of NO by NOS-2 in human macrophages including microglial cells remains controversial (Albina, 1995; Ding et al., 1997; Zhao et al., 1998). The large amount of NO generated by microglial and astroglial NOS-2 over a long period of time is implicated in the unspecific immune defense in brain (Murphy and Grzybicki, 1996; Minghetti and Levi, 1998). On the other hand, both astrocytes and microglia may be the source of potentially cytotoxic NO levels under pathological conditions (Meda et al., 1995; Chao et al., 1996). Involvement of glial NOS-2-derived NO in demyelinating and neurodegenerative diseases and in cerebral ischemia has been reviewed recently (Heales et al., 1999; Murphy, 2000).

5. Arginine in the brain

Most features of arginine metabolism outlined above (see Sections 2 and 3) are encountered when turning the

attention to nervous tissue and the brain in particular. However, until recently a lack of data was obvious as soon as individual neural cell populations were focussed upon. The complex peripheral inter-organ cooperation may have its counterpart in a similarly complex inter-cellular cooperation within the one organ brain, and a fine-tuned 'teamwork' between different types of glial cells as well as between glia and neurons in arginine synthesis and catabolism has to be taken into account. A precise localisation at the cellular level not only of participating enzymes but also of various transport capacities is necessary in order to test this hypothesis. In recent years, a number of studies with cell cultures and in situ have attempted to elucidate the role of individual cells in brain arginine metabolism.

5.1. Metabolism of arginine

5.1.1. Mitochondrial enzymes of the urea cycle

Data on urea cycle metabolites (Table 1) as well as enzymes in brain have been gathered early (Ratner et al., 1960; Buniatian and Davtian, 1966). Activities of the mitochondrial enzymes of the urea cycle, CPS I and OTC (Fig. 1), were not detected in rodent CNS tissue; consequently brain and its constituent cell populations are not capable of de novo synthesis of arginine, and urea formation cannot be the way of disposal of waste nitrogen in the rat brain (Jones et al., 1961; Buniatian, 1971). In contrast, low activities of CPS I and OTC (3% and 0.2% of liver activity, respectively) have been reported for the brain of the newborn infant (Glick et al., 1976); however, the contribution of the organ to total nitrogen disposal is insignificant. Indeed, it is accepted that CNS tissue has its own way of detoxifying potentially harmful ammonia, i.e. predominantly via the reaction of glutamine synthetase, an enzyme localized exclusively in glial cells (Wiesinger, 1995; Hertz et al., 1999).

Table 1
Concentrations of urea cyle intermediates and related compounds in brain, plasma and CSF

	Brain (μmol/g wet weight)	Plasma (μM)	CSF (μM)
Arginine	0.07–0.17[a]	80.9[b]	22.4[b]
	0.12–0.19[c]	76[d]	20.1[d]
	0.128[e]		
Citrulline	0.02–0.1[a]		14.2–21.6[f]
			1.5–2.7[f]
		29[d]	5.7[d]
Urea	3.7–4.9[g]		
NMMA	0.00014[h]		
	0.0035[i]		
ADMA	0.007[h]		
	0.0033[i]		

[a] Whole brain, common laboratory animals (Perry, 1982).
[b] Human (Wood, 1982).
[c] Rat brain, different areas (Sadasivudu and Rao, 1976).
[d] Human, reported in Shih (1978).
[e] Mouse brain (Ratnakumari et al., 1996).
[f] Human, data from several authors (Gjessing et al., 1972).
[g] Rat brain, data from several authors (Buniatian, 1971).
[h] Rat brain, mean value from four different areas (Ueno et al., 1992).
[i] Rat brain (Kimoto et al., 1993).

With the knowledge of lack of a full urea cycle the finding of substantial amounts of citrulline in brain (Table 1) remained enigmatic for a long time. It is clear today that citrulline is coproduct of neural NO synthesis and may be part of a neural citrulline–NO cycle in brain (Section 5.3.4). Nevertheless, it should be worthwhile to test for expression of however small amounts of CPS I or OTC proteins with today's sensitive immunochemical methods and for the respective messages with molecular biology techniques such as in situ hybridization or polymerase chain reaction. All these methods have been applied over the last few years in order to establish the cellular localization of the extramitochondrial enzymes of the urea cycle the activities of which had already been described in the brain in the early reports (Ratner et al., 1960; Buniatian, 1971; Sadasivudu and Rao, 1976).

5.1.2. Neuronal localisation of argininosuccinate synthetase

A thorough and detailed study of localization of ASS in formaldehyde-fixed rat brain sections was presented by Nakamura et al. (1991a) using a rabbit antiserum generated against purified rat liver ASS. Strong ASS-like immunoreactivity was detected in neurons in the septal area, basal forebrain, hypothalamus, amygdala, basal nucleus of Meinert, raphe nuclei, area postrema, and other parts of the brain. Most of these neurons were small or medium in size and characterized as interneurons. In the septal area, hypothalamus and area postrema, among others, also neuropile staining was observed, whereas no staining was detected in large white matter structures such as corpus callosum or the anterior commissure.

A predominantly neuronal expression of ASS was confirmed in a study which attempted a colocalization of ASS and NOS in formaldehyde-fixed rat brain sections using an antiserum generated against human liver ASS and the NADPH-diaphorase technique for detection of NOS (Arnt-Ramos et al., 1992). The overall distribution of ASS-positive neurons was similar to the one described before (Nakamura et al., 1991a), and the expression pattern of NOS was comparable to the one reported in a study of its own (Vincent and Kimura, 1992). Colocalization of ASS and NOS was restricted to selected neurons, e.g. in the forebrain and the striatum. Many of these double-labelled cells showed also citrulline-like immunoreactivity (Pasqualotto et al.,

1991). However, the majority of neurons contained only one of the enzymes, and ASS-immunoreactive neurons by far outnumbered NOS-positive ones. For example, in the cerebellum, the brain area with the highest concentration of NOS, NADPH-diaphorase activity was detected notably in the basket and granule cells of the cerebellar cortex; these cells are clearly distinct from the ASS-positive neurons beneath the Purkinje cell layer, presumably Lugaro cells (Arnt-Ramos et al., 1992). In this latter study ASS-immunoreactivity was not reported for glial cells, a finding which seems to be supported by the lack of ASS transcript in these cells in vivo (Braissant et al., 1999a).

ASS-positive neurons were identified as interneurons in the cat hypothalamus (Isayama et al., 1997), and between 20 and 30% of these neurons (depending on the exact location) were also stained with the NADPH-diaphorase technique and thus presumably express NOS. On the other hand, only in 10–26% of NOS-positive neurons ASS-immunoreactivity was detected, and the authors suggest that neurons expressing NOS but lacking ASS may obtain arginine from surrounding astrocytes. A differential neuronal expression of ASS and NOS was also reported for the dorsal horn of the rat spinal cord, whereas in the central canal region and the nucleus intermediolateralis NOS and ASS were colocalized in a substantial number of neurons (Nakamura, 1997).

Taken together, the data summarized above suggested that ASS is predominantly if not exclusively a neuronal enzyme. Differential localization of ASS and NOS implies that synthesis of arginine and consumption of the amino acid during generation of NO take place in different neurons. Consequently, neglecting a potential participation of glial cells (Section 5.4), an interneuronal trafficking of urea cycle intermediates has to occur.

5.1.3. Neuronal localisation of argininosuccinate lyase

An even more complicated picture of CNS arginine metabolism emerged from studies on the localization of ASL. All combinations conceivable were detected when the expression of NOS, ASS and ASL was compared. For example, in the rat spinal cord nucleus intermediolateralis, a high number of NOS-positive neurons expressed ASL (30–60%), whereas only a small portion of NOS-positive neurons was ASS-positive (10–20%; Nakamura, 1997). An earlier study had already arrived at the conclusion that in the rat olfactory bulb and cerebellar cortex ASL is generally expressed in neurons which are different from the ones expressing ASS (Nakamura et al., 1990). It is noteworthy that ASL-immunoreactivity was evenly distributed in rat brain suggesting a ubiquitous expression of the enzyme whereas expression of ASS was more restricted. As an example, the differential distribution of ASS and ASL led to the conclusion that, in the cerebellar cortex, AS is generated in the ASS-containing basket and stellate cells and is transported to the Golgi epithelial cells which express ASL and thus are able to produce arginine (Nakamura et al., 1990). In the rat accessory olfactory bulb the granule cells express NOS and ASL, but are lacking ASS (Nakamura et al., 1999). The authors suggest efficient generation of NO in these cells, however NO synthesis may critically depend on supply of AS from neighbouring cells, e.g. mitral or tufted cells which express the enzymes of the urea cycle but not NOS. Such an interneuronal trafficking of compounds involved in the synthesis of NO (Section 5.1.2) may be a way of regulating the availability of this signaling molecule by cellular compartmentalization of its precursors but necessitates the transport not only of citrulline and arginine, but also of AS across plasma membranes. Notwithstanding the complex expression pattern a predominantly neuronal localisation of ASL in brain was inferred from the data on protein expression. In contrast, ASL transcript was detected in neurons as well as in astrocytes throughout the rat brain (Braissant et al., 1999a).

The finding of ASS expression in neurons lacking ASL led the authors to the proposal that AS has a function of its own, probably as a neuromodulator (Nakamura et al., 1991b). The hypothesis appears to be substantiated by findings that AS in concentrations as low as 10^{-13} M reduced the increase in intracellular calcium evoked by glutamate action on quisqualate-type receptors in neurons isolated from immature rat cerebellum (Nakamura et al., 1991b). In these studies, AS could not be replaced by arginine. Transport mechanisms for AS will be discussed (Section 5.2).

5.1.4. Argininosuccinate synthetase in glial cells

Nakamura's work had led to the conclusion that ASS is predominantly a neuronal enzyme although the authors concede that 'immunoreactive material was observed… in the glial cells scattered in some tracts', and weak staining was described for glial cells of cerebellar white matter (Nakamura et al., 1991a). No attempt of a cellular resolution of the glial cells was made. An even stricter statement was given by Arnt-Ramos et al. (1992). Whereas in this study ASS-immunoreactivity was detected in neurons throughout the brain, 'glial cells were not stained' (Arnt-Ramos et al., 1992).

The finding of an almost exclusive presence of ASS in neurons and lack of the enzyme in glial cells was questioned later on. First hints on the ability of astrocytes to express ASS had already emerged from the studies by Yudkoff and coworkers. When ^{15}N-labelled aspartate was fed to astroglial cultures label was incorporated into arginine (Yudkoff et al., 1987). In a more direct attempt ASS enzyme activity was measured in cultured astrocytes from neonatal rat brain and was

shown to increase upon incubation of the cells with dexamethasone or dibutyryl cyclic AMP although ASS mRNA was undetectable in either case (Jackson et al., 1996). Therefore, it cannot be concluded unambiguously if the presence of glucocorticoids and morphological differentiation in the brain are factors which activate pre-formed ASS or induce the enzyme at the transcriptional level.

In contrast, a clear induction of ASS protein by NOS-inducing immunostimulants was demonstrated for glial cells in several experimental paradigms using monospecific antisera generated against peptides representing partial sequences of mouse liver ASS. Weak immunoreactivity was detected in homogenates of glioma cells C6-BU-1 (Schmidlin et al., 1997a) or pure mouse astroglial cultures (Schmidlin and Wiesinger, 1998). In both cell culture systems, ASS immunoreactivity increased strongly when the cells had been preincubated with immunostimulants (LPS and/or IFN-γ). The findings of both studies demonstrated for the first time that ASS protein is present in glial cells and that it is induced under conditions which elicit persistent generation of NO. This was confirmed in a recent study on the C6 parent cell line at both the mRNA and protein level where a mixture of LPS, IFN-γ and tumor necrosis factor α was used to stimulate the cells (Zhang et al., 1999). Increased ability of intracellular recycling of NOS coproduct citrulline to arginine may be of particular importance in the glioma cells which, in contrast to primary astroglial cells, are unable to up-regulate arginine import from the extracellular environment (Schmidlin and Wiesinger, 1994). Expression of ASS transcript was also reported for astrocytes (and neurons) in aggregating cell cultures from fetal rat telencephalic cortex; mRNA signal in astrocytes (but not neurons) was increased after prolonged treatment of the cultures with NH_4Cl thus simulating hyperammonemia due to disorders of the urea cyle or liver failure (Braissant et al., 1999b).

Functional expression of ASS and a role of the enzyme in channeling extracellular citrulline into the NO pathway were demonstrated for mouse astroglial cells, since substantial amounts of nitrite, an indicator of NO production, were detected when the cells had been stimulated in arginine-free medium in the presence of citrulline (Schmidlin and Wiesinger, 1998). Several other findings are worth noting. Ornithine could not substitute for citrulline suggesting indeed the absence (or inactivity) of OTC in astrocytes (Section 5.1.1). Inhibition of ASS by α-methyl-D,L-aspartate (Ratner, 1973) reduced drastically generation of NO also in arginine-containing medium, a clear hint at the importance of intracellular recycling of NOS coproduct citrulline to NOS substrate arginine in astrocytes. Aspartate, which is cosubstrate of ASS, is most likely generated by intracellular transamination, since NO production was abolished in the presence of (aminooxy)acetic acid, an inhibitor of aminotransferases (Palaiologos et al., 1988).

A complex expression pattern emerged when colocalization of ASS and NOS-2 was attempted in mixed glial cultures (Schmidlin and Wiesinger, 1998). After stimulation ASS-immunoreactivity with varying intensities was detectable in astroglial cells almost exclusively while expression of NOS-2 was confined to microglial cells. The implication of such a separation of arginine-producing and arginine-consuming pathways on intercellular trafficking of substrates will be discussed below (Section 5.4; see also Section 5.1.2).

From the experiments with primary cultures it was clear that ASS is expressed in glial cells, however, at levels which are at the limits of detection with the antisera used. Strong ASS immunoreactivity was induced after incubation of the cells with immunostimulants. It suggested itself that earlier studies had failed to detect ASS in glial cells in situ since brain sections of healthy animals had been used (Nakamura et al., 1991a; Arnt-Ramos et al., 1992). Indeed, when proinflammatory conditions were generated by injecting a mixture of LPS and IFN-γ into rat striatum strong induction of ASS immunoreactivity occurred in glial cells (Heneka et al., 1999). A detailed quantitative analysis revealed that ASS was almost exclusively localized in reactive microglial cells. It is quite intriguing that ASS immunoreactivity was also found in sham-injected animals in microglial cells with the typical round or oval morphology of activated microglia. Obviously induction of ASS is coupled to the activation of the cells without being dependent on the stimulus which leads to activation, be it the trauma set by the injection alone or the release of proinflammatory agents. Only occasionally colocalization of ASS with astroglial marker glial fibrillary acidic protein was observed (Heneka et al., 1999). This discrepancy to the results obtained in glial cell cultures still has to be explored further, and hypotheses based on the states of differentiation of astroglial cells, on the more complex cytokine pattern in situ or on differences in the time course of induction may be tested experimentally. The same levels of ASS immunoreactivity were detected in cortical and striatal neurons of control and injected animals with comparable staining intensities. By morphological considerations these neurons were identified as interneurons thus confirming a constitutive expression of the enzyme in this cell type (Nakamura et al., 1991a). In contrast to the animal model, a clear colocalization of ASS and NOS-2 was found in astrocytes as well as microglial cells in the brains of Alzheimer patients whereas neither enzyme was detectable in glial cells of control brains (Heneka et al., 2001). Thus, a pathway auxiliary to generation of nitric oxide is expressed in human glial cells under pathophysiological conditions.

5.1.5. Argininosuccinate lyase in glial cells

The biochemical data had demonstrated indirectly that ASL is active in (astro)glial cells (Yudkoff et al., 1987). The previous report by Nakamura et al. (1990) gave no indication of ASL protein being present in glial cells in brain; however, in the rat accessory olfactory bulb ASL-immunoreactivity was reported for small white matter cells considered as glial cells by the authors (Nakamura et al., 1999). Expression of ASL transcript was detected in astrocytes throughout the rat brain at low levels (Braissant et al., 1999a). ASL activity and mRNA were reported for astroglial primary cultures and were found to be increased after treatment of the cells with dexamethasone or dibutyryl cyclic AMP, i.e. under the influence of glucocorticoids and during differentiation of the cells (Jackson et al., 1996). ASL transcript was increased in astrocytes of hyperammonemic aggregating fetal rat brain cultures (Braissant et al., 1999b). In order to explore in more depth changes in ASL expression levels antisera against peptide sequences of the rat brain enzyme were recently generated in the author's laboratory and shown to be well suited for detection of the protein in neural cell homogenates (Bolla et al., 1999). ASL immunoreactivity was detected in a variety of glial cell preparations, however, in contrast to ASS, expression of ASL appeared not to be influenced by incubation of the cells with proinflammatory stimulants. This was confirmed in the recent study on the expression of the citrulline-NO cycle in the glioma cell line C6 (Zhang et al., 1999).

5.1.6. Arginase and arginine catabolism

In the early report on enzymes of arginine metabolism in the rat brain arginase activity was detected in the cerebellum, cerebral cortex, and brain stem (Sadasivudu and Rao, 1976). Later on, arginase immunoreactivity was studied in the main olfactory bulb, the cerebellar cortex, and the facial motor nucleus, and was localized in neurons exclusively (Nakamura et al., 1990). Because an antiserum against rat liver arginase was used in these studies, the authors supposedly detected arginase I immunoreactivity. The arginase-positive neurons in most cases did not express ASS, and some of them did not express ASL either. For example, in the cerebellar cortex the Purkinje cells exhibited arginase immunoreactivity but lacked ASS and ASL. Therefore, substrate arginine for arginase has to be derived from neighbouring structures, presumably ASL-expressing Golgi cells (Nakamura et al., 1990). Again, interneuronal trafficking of arginine metabolites is a prerequisite for any function that cytosolic arginase fulfills in the particular neurons. Mitochondrial arginase II mRNA was detected throughout the rat brain both in neurons and in glial cells, however, at varying levels (Braissant et al., 1999a). Notably the message was not found in Purkinje cells, but was identified in oligodendrocytes of cerebellar white matter. This is the first report of arginine catabolism in the intact myelin sheath. In contrast, ASS protein and ASL transcript as indicators of arginine synthesis were not found in oligodendrocytes in mixed glial cultures (Schmidlin and Wiesinger, 1998) and in situ (Braissant et al., 1999a), respectively.

Arginine in brain may also be catabolised by arginine:glycine amidinotransferase, the first enzyme in the biosynthetic pathway leading to creatine (Sadasivudu and Indira, 1974). Recently, NMR evidence for creatine synthesis in brain astroglial cells was obtained (Dringen et al., 1998b). The further fate of ornithine produced either by arginase or arginine:glycine amidinotransferase in brain is an unresolved issue, and roles in polyamine synthesis and as a precursor for glutamate and γ-aminobutyric acid have been discussed (Seiler and Daune-Anglard, 1993; Bernstein and Müller, 1999; see Section 5.4).

5.1.7. Agmatine and methylarginines

Agmatine and membrane-associated ADC are present in bovine and rat brain and a function for agmatine as neuromodulator has been proposed (Li et al., 1994; Yang and Reis, 1999; Regunathan and Reis, 2000; see Section 2.1). Ornithine decarboxylase has also been implicated in the decarboxylation of arginine in rodent brain (Gilad et al., 1996a). Cultured astroglial cells express ADC and contain substantial amounts of agmatine, and astroglial synthesis of agmatine is stimulated by IFN-γ (Regunathan et al., 1995). The hypothesis that astrocytes are major sites of synthesis and storage of agmatine awaits further experiments since the authors concede that the high medium concentration of arginine may obscure results derived from culture experiments. The glial function of agmatine has also to be clarified: regulation of NO synthesis, autocrine regulation of glial receptors binding agmatine (Regunathan et al., 1995), or synthesis for the benefit of surrounding neurons have to be taken into account. Agmatine can also be catabolized in brain, and an agmatinase-like activity has been described (Gilad et al., 1996c). However, it is not clear if hydrolysis of agmatine is catalyzed by a brain agmatinase or by arginase; both activities would yield urea and putrescine, the latter being the precursor for the polyamines. Either polyamine synthesis or inhibition of NOS may explain the neuroprotective effect of agmatine in models of brain injury (Gilad et al., 1996b).

The methylated arginine analogues NMMA and ADMA have been found in brain tissue, notably in cerebellum, hippocampus, hypothalamus, and olfactory bulb (Ueno et al., 1992), all of which also express NOS which is inhibited by NMMA (Section 3.2). Because methylated proteins in the brain are not turned over rapidly, the source of NMMA and ADMA in this

Table 2
Identification of (A) transport systems[a] and (B) transcripts[b]

System	Transporter	Reference
(A)		
Primary neurons		
Glutamatergic	y^+	Westergaard et al., 1993
Gabaergic	y^+	Westergaard et al., 1993
Primary neurons, hindbrain	y^+	Stevens and Vo, 1998
Neuron-enriched hypothalamic cultures	y^+	Wayte et al., 1996
Cell lines with neuronal properties		
NG108-15[c]	y^+	Schmidt et al., 1995
108CC15[c]	y^+	Schmidlin et al., 1997b
Brain synaptosomes (cortex)	y^+	Lopes et al., 1994
Brain synaptosomes (cortex)	Ly^+	Tan and Ng, 1995
Cerebellar and cortical synaptosomes	y^+	Collard, 1995; Aldridge and Collard, 1996
Cerebellar synaptosomes	y^+	Rao and Butterworth, 1996
Primary astroglial cells (mouse)	y^+	Schmidlin and Wiesinger, 1994
Primary astroglial cells (rat)	y^+	Stevens et al., 1996
Primary mixed glial cells (rat)	y^+	Schmidlin and Wiesinger, 1994
Glial cell lines		
C6-BU-1 (astroglial)	y^+	Schmidlin and Wiesinger, 1994
N11 (microglial)	y^+, $B^{0,+}$	Schmidlin et al., 1997b
Microvessels (in situ)	y^+	Stoll et al., 1993
Choroid plexus	y^+	Segal et al., 1990; Stuhlmiller and Boje, 1995
	Transcript	
(B)		
Microvessels and choroid plexus	CAT-1	Stoll et al., 1993
Neurons, rat primary cultures	CAT-1	Stevens and Vo, 1998
Neurons and astrocytes, rat brain	CAT-1	Braissant et al., 1999a
	CAT-2	Braissant et al., 1999a
Astrocytes, rat primary cultures	CAT-2[d]	Stevens et al., 1996
Mouse brain	CAT-3	Ito and Groudine, 1997
Rat brain	CAT-3	Hosokawa et al., 1997
Neurons, rat brain	CAT-3	Hosokawa et al., 1999
	CAT-3	Braissant et al., 1999a

[a] For arginine in brain, neural cell cultures and synaptosomal preparations by kinetic studies.
[b] For cationic amino acid transporters (CATs) in brain and neural cell cultures.
[c] Same cell line with different designations.
[d] CAT-2 splice variant, not CAT-2a.

tissue under normal conditions is not clear. However, an up-regulation of protein degradation under pathological circumstances may necessitate increased degradation of methylarginines as was indeed found after motor nerve injury (Nakagomi et al., 1999)

5.2. Transport of arginine, citrulline and argininosuccinate in neural cells

Transport of arginine has been characterized as being mediated by system y^+ in a variety of neural cell cultures and synaptosomal preparations (Table 2). The presence of less specific systems in cultured brain cells has been reported occasionally (Table 2). System y^+ appears also to mediate uptake of arginine in the capillary endothelial cells of the microvessels and in the choroid plexus epithelium (Segal et al., 1990; Stoll et al., 1993; Stuhlmiller and Boje, 1995) thus rendering kidney-derived peripheral arginine available to the brain parenchyma and the cerebrospinal fluid. Arginine is also transported by the heterodimeric system 4F2hc/y^+LAT2. Transcripts of both subunits have been detected in brain, but also in cultured neurons and astrocytes (Bröer et al., 2000). Arginine efflux by this transporter will be discussed below (Section 5.4). It should be noted that system y^+ mediates uptake of arginine into mitochondria as was deduced from transport studies with preparations from rat cerebral hemispheres which are enriched in mitochondria of glial cells (Dolinska and Albrecht, 1998). These findings are of importance since arginine may undergo two different transformations within the mitochondrial matrix where arginase II as well as ADC are located (Sections 5.1.6 and 5.1.7).

An alternative means of uptake of arginine into neurons expressing NOS-1 was suggested recently on the grounds of a detailed study on the neural localization of NBAT, a member of the $b^{0,+}$ family of transporters for neutral and basic amino acids (Pickel et al., 1999). NBAT had been identified earlier in enteric neurons of the small intestine, in neurons of autonomic nuclei of the brainstem, and in chromaffin cells of the adrenal medulla (Nirenberg et al., 1995), and a role for NBAT in providing these cells with L-tyrosine for catecholamine biosynthesis was proposed (Tam and Roth, 1997). However, NBAT was also present in neurons lacking the enzymes for catecholamine synthesis. In the more recent study (Pickel et al., 1999) NBAT immunoreactivity was shown to be colocalized with that of NOS-1 in the somata as well as dendrites of numerous striatal and cortical neurons in the rat forebrain. Nevertheless, a large number of neurons containing NOS-1 but lacking NBAT was also detected. A transport protein homologuous to NBAT, rBAT cloned from rabbit kidney, was localized at intracellular structures in hypothalamic neurons (Hisano et al., 1996).

Only scarce data exist on the regulation of arginine uptake in neural cells. Up-regulation of arginine transport in brain cells under proinflammatory conditions,

i.e. upon incubation with LPS, was demonstrated for the first time in cultured astroglial cells (Schmidlin and Wiesinger, 1994, 1995). Up-regulation occurred in parallel to the time-course of generation of NO and was abolished in the presence of cycloheximide, suggesting that de novo synthesis of transporter protein was the underlying mechanism. However, induction of NOS-2 and stimulation of arginine transport exhibited different inhibition patterns and different EC_{50} values of LPS suggesting different signaling pathways (Schmidlin and Wiesinger, 1995). In contrast, rates of arginine uptake were not changed when cells with microglial or neuronal properties had been incubated with LPS (Schmidlin et al., 1997b). In neither cell line LPS alone was able to induce generation of NO. Lack of induction of NOS by LPS/IFN-γ corresponded well with constitutive expression of y^+ transport activity in neuronal primary cultures (Stevens and Vo, 1998). Arginine transport in astrocytes and glioma cells C6 was down-regulated by prolonged exposure to noradrenaline, isoproterenol or dibutyryl cyclic AMP (Feinstein and Rozelman, 1997).

Several reports indicated that arginine transport in neural cells is altered under pathological conditions. Hepatic encephalopathy is characterized by neural accumulation of ammonia and mangenese, since these substances are not removed by the hepatobiliary route after liver failure (Hazell and Butterworth, 1999). Arginine uptake was stimulated by a 40–60% increase in transport velocity in synaptosomes isolated from the brains of portocaval-shunted rats, an animal model of HE (Rao et al., 1997). This increase in V_{max} was most probably caused by the elevated brain levels of ammonia in HE as hyperammonemia induced in sham-operated rats had the same effect on synaptosomal arginine transport (Rao et al., 1997), and high concentrations of extracellular ammonia stimulated arginine transport in synaptosomes (Rao et al., 1997) and in cultured astrocytes, the cell type which is most vulnerable in HE (Hazell and Norenberg, 1998). However, it should be emphasized that in synaptosomes a short-time exposure (up to 10 min) with ammonia was sufficient for stimulation of arginine transport. It is still more intriguing that in cultured astrocytes K_M values of arginine uptake were elevated after prolonged treatment with ammonia or manganese ions (Hazell and Norenberg, 1998). No mechanistic explanations have been given in either case. Nevertheless, elevated levels of ammonia and manganese may contribute to the pathogenesis of HE by their influence on arginine uptake and, consequently, NO synthesis.

Infection of various astroglial cultures with *ts*1, a neuroimmunopathogenic mutant of Moloney murine leukemia virus, reduced arginine uptake (Lin et al., 1997) probably by interaction of the viral envelope proteins with CAT-1 which is identical with an ecotropic retrovirus receptor (Kim et al., 1991). Reduction of glial arginine content might be important for *ts*1 pathogenesis because the disease develops in growing mice which are sensitive to arginine depletion (Lin et al., 1997).

It is highly probable that y^+ is also operating in neural cells in situ since several transcripts related to this transport system have been detected in brain (Table 2). Transcript CAT-3 appears to be brain specific (Ito and Groudine, 1997; Hosokawa et al., 1997), however, may be induced and functionally compensate for other CAT proteins in knockout peripheral cell lines (Nicholson et al., 1998). Although only a limited amount of data is available at present, a discrete regional and cellular distribution of CAT transcripts appears probable. CAT-3 transcripts were localized mainly along the midbrain-thalamus-hypothalamus axis in the adult rodent brain (Ito and Groudine, 1997; Hosokawa et al., 1999), whereas CAT-1 appears to be enriched in regions with barrier properties such as microvessels and choroid plexus (Stoll et al., 1993), but was also found in neurons and glial cells (Braissant et al., 1999a). At the cellular level neither capillary endothelial nor glial cells yielded hybridisation signals with a CAT-3 cRNA probe whereas neurons were strongly positive (Braissant et al., 1999a; Hosokawa et al., 1999). It cannot be taken for granted that detection of an mRNA implies expression of the corresponding protein (Luss et al., 1997), and unequivocal results can only be obtained by immunohistochemical methods. Indeed, an antiserum prepared against a rat CAT-3 peptide sequence revealed only weak immunostaining in the ventromedial part of the rat brain despite the strong hybridisation signal in this area (Hosokawa et al., 1999). Good to excellent correspondence of immunohistochemistry and cRNA hybridisation was observed in the pyramidal cell layer of the cerebral cortex and hippocampus, the mitral cell layer of the olfactory bulb and the granular and Purkinje cell layers of the cerebellum. Immunoreactivity was confined to neuronal cell bodies (Hosokawa et al., 1999) thus confirming the idea that CAT-3 is a neuron-specific arginine transporter. In accordance with the notion that CAT-2 splice variants are responsible for induced increase of arginine transport a particular splice variant of CAT-2 was detected in cultured astroglial cells upon incubation of the cells with immunostimulants (Stevens et al., 1996).

The highly differential expression of ASS, ASL, and NOS isoforms in neural cells (Section 5.1) necessitates not only the exchange of arginine, but also of citrulline across glial and neuronal plasma membranes. All types of brain cells investigated, i.e., astrocytes, microglial cells, and neurons, are capable of

transporting citrulline with K_M values ranging from 0.4 to 3.4 mM, and system L for large neutral amino acids has been proposed for mediating neural citrulline transport (Schmidlin et al., 2000). Citrulline transport notably was not found to be regulated by immunostimulants, in accordance with the known properties of system L (Christensen, 1990). Although data from peripheral (Baydoun et al., 1994) and CNS cell systems (Schmidlin et al., 2000) are limited it is of significance that citrulline does not appear to use a transporter of its own. This has two important consequences: citrulline transport cannot be regulated by a specific mechanism, and citrulline has to compete with other substrates of system L which are transported with K_M values in the low μM range (Kanai et al., 1998) and the concentrations of which exceed that of citrulline by far. Therefore, utilization of extracellular citrulline as part of a neural citrulline–NO cycle depends on the local concentration of all neutral amino acids present.

This problem is still more obvious for AS which has to be shuttled between cells expressing exclusively ASS or ASL and lacking the respective other enzyme. Furthermore, if AS is indeed a neuromodulator (Nakamura et al., 1991b), a means of release of the compound into the synaptic cleft has to exist. However, data on the transport of AS across cell membranes or on its vesicular storage and release have not been reported so far. Diffusion through the lipid bilayer (Isayama et al., 1997) can be excluded on grounds of the charged nature of the complex molecule and is the least plausible way of directing a compound into the synaptic cleft. Release or uptake may be mediated by carrier proteins, and AS may use transporters of molecules with similar structure or structural components such as carriers of anionic or cationic amino acids. As in the case of citrulline there is always competition among carrier substrates, and this poses a particular problem when one considers the high concentrations of the respective amino acids in the brain. Preliminary experiments in the author's laboratory exclude the transport systems for anionic or cationic amino acids as being able to accept AS as substrate since uptake neither of glutamate nor of arginine in cultured astrocytes was inhibited by a large excess of AS (Wiesinger, H., Vogel, D., unpublished results). The recent finding that in the presence of AS the synthesis of astroglial glutathione from extracellular cysteinylglycine was diminished may point to the participation of a peptide transporter in the uptake of AS in astrocytes (Wiesinger, H., Dringen, R., unpublished results; see also Dringen et al., 1998a). However, more data, preferably obtained with radioactively labelled AS, are necessary to arrive at a hypothesis of the mechanism of release and transmembrane exchange of AS. The results then may shed light on the role of AS in intercellular compartmentalization of urea cycle in brain or even as a potential neuromodulator.

5.3. Arginine and the regulation of NO synthesis in the brain

Many of the findings on brain arginine metabolism summarized in the previous sections were descriptional, and implications for the role of arginine in the regulation of neural NO synthesis are in many cases circumstantial. In the following it is attempted to bring together data that point to a function of arginine or its derivatives in the control of NO synthesis in nervous tissue.

5.3.1. Generation of superoxide

As was already pointed out all isoforms of NOS can generate the superoxide radical, O_2^-, when the substrate arginine is not present in sufficient quantity (Section 3.1). When cultured cerebellar granule neurons were depleted of arginine a rapid burst of superoxide was observed upon stimulation of the cells with NMDA (Culcasi et al., 1994). The time course of O_2^- generation was different from NMDA-dependent superoxide production of granule cells cultured in arginine-containing medium a process which only marginally may involve NOS (Lafon-Cazal et al., 1993). The generation of O_2^- was blocked after addition of N^G-nitroarginine and did not occur when cells were repleted with arginine, manipulations which suppressed also NMDA-induced cell death. Thus an example was presented that activation of NOS-1 in the presence of suboptimal concentrations of arginine leads to neurotoxicity via generation of O_2^-, and the necessity of adequate substrate supply of neuronal NOS-1 was established. Because to our present knowledge neither arginine synthesis nor arginine transport can be up-regulated in neurons (Sections 5.1 and 5.2), the environment has to be included in any consideration of proper neuronal NO synthesis. Indeed, a mechanism may exist by which arginine depletion in neurons is prevented with the help of neighbouring astrocytes (Section 5.4). On the other hand, interleukin-1β-stimulated hypothalamic astrocytes also have been shown to release superoxide under arginine deprivation (Tolias et al., 1999). It is clear from these studies that in neural cells substrate deprivation of NOS is a regulatory factor in the generation of NO with potentially detrimental consequences.

5.3.2. Agmatine and methylarginines

A role of agmatine as potential endogenous inhibitor of NO synthesis in brain has been proposed (Galea et al., 1996), and agmatine treatment protected neurons in models of brain injury (Gilad et al., 1996b). Because concentrations of agmatine measured in normal tissues are far below the concentrations of arginine (Galea et al., 1996) effective regulation of NOS seems improbable. However, the situation may change under pathophysiological conditions leading to high local

concentrations of agmatine. Indeed, an upregulation of arginine decarboxylation by IFN-γ occurred in cultured astrocytes (Regunathan et al., 1995) and may influence NO synthesis in the glial cells, but also in neighbouring neurons which possess an agmatine uptake system (Sastre et al., 1997).

It is intriguing that in the rat brain the highest concentrations of methylarginines are found in the cerebellum and olfactory bulb (Ueno et al., 1992), regions with prominent NOS activity (Bredt et al., 1990). Similarly, in the bovine brain the distribution of methylarginines parallels the expression of NOS (Ueno et al., 1992). Again, in order to assess the potential of the methylated arginine analogues as endogenous inhibitors of neural NO synthesis intracellular concentrations must be known. Unfortunately, equivocal data on the content of the compounds in brain tissue can be taken from the literature (Table 1). The values reported by Ueno et al. (1992) are less than one hundredth of the average tissue concentration of arginine (Table 1). On the other hand, concentrations for NMMA and ADMA in brain given by Kimoto et al. (1993) would amount to 2–5% of the concentration of free arginine, and the molecules could clearly act as inhibitors of NO synthesis (Section 3.2); adequate degradation may become mandatory. DDAH immunoreactivity was detected in cultured neurons (Fig. 3), and DDAH transcript was expressed at high concentration in cells of the choroid plexus and in neurons in many parts of the brain, most prominently in cerebral cortex and thalamus, weakly, however, in the hypothalamus and not at all in the cerebellum (Nakagomi et al., 1999). Thus, an inverse relationship between the presence of DDAH and the concentration of methylarginines seems probable. Expression of NOS-1 as well as DDAH was up-regulated in hypoglossal motor neurons after axotomy; since also protein degradation is enhanced in this case the capacity to metabolize protein-derived methylarginines may indeed be necessary for a proper functioning of NOS (Nakagomi et al., 1999). DDAH is also expressed in cultured astroglial cells, and the level of expression does not appear to be influenced by immunostimulants (Fig. 3; Wiesinger, H., Kimoto, M., Ogawa, T., unpublished results); however, data about protein turnover in immune-stimulated astrocytes are lacking.

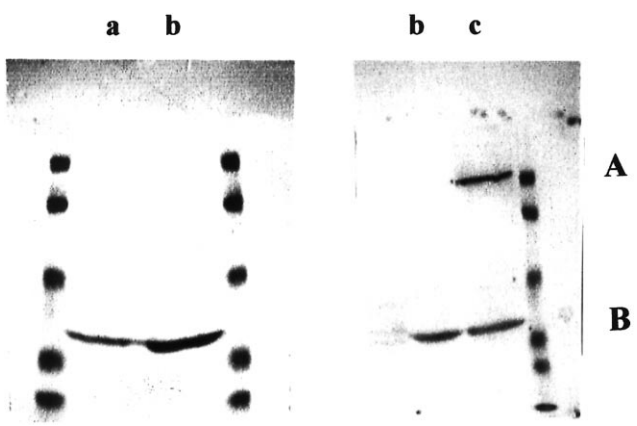

Fig. 3. Detection of NOS-2 (A) and DDAH (B) by Western blot analysis in homogenates of cultured neurons (a) and astroglial cells (b,c) from rat brain. The astroglial cells were incubated for 24 h in the absence (b) or presence (c) of LPS (50 μg/ml) and IFN-γ (100 U/ml). One hundred μg of homogenate protein were separated on a 10% sodium dodecylsulfate-polyacrylamide gel and transferred to nitrocellulose by immunoblotting. A monoclonal antibody against DDAH generated by T. Ogawa and M. Kimoto, formerly Tokushima University, Japan (Kimoto et al., 1993, 1995), and a commercially available antibody against NOS-2 were used for detection of the proteins. Markers correspond to the following molecular masses (in kDa; right panel, from top): 97.4, 66, 45, 31, 21.5, 14.5.

5.3.3. Arginine-supported neural functions

It is generally accepted that concentration-dependent effects of arginine on physiological functions may be indicative of the involvement of NO in the system under investigation. It stands to reason that in arginine-depleted culture or slice systems refeeding of arginine results in an increase of NO-mediated functions as was shown already in the early work on NMDA receptor-stimulated generation of NO (Garthwaite et al., 1989). Nevertheless, the results demonstrate that in the absence of intracellular stores of arginine recycling of NOS coproduct citrulline is insufficient despite the fact that ASS as rate-limiting enzyme of the urea cycle is constitutively expressed in neurons (Section 5.1.2). Discrepancies in the amounts of enzymes or in the kinetics of NOS-1 and ASS under low substrate concentrations may explain the findings. In addition, in the complex slice system the differential distribution of NOS, ASS and ASL (Sections 5.1.2 and 5.1.3) and the need of intercellular trafficking of the substrates would slow down considerably the overall synthesis of arginine from citrulline and indeed deprive NOS of its substrate.

Without manipulations to deplete intracellular arginine stores the amino acid is present and/or recycled in sufficient quantity to sustain Ca^{2+}-dependent generation of NO (Hecker et al., 1990; Mitchell et al., 1990). However, in other cases intracellular pools of arginine are either insufficient or inaccessible for NOS since in many in vivo experiments synthesis of NO — as determined by its physiological effect — is enhanced after administration of arginine suggesting that generation of NO takes place under suboptimal substrate supply. In the central nervous system this appears to hold for Ca^{2+}-dependent NO synthesis by NOS-1 and/or NOS-3 because application of arginine in vivo was found to facilitate sensory synaptic transmission in the thalamus (Do et al., 1994) and to increase cerebral blood flow in several experimental paradigms (He et al., 1995; Reutens et al., 1997). Again, one has to remember the complex differential expression of NOS and the

enzymes of the urea cycle in neurons and glial cells, and most NOS-containing neurons in the CNS have not the capacity to recycle citrulline to arginine (Section 5.1.2). In contrast, enteric neurons appear to possess the full machinery for generating arginine from citrulline since they sustain NO-dependent neurotransmission when arginine transport inhibitors and citrulline are present simultaneously (Shuttleworth et al., 1995, 1997). Similarly, perivascular nerves of cerebral arteries can convert citrulline to arginine during NO-induced cerebral vasodilation (Chen and Lee, 1995; Yu et al., 1997).

5.3.4. Citrulline-NO cycle in the nervous system

As pointed out above already short-term generation of NO by constitutive NOS may depend on the availability of extracellular arginine. Generation of NO in high quantities for a long period of time after induction of NOS-2 is even more stringently coupled to an adequate supply of substrate which in vivo may depend on the availability of circulating arginine (Bune et al., 1995). In many cases supply of arginine seems to be secured by an up-regulation of arginine transport as well as synthesis concomittant with induction of NOS-2. Intracellular recycling of citrulline may provide arginine for Ca^{2+}-dependent NO synthesis (Section 5.3.3). However, throughout the recent literature the term 'citrulline–NO cycle' has been used whenever the NOS inducing stimulants also increased ASS transcript or protein expression implying an up-regulation of the rate-limiting step in the recycling of citrulline to arginine (Section 2.1). ASS protein is increased from a barely detectable level to considerable amounts in cultured astroglial after stimulation with LPS and IFN-γ (Schmidlin and Wiesinger, 1998), and in NOS-2-positive astrocytes in the brains of Alzheimer patients strong ASS-immunoreactivity was detected (Heneka et al., 2001). Therefore, this particular population of glial cells is enabled to utilize the citrulline-NO cycle for providing arginine for induced NO synthesis. However, NOS-2 expression in astroglial cells may be down-regulated in the presence of other immune-competent cells, and in mixed glial cultures ASS expression was confined to astrocytes whereas NOS-2 was almost exclusively localized in microglial cells (Schmidlin and Wiesinger, 1998). It remains to be clarified if in this instance an intercellular citrulline–NO cycle is operating between two glial cell populations (Schmidlin et al., 2000) and if the expression pattern of such a cycle in vivo is a complex function of space and time (Heneka et al., 1999).

All evidence that a particular CAT is involved in regulation of NO synthesis is circumstantial and inferred from a simultaneous induction of CAT and NOS isoforms (e.g. Hattori et al., 1999). Only one study so far attempted to elucidate the importance of arginine uptake for induced NO synthesis in brain cells, i.e. cultured astrocytes (Stevens et al., 1996). However, contrary to the titel of the study it is not demonstrated unequivocally that the particular CAT-2 isoform induced by LPS/IFN-γ supplies arginine selectively to NOS-2. Since induction of NOS-2 and CAT-2B cannot be uncoupled any NO production measured after several hours may also be due to arginine supplied by CAT-1 which is present in the astroglial cells. In general, statements about arginine uptake being 'rate-limiting' for generation of NO have to be discussed critically when NO was determined by an integral method over many hours. This drawback was overcome in an excellent recent paper in which for the first time true kinetic experiments on the role of CATs in arginine-dependent NO synthesis were performed (Closs et al., 2000). The findings that for a short time macrophages can generate NO without supply of arginine and that maximal CAT-2B expression can be seen when NOS-2 protein has decreased already considerably may argue against a role of CAT-2B in substrate supply for NOS-2 (Closs et al., 2000). Clearly, carefully designed experiments preferably with animals in which one or several of the CAT genes are deleted are necessary in order to dissect unambiguously the contributions of arginine transport and the citrulline–NO cycle to arginine supply of NOS, not the least in immune-stimulated brain cells. In this context it has to be considered that urea cycle enzymes may be organized in a multienzyme system allowing effective channeling of the intermediates in a spezialized 'metabolon' which is difficult to enter from the cytosol (Cheung et al., 1989; Watford, 1989). It is a totally unexplored issue if such a supramolecular organization is present in brain cells and if a citrulline–NO cycle constitutes a metabolon of its own.

5.4. Arginine and astrocytes

As demonstrated above the intracellular availability of arginine may regulate specificity as well as quantity of NOS output. In the complex organ brain, where does this arginine originate? Arginine released from the kidney enters the brain parenchyma via transport system y^+ localized in the endothelial cells of the blood-brain barrier (Stoll et al., 1993). Additional arginine may derive from protein degradation or from citrulline which in turn may have been extracted from the blood or may stem from intraparenchymal NOS activity. Arginine appears to be distributed unevenly among the major brain cell populations. L-Arginine immunoreactivity in the rat brain and spinal cord was mainly localized in astrocytes whereas oligodendrocytes were not immunoreactive (Aoki et al., 1991b). Some neurons in the cerebellum showed weak immunoreactivity, several fibers in the brain stem and spinal cord, however, were prominently stained. Notably Bergmann glial cells in the cerebellum were immunopositive whereas neigh-

bouring basket and Purkinje cells are lacking arginine. Arginine immunoreactivity was also seen in astrocytic processes wrapping endothelial cells of the vasculature (Aoki et al., 1991b). In the peripheral nervous system arginine was detected in glial components ensheathing neurons (Aoki et al., 1991a). In contrast, citrulline immunoreactivity was described for NADPH diaphorase-positive neurons exclusively, although in some areas such as the striatum many more cells expressed NADPH diaphorase without being labelled by anti-citrulline antiserum (Pasqualotto et al., 1991). It is tempting to speculate that the latter are neurons which also express ASS (Section 5.1.2), whereas the former ones obviously lack the capacity to recycle citrulline. A simultaneous immunohistochemical localization of arginine and citrulline was attempted in the rat neurohypophysis, and the results clearly demonstrated a differential localisation of arginine and citrulline in pituicytes, i.e. members of the astroglial family, and neurons, respectively (Pow, 1994). A glial localisation of arginine was also demonstrated in the ventroposterior thalamic nucleus of the rat (Kharazia et al., 1997). In cultured astrocytes, the concentration of arginine (45.6 nmol/mg of protein) is surpassed only by the concentrations of taurine and glutamine (Yudkoff et al., 1989).

Indeed astrocytes are well equipped for accumulating arginine since they express the transport as well as the recycling machinery for arginine (Sections 5.1.4 and 5.2). Both uptake and recycling are upregulated when astrocytes are stimulated with LPS and/or proinflammatory cytokines. Although system y^+ operates independently of the gradient of Na^+-ions arginine as a cation at physiological pH can accumulate within the cell due to the favourable membrane potential. In addition, astrocytes express pyruvate carboxylase, an enzyme which is lacking in neurons (Cesar and Hamprecht, 1995). Thus, astrocytes possess a biochemical pathway by which oxaloacetate, precursor of ASS cosubstrate aspartate, is provided within the same cell whereas neurons are dependent on neighbouring glial cells for this anaplerotic reaction. This may be another reason why in some NOS-positive neurons citrulline accumulates and can be detected by immunohistochemistry. Aspartate immunoreactivity was also localized in the glial compartment (pituicytes) in the neurohypophysis (Pow, 1994).

The finding of enriched arginine-immunoreactivity in astrocytes is difficult to reconcile with the fact that in the adult rat brain these cells exhibit prominent immunostaining for polyamines (Laube and Veh, 1997). A prerequisite for polyamine synthesis is the degradation of arginine to ornithine which is then decarboxylated to putrescine. Arginase is present in astrocytes (Section 5.1.6) and in contrast to hepatocytes may have the only function to generate ornithine. Urea would thus be a byproduct (and, indeed, is not necessary for nitrogen disposal in brain; see Section 5.1.1) which is not allowed to accumulate and disturb the osmotic balance. Disposal of urea during synthesis of polyamines may therefore be the function of urea transporter UT3 whose transcript was recently discovered in astrocytes in culture and in vivo throughout the rat brain (Berger et al., 1998). For a proper consideration of the problem it should be mentioned that under normal conditions ornithine decarboxylase is predominantly expressed in neurons (Bernstein and Müller, 1999) and that glial cells possess high affinity uptake systems for polyamines (Khan et al., 1990; Li and Wiesinger, 1996). However, in separate models of reactive gliosis an up-regulation of astroglial ornithine decarboxylase protein and UT3 transcript, respectively, was observed (Berger et al., 1998; Bernstein and Müller, 1999).

The data described above suggest an intimate relationship between a particular set of neurons and astrocytes, with arginine being transported from the glial cells to the neurons for effective synthesis of NO. This proposal is supported by functional data gathered during the last couple of years. Arginine was released in the ventrobasal thalamus in vivo upon sensory afferent stimulation (Do et al., 1994, 1996). Subsequently evidence was obtained that the signal for arginine release is glutamate acting on non-NMDA receptors and that the source of arginine are the astrocytes: ionotropic non-NMDA receptors are present on glial cells, and arginine efflux can be induced in primary astrocytes upon incubation with glutamate (Grima et al., 1997). Moreover, in a slice system the availability of arginine mobilized by the activation of glial non-NMDA receptors determined the rate of neuronal NO synthesis (Grima et al., 1998). Thus glutamate activates NOS-1 in neurons by increasing the concentration of cytosolic Ca^{2+} and simultaneously activates astrocytes to release arginine which is taken up by neurons for serving their NO synthesis. Astroglial arginine release may ultimately be embedded in an even more intricate network of glial-neuronal cooperation in energy metabolism, glutamatergic neurotransmission, and regulation of local cerebral blood flow (Fillenz et al., 1999). Although not demonstrated it is conceivable that NOS coproduct citrulline returns to the astroglial cells and is recycled to arginine. A neuronal-glial citrulline–NO cycle with intercellular trafficking of arginine and citrulline may be proposed (Fig. 4), as was done for the particular system pituicytes-neurons in the neurohypophysis (Pow, 1994). Such a compartmentalization has the advantage of separating pools of arginine which can enter a number of metabolic pathways, and of keeping AS away from NOS which is inhibited by this compound (Gold et al., 1989). The proposal of an interglial NO–citrulline cycle between astrocytes and microglial cells upon immunostimulation (Schmidlin and Wiesinger, 1998; Schmidlin

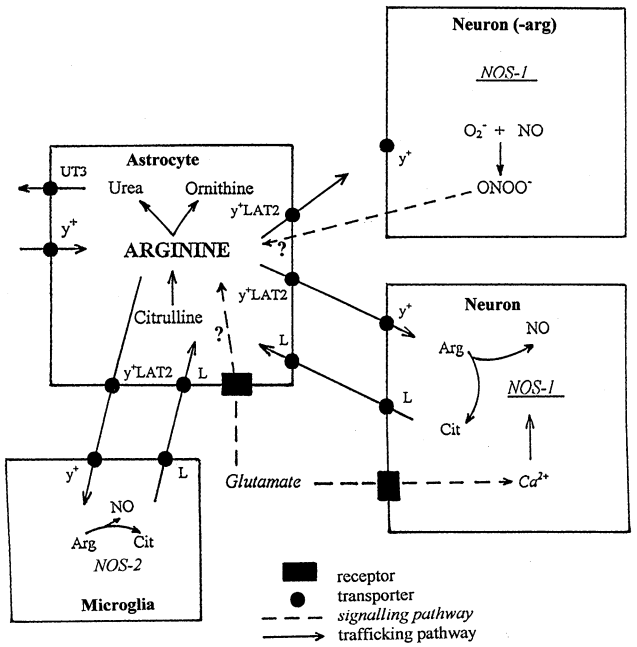

Fig. 4. Schematic representation of the hypothetical role of astrocytes as arginine stores for neighbouring neurons and microglial cells. Interneuronal metabolite trafficking, an intracellular citrulline–NO cycle, e.g. in the microglial cells, and arginine consumption for the generation of NO within the astroglial cells have also to be considered. Nothing is known about the pathways triggering arginine release ('?'). The scheme is based on the author's own work and on several reports cited in the text. For participating enzymes, see text and Fig. 1.

et al., 2000; Fig. 4) clearly has to be substantiated by functional data. In contrast, in preparations of avian retinal cells it was demonstrated unequivocally that arginine released from the glial cells upon stimulation with K^+ is taken up by cocultured neurons; in addition, while arginine uptake occurred in both cell types, citrulline as well as NOS-1 immunoreactivity were only present in the neurons (Pow and Crook, 1997; Cossenza and Paes de Carvalho, 2000).

A further striking example of stimulus-dependent release of arginine from astroglial cells was reported. Peroxynitrite is formed after generation of O_2^- by NOS-1 under suboptimal supply with arginine (Beckman and Koppenol, 1996; Sections 3.1 and 5.3.1); when given to cultured astrocytes $ONOO^-$ induced efflux of arginine in a concentration-dependent manner (Vega-Agapito et al., 1999). This may be a mechanism by which neurons secure a sufficient amount of arginine for their NOS-1 in order to avoid excessive formation of neurotoxic $ONOO^-$ and to enable neuronal NOS-1 to function properly in the synthesis of NO.

The recent studies demonstrated that glutamate as well as peroxynitrite are signaling molecules which in vivo most probably are derived from neurons and act upon astrocytes to induce arginine release from intracellular stores. The mechanism of release, however, remained obscure. An explanation is offered by the recent finding that the transcripts of both subunits of the complex transporter 4F2hc/y^+LAT2 are present in astrocytes (Bröer et al., 2000). Mediation of arginine efflux may be the main physiological function of transporter subunit y^+LAT2 similar to its counterpart in the kidney, y^+LAT1 (Torrents et al., 1998). It remains to be determined if y^+LAT2 is activated by signals such as glutamate, K^+, or $ONOO^-$, and maybe even is up-regulated by immunostimulants.

6. Concluding remarks

The renewed interest in arginine after the discovery of its role in the synthesis of the biologically important signaling and effector molecule NO is reflected in a renaissance of studies on metabolism and transport of the amino acid in the nervous system. However, experimental approaches to elucidate the interplay between arginine and generation of NO in nervous tissue are hampered by the complex cellular composition particularly of the brain. As a consequence, many results on the role of neural arginine have been obtained from model systems such as cell cultures and brain slices, and descriptional data on mRNA or protein expression prevail. It is a challenge for the future to design experiments which will elucidate in detail the roles that the enzymes and transport systems reported to be present in neural cell populations play under normal and pathophysiological conditions. Nevertheless, evidence has been gathered over the last few years that intercellular cooperation in uptake, synthesis, storage and release of arginine or its metabolites is mandatory for proper execution of neural NO synthesis. Neuron–neuron, neuron–glia and interglial trafficking of components of the citrulline–NO cycle and a prominent role of astrocytes as arginine suppliers for other brain cells may be deduced. Any considerations of therapeutic intervention, be it towards attenuation or enhancement of neural NO synthesis, have to be based on the present knowledge and on future refined elucidation of the intercellular complexity of neural arginine metabolism.

Acknowledgements

The author is indebted to Dr R. Dringen, Tübingen, for critical reading of the manuscript, and wants to express his thanks to Drs T. Ogawa, now Kyoto University, Japan, and M. Kimoto, now Okayama University, Japan, for their generous gift of monoclonal antibody against DDAH. The author's own work was financially supported by the Deutsche Forschungsgemeinschaft (grants Wi 657/4-1 to 4-4) and the Fonds der Chemischen Industrie which is gratefully acknowledged.

References

Albina, J.E., 1995. On the expression of nitric oxide synthase by human macrophages. Why no NO? J. Leucoc. Biol. 58, 643–649.

Albina, J.E., Mills, C.D., Barbul, A., Thirkill, C.E., Henry, W.L., Mastrofrancesco, B., Caldwell, M.D., 1988. Arginine metabolism in wounds. Am. J. Physiol. 254, E459–E467.

Aldridge, C.R., Collard, K.J., 1996. The characteristics of arginine transport by rat cerebellar and cortical synaptosomes. Neurochem. Res. 21, 1539–1546.

Aoki, E., Semba, R., Kashiwamata, S., 1991a. Evidence for the presence of L-arginine in the glial components of the peripheral nervous system. Brain Res. 559, 159–162.

Aoki, E., Semba, R., Mikoshiba, K., Kashiwamata, S., 1991b. Predominant localization in glia cells of free L-arginine. Immunocytochemical evidence. Brain Res. 547, 190–192.

Arbones, M.L., Ribera, J., Agullo, L., Baltrons, M.A., Casanovas, A., Rvereos-Moreno, V., Garcia, A., 1996. Characteristics of nitric oxide synthase type-I in rat cerebellar astrocytes. Glia 18, 224–232.

Arnt-Ramos, L.R., O'Brien, W.E., Vincent, S.R., 1992. Immunohistochemical localization of argininosuccinate synthetase in the rat brain in relation to nitric oxide synthase-containing neurons. Neuroscience 51, 773–789.

Barbul, A., 1986. Arginine: biochemistry, physiology, and therapeutic implications. J. Parent. Ent. Nutr. 10, 227–238.

Baydoun, A.R., Mann, G.E., 1994. Selective targeting of nitric oxide synthase inhibitors to system y^+ in activated macrophages. Biochem. Biophys. Res. Commun. 200, 726–731.

Baydoun, A.R., Bogle, R.G., Pearson, J.D., Mann, G.E., 1994. Discrimination between citrulline and arginine transport in activated murine macrophages: inefficient synthesis of NO from recycling of citrulline to arginine. Br. J. Pharmacol. 112, 487–492.

Baydoun, A.R., Wileman, S.M., Wheeler-Jones, C.P.D., Marber, M.S., Mann, G.E., Pearson, J.D., Closs, E.I., 1999. Transmembrane signalling mechanisms regulating expression of cationic amino acid transporters and inducible nitric oxide synthase in rat vascular smooth muscle cells. Biochem. J. 344, 265–272.

Beckman, J.S., Koppenol, W.H., 1996. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. Am. J. Physiol. 271, C1424–C1437.

Berger, U.V., Tsukaguchi, H., Hediger, M.A., 1998. Distribution of mRNA for the facilitated urea transporter UT3 in the rat nervous system. Anat. Embryol. 197, 405–414.

Bernstein, H.-G., Müller, M., 1999. The cellular localization of the L-ornithine decarboxylase/polyamine system in normal and diseased central nervous systems. Prog. Neurobiol. 57, 485–505.

Blottner, D., Grozdanovic, Z., Gossrau, R., 1995. Histochemistry of nitric oxide synthase in the nervous system. Histochem. J. 27, 785–811.

Bogle, R.G., Baydoun, A.R., Pearson, J.D., Moncada, S., Mann, G.E., 1992. L-Arginine transport is increased in macrophages generating nitric oxide. Biochem. J. 284, 15–18.

Bogle, R.G., MacAllister, R.J., Whitley, G.S.J., Vallance, P., 1995. Induction of N^G-monomethyl-L-arginine uptake: a mechanism for differential inhibition of NO synthases? Am. J. Physiol. 269, C750–C756.

Bolla, T., Kalbacher, H., Vogel, D., Wiesinger, H., 1999. Argininosuccinate lyase: generation of antisera against peptide sequences of the rat brain enzyme and immunochemical studies on glial cells. Biol. Chem. 380, S95.

Boucher, J.L., Moali, C., Tenu, J.P., 1999. Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization. Cell. Mol. Life Sci. 55, 1015–1028.

Braissant, O., Gotoh, T., Loup, M., Mori, M., Bachmann, C., 1999a. L-Arginine uptake, the citrulline–NO cycle and arginase II in the rat brain: an in situ hybridization study. Mol. Brain Res. 70, 231–241.

Braissant, O., Honegger, P., Loup, M., Iwase, K., Takiguchi, M., Bachmann, C., 1999b. Hyperammonemia: regulation of argininosuccinate synthetase and argininosuccinate lyase genes in aggregating cell cultures of fetal rat brain. Neurosci. Lett. 266, 89–92.

Bredt, D.S., Hwang, P.M., Snyder, S.H., 1990. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 347, 768–770.

Bredt, D.S., Glatt, C.E., Hwang, P.M., Fotuhi, M., Dawson, T.M., Snyder, S.H., 1991. Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. Neuron 7, 615–624.

Brenman, J.E., Chao, D.S., Gee, S.H., McGee, A.W., Craven, S.E., Santillano, D.R., Wu, Z., Huang, F., Xia, H., Peters, M.F., Froehner, S.C., Bredt, D.S., 1996. Interaction of nitric oxide synthase with the post-synaptic density protein PSD-95 and α-1-syntrophin mediated by PDZ domains. Cell 84, 757–767.

Bröer, A., Wagner, C.A., Lang, F., Bröer, S., 2000. The heterodimeric amino acid transporter 4F2hc/y + LAT2 mediates arginine efflux in exchange with glutamine. Biochem. J. 349, 787–795.

Buga, G.M., Singh, R., Pervin, S., Rogers, N.E., Schmitz, D.A., Jenkinson, C.P., Cederbaum, S.D., Ignarro, L.J., 1996. Arginase activity in endothelial cells: inhibition by N^G-hydroxy-L-arginine during high-output NO production. Am. J. Physiol. 271, H1988–H1998.

Bult, H., Boeckxstaens, G.E., Pelckmans, P.A., Jordaens, F.H., Van Maercke, Y.M., Herman, A.G., 1990. Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. Nature 345, 346–347.

Bune, A.J., Shergill, J.K., Cammack, R., Cook, H.T., 1995. L-Arginine depletion by arginase reduces nitric oxide production in endotoxic shock: an electron paramagnetic resonance study. FEBS Lett. 366, 127–130.

Buniatian, H.C., 1971. The urea cycle. In: Lajtha, A. (Ed.), Handbook of Neurochemistry, vol. V. Plenum Press, New York, pp. 235–247.

Buniatian, H.C., Davtian, M.A., 1966. Urea synthesis in brain. J. Neurochem. 13, 743–753.

Caggiano, A.O., Kraig, R.P., 1998. Neuronal nitric oxide synthase expression is induced in neocortical astrocytes after spreading depression. J. Cerebr. Blood Flow Metab. 18, 75–87.

Caivano, M., 1998. Role of MAP kinase cascades in inducing arginine transporters and nitric oxide synthetase in RAW264 macrophages. FEBS Lett. 429, 249–253.

Cesar, M., Hamprecht, B., 1995. Immunocytochemical examination of neural rat and mouse primary cultures using monoclonal antibodies raised against pyruvate carboxylase. J. Neurochem. 64, 2312–2318.

Chabrier, P.-E., Demerle-Pallardy, C., Auguet, M., 1999. Nitric oxide synthases: targets for therapeutic strategies in neurological diseases. Cell. Mol. Life Sci. 55, 1029–1035.

Chao, C.C., Hu, S., Sheng, W.S., Bu, D., Bukrinsky, M.I., Peterson, P.K., 1996. Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism. Glia 16, 276–284.

Chen, F.-Y., Lee, T.J.-F., 1995. Arginine synthesis from citrulline in perivascular nerves of cerebral artery. J. Pharmacol. Exp. Therapeut. 273, 895–901.

Cheung, C.-W., Cohen, N.S., Raijman, L., 1989. Channeling of urea cycle intermediates in situ in permeabilized hepatocytes. J. Biol. Chem. 264, 4038–4044.

Christensen, H.N., 1990. Role of amino acid transport and countertransport in nutrition and metabolism. Physiol. Rev. 70, 43–77.

Clarke, S., 1993. Protein methylation. Curr. Opin. Cell Biol. 5, 977–983.

Closs, E.I., Mann, G.E., 1999. Identification of carrier systems in plasma membranes of mammalian cells involved in transport of L-arginine. Methods Enzymol. 301, 78–91.

Closs, E.I., Lyons, C.R., Kelly, C., Cunningham, J.M., 1993. Characterization of the third member of the MCAT family of cationic amino acid transporters. Identification of a domain that determines the transport properties of the MCAT proteins. J. Biol. Chem. 268, 20796–20800.

Closs, E.I., Basha, F.Z., Habermeier, A., Förstermann, U., 1997. Interference of L-arginine analogues with L-arginine transport mediated by the y^+ carrier hCAT-2B. Nitric Oxide 1, 65–73.

Closs, E.I., Scheld, J.-S., Sharafi, M., Förstermann, U., 2000. Substrate supply for nitric-oxide synthase in macrophages and endothelial cells: role of cationic amino acid transporters. Mol. Pharmacol. 57, 68–74.

Collard, K.J., 1995. On the role of nitric oxide as a cellular messenger in brain. Mol. Cell. Biochem. 149/150, 249–256.

Colosanti, M., Persichini, T., Fabrizi, C., Cavalieri, E., Venturini, G., Ascenzi, P., Lauro, G.M., Suzuki, H., 1998. Expression of a NOS-III-like protein in human astroglial cell culture. Biochem. Biophys. Res. Commun. 252, 552–555.

Cook, H.T., Jansen, A., Lewis, S., Largen, P., O'Donnell, M., Reaveley, D., Cattell, V., 1994. Arginine metabolism in experimental glomerulonephritis: interaction between nitric oxide synthase and arginase. Am. J. Physiol. 267, F646–F653.

Cossenza, M., Paes de Carvalho, R., 2000. L-Arginine uptake and release by cultured avian retinal cells: differential cellular localization in relation to nitric oxide synthase. J. Neurochem. 74, 1885–1894.

Culcasi, M., Lafon-Cazal, M., Pietri, S., Bockaert, J., 1994. Glutamate receptors induce a burst of superoxide via activation of nitric oxide synthase in arginine-depleted neurons. J. Biol. Chem. 269, 12589–12593.

Dawson, T.M., Bredt, D.S., Fotuhi, M., Hwang, P.M., Snyder, S.H., 1991. Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. Proc. Natl. Acad. Sci. 88, 7797–7801.

Dawson, V.L., Dawson, T.M., Bartley, D.A., Uhl, G.R., Snyder, S.H., 1993. Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. J. Neurosci. 13, 2651–2661.

De Jonge, W.J., Dingemanse, M.A., De Boer, P.A.J., Lamers, W.H., Moormann, A.F.M., 1998. Arginine-metabolizing enzymes in the developing rat small intestine. Pediatr. Res. 43, 442–451.

Deves, R., Boyd, C.A.R., 1998. Transporters for cationic amino acids in animal cells: discovery, structure, and function. Physiol. Rev. 78, 487–545.

Dhanakoti, N., Brosnan, M.E., Herzberg, G.R., Brosnan, J.T., 1992. Cellular and subcellular localization of enzymes of arginine metabolism in rat kidney. Biochem. J. 282, 369–375.

Dinerman, J.L., Dawson, T.M., Schell, M.J., Snowman, A., Snyder, S.H., 1994. Endothelial nitric oxide synthase localized to hippocampal pyramidal cells: implications for synaptic plasticity. Proc. Natl. Acad. Sci. USA 91, 4214–4218.

Ding, M., St. Pierre, B.A., Parkinson, J.F., Medberry, P., Wong, J., Roger, N.E., Ignarro, L.J.and, Merrill, J.E., 1997. Inducible nitric-oxide synthase and nitric oxide production in human fetal astrocytes and microglia. J. Biol. Chem. 272, 11327–11335.

Do, K.-Q., Binns, K.E., Salt, T.E., 1994. Release of the nitric oxide precursor, arginine, from the thalamus upon sensory afferent stimulation, and its effect on thalamic neurons in vivo. Neuroscience 60, 581–586.

Do, K.Q., Benz, B., Grima, G., Gutteck-Amsler, U., Kluge, I., Salt, T., 1996. Nitric oxide precursor arginine and *S*-nitrosoglutathione in synaptic and glial function. Neurochem. Int. 29, 213–224.

Dolinska, M., Albrecht, J., 1998. L-Arginine uptake in rat cerebral mitochondria. Neurochem. Int. 33, 233–236.

Donati, G., Pournaras, C.J., Munoz, J.-L., Poitry, S., Poitry-Yamate, C.L., Tsacopoulos, M., 1995. Nitric oxide controls arteriolar tone in the retina of the miniature pig. Invest. Ophtalmol. Vis. Sci. 36, 2228–2237.

Dringen, R., Hamprecht, B., Bröer, S., 1998a. The peptide transporter PepT2 mediates the uptake of the glutathione precursor CysGly in astroglia-rich primary cultures. J. Neurochem. 71, 388–393.

Dringen, R., Verleysdonk, S., Hamprecht, B., Willker, W., Leibfritz, D., Brand, A., 1998b. Metabolism of glycine in primary astroglial cells: synthesis of creatine, serine, and glutathione. J. Neurochem. 70, 835–840.

Dun, N.J., Dun, S.L., Forstermann, U., Tseng, L.F., 1992. Nitric oxide synthase immunoreactivity in the spinal cord. Neurosci. Lett. 147, 217–220.

Egberongbe, Y.I., Gentleman, S.M., Falkai, P., Bogerts, B., Polak, J.M., Roberts, G.W., 1994. The distribution of nitric oxide synthase immunoreactivity in the human brain. Neuroscience 59, 561–578.

Eliasson, M.J.L., Blackshaw, S., Schell, M.J., Snyder, S.H., 1997. Neuronal nitric oxide synthase alternatively spliced forms: prominent functional localizations in the brain. Proc. Natl. Acad. Sci. USA 94, 3396–3401.

Endoh, M., Maiese, K., Wagner, J.A., 1994. Expression of the neural form of nitric oxide synthase by CA1 hippocampal neurons and other central nervous system neurons. Neuroscience 63, 679–689.

Eremin, O., 1997. L-Arginine: Biological Aspects and Clinical Application. Springer, New York.

Feinstein, D.L., Rozelman, E., 1997. Norepinephrine suppresses L-arginine uptake in rat glial cells. Neurosci. Lett. 223, 37–40.

Fillenz, M., Lowry, J.P., Boutelle, M.G., Fray, A.E., 1999. The role of astrocytes and noradrenaline in neuronal glucose metabolism. Acta Physiol. Scand. 167, 275–284.

Flodström, M., Niemann, A., Bedoya, F.J., Morris, S.M., Jr, Eizirik, D.L., 1995. Expression of the citrulline-nitric oxide cycle in rodent and human pancreatic β-cells: induction of argininosuccinate synthetase by cytokines. Endocrinology 136, 3200–3206.

Frank, S., Madlener, M., Pfeilschifter, J., Werner, S., 1998. Induction of inducible nitric oxide synthase and its corresponding tetrahydrobiopterin-cofactor-synthesizing enzyme GTP-cyclohydrolase I during cutaneous wound repair. J. Invest. Dermatol. 111, 1058–1064.

Fukuto, J.M., Chaudhuri, G., 1995. Inhibition of constitutive and inducible nitric oxide synthase: potential selective inhibition. Annu. Rev. Pharmacol. Toxicol. 35, 165–194.

Galea, E., Regunathan, S., Eliopoulos, V., Feinstein, D.L., Reis, D.J., 1996. Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. Biochem. J. 316, 247–249.

Garcia-Nogales, P., Almeida, A., Fernadez, E., Medina, J.M., Bolanos, J.P., 1999. Induction of glucose-6-phosphate dehydrogenase by lipopolysaccharide contributes to preventing nitric oxide-mediated glutathione depletion in cultured rat astrocytes. J. Neurochem. 72, 1750–1758.

Garthwaite, J., Boulton, C.L., 1995. Nitric oxide signaling in the central nervous system. Annu. Rev. Physiol. 57, 683–706.

Garthwaite, J., Charles, S.L., Chess-Williams, R., 1988. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in brain. Nature 336, 385–388.

Garthwaite, J., Garthwaite, G., Palmer, R.M.J., Moncada, S., 1989. NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. Eur. J. Pharmacol. 172, 413–416.

Gilad, G.M., Gilad, V.H., Rabey, J.M., 1996a. Arginine and ornithine decarboxylation in rodent brain: coincidental changes during development and after ischemia. Neurosci. Lett. 216, 33–36.

Gilad, G.M., Salame, K., Rabey, J.M., Gilad, V.H., 1996b. Agmatine treatment is neuroprotective in rodent brain injury models. Life Sci. 58, 41–46.

Gilad, G.M., Wollam, Y., Iaina, A., Rabey, J.M., Chernihovsky, T., Gilad, V.H., 1996c. Metabolism of agmatine into urea but not into nitric oxide in rat brain. NeuroReport 7, 1730–1732.

Gjessing, L.R., Gjesdahl, P., Sjaastad, O., 1972. The free amino acids in human cerebrospinal fluid. J. Neurochem. 19, 1807–1808.

Glick, N.R., Snodgrass, P.J., Schafer, I.A., 1976. Neonatal argininosuccinic aciduria with normal brain and kidney but absent liver argininosuccinate lyase activity. Am. J. Hum. Gen. 28, 22–30.

Gold, M.E., Wood, K.S., Buga, G.M., Byrns, R.E., Ignarro, L.J., 1989. L-Arginine causes whereas L-argininosuccinic acid inhibits endothelium-dependent vascular smooth muscle relaxation. Biochem. Biophys. Res. Commun. 161, 536–543.

Gotoh, T., Mori, M., 1999. Arginase II downregulates nitric oxide (NO) production and prevents NO-mediated apoptosis in murine macrophage-derived RAW 264.7 cells. J. Cell Biol. 144, 1–8.

Gotoh, T., Sonoki, T., Nagasaki, A., Terada, K., Takiguchi, M., Mori, M., 1996. Molecular cloning of cDNA for nonhepatic mitochondrial arginase (arginase II) and comparison of its induction with nitric oxide synthase in a murine macrophage-like cell line. FEBS Lett. 395, 119–122.

Gotoh, T., Araki, M., Mori, M., 1997. Chromosmal localization of the human arginase II gene and tissue distribution of its mRNA. Biochem. Biophys. Res. Commun. 233, 487–491.

Grant, S.K., Green, B.G., Stiffey-Wilusz, J., Durette, P.L., Shah, S.K., Kozarich, J.W., 1998. Structural requirements for human inducible nitric oxide synthase substrates and substrate analogue inhibitors. Biochemistry 37, 4174–4180.

Grima, G., Benz, B., Do, K.Q., 1997. Glutamate-induced release of the nitric oxide precursor, arginine, from glial cells. Eur. J. Neurosci. 9, 2248–2258.

Grima, G., Cuenod, M., Pfeiffer, S., Mayer, B., Do, K.Q., 1998. Arginine availability controls the *N*-methyl-D-aspartate-induced nitric oxide synthesis: involvement of a glial–neuronal arginine transfer. J. Neurochem. 71, 2139–2144.

Gross, S.S., Wolin, M.S., 1995. Nitric oxide: pathophysiological mechanisms. Annu. Rev. Physiol. 57, 737–769.

Grozdanovic, Z., Baumgarten, H.G., Brüning, G., 1992. Histochemistry of NADPH-diaphorase, a marker for neuronal nitric oxide synthase, in the peripheral autonomous nervous system of the mouse. Neuroscience 48, 225–235.

Guder, W.G., Morel, F., 1992. Biochemical characterization of individual nephron segments. In: Windhager, E.E. (Ed.), Handbook of Physiology, Section 8: Renal Physiology, vol. II. Oxford University Press, Oxford, pp. 2119–2164.

Hammermann, R., Bliesener, N., Mössner, J., Klasen, S., Wiesinger, H., Wessler, I., Racke, K., 1998. Inability of rat alveolar macrophages to recycle L-citrulline to L-arginine despite induction of argininosuccinate synthetase mRNA and protein, and inhibition of nitric oxide synthesis by exogenous L-citrulline. Naunyn-Schmiedeberg's Arch. Pharmacol. 358, 601–607.

Hattori, Y., Campbell, E.B., Gross, S.S., 1994. Argininosuccinate synthetase mRNA and activity are induced by immunostimulants in vascular smooth muscle cells. Role in the regeneration of arginine for nitric oxide synthesis. J. Biol. Chem. 269, 9405–9408.

Hattori, Y., Shimoda, S.-I., Gross, S.S., 1995. Effect of lipopolysaccharide treatment in vivo on tissue expression of argininosuccinate synthetase and argininosuccinate lyase mRNAs: relationship to nitric oxide synthase. Biochem. Biophys. Res. Commun. 215, 148–153.

Hattori, Y., Kasai, K., Gross, S.S., 1999. Cationic amino acid transporter gene expression in cultured vascular smooth muscle cells and in rats. Am. J. Physiol. 276, H2020–H2028.

Hazell, A.S., Butterworth, R.F., 1999. Hepatic encephalopathy: an update of pathophysiologic mechanisms. Proc. Soc. Exp. Biol. Med. 222, 99–112.

Hazell, A.S., Norenberg, M.D., 1998. Ammonia and manganese increase arginine uptake in cultured astrocytes. Neurochem. Res. 23, 869–873.

He, Z., Ibayashi, S., Nagao, T., Fujii, K., Sadoshima, S., Fujishima, M., 1995. L-Arginine ameliorates cerebral blood flow and metabolism and decreases infarct volume in rats with cerebral ischemia. Brain Res. 699, 208–213.

Heales, S.J.R., Bolanos, J.P., Stewart, V.C., Brookes, P.S., Land, J.M., Clark, J.B., 1999. Nitric oxide, mitochondria and neurological disease. Biochim. Biophys. Acta 1410, 215–228.

Hecker, M., Sessa, W.C., Harris, H.J., Änggard, E.E., Vane, J.R., 1990. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: cultured endothelial cells recycle L-citrulline to L-arginine. Proc. Natl. Acad. Sci. USA 87, 8612–8616.

Heneka, M.T., Löschmann, P.-A., Gleichmann, M., Weller, M., Schulz, J.B., Wüllner, U., Klockgether, T., 1998. Induction of nitric oxide synthase and nitric oxide mediated apoptosis in neuronal PC 12 cells after stimulation with tumor necrosis factor-α/lipopolysaccharide. J. Neurochem. 71, 88–94.

Heneka, M.T., Schmidlin, A., Wiesinger, H., 1999. Induction of argininosuccinate synthetase in rat brain glial cells after striatal microinjection of immunostimulants. J. Cerebr. Blood Flow Metab. 19, 898–907.

Heneka, M.T., Wiesinger, H., Dumitrescu, L., Wüllner, U., Löschmann, P.-A., Feinstein, D.L., Klockgether, T., 2001. Argininosuccinate synthetase is colocalized with inducible nitric oxide synthase in brains of Alzheimer patients. Neuropathology (submitted for publication).

Hertz, L., Dringen, R., Schousboe, A., Robinson, S.R., 1999. Astrocytes: glutamate producers for neurons. J. Neurosci. Res. 57, 417–428.

Hewett, J.A., Hewett, S.J., Winkler, S., Pfeiffer, S.E., 1999. Inducible nitric oxide synthase expression in cultures enriched for mature oligodendrocytes is due to microglia. J. Neurosci. Res. 56, 189–198.

Hey, C., Boucher, J.L., Vadon-Le Goff, S., Ketterer, G., Wessler, I., Racke, K., 1997. Inhibition of arginase in rat and rabbit alveolar macrophages by N^{ω}-hydroxy-D,L-indospicine, effects on L-arginine utilization by nitric oxide synthase. Br. J. Pharmacol. 121, 395–400.

Hibbs, J.B., Jr, Vavrin, Z., Taintor, R.R., 1987. L-Arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. J. Immunol. 138, 550–565.

Hisano, S., Haga, H., Miyamoto, K.-I., Takeda, E., Fukui, Y., 1996. The basic amino acid transporter (rBAT)-like immunoreactivity in paraventricular and supraoptic magnocellular neurons of the rat hypothalamus. Brain Res. 710, 299–302.

Hope, B.T., Michael, G.J., Knigge, K.M., Vincent, S.R., 1991. Neuronal NADPH diaphorase is a nitric oxide synthase. Proc. Natl. Acad. Sci. USA 88, 2811–2814.

Hori, K., Burd, P.R., Furuke, K., Kutza, J., Weih, K.A., Clouse, K.A., 1999. HIV-1 infected macrophages induce iNOS and NO production in astrocytes. Blood 93, 1843–1850.

Hosokawa, H., Sawamura, T., Kobayashi, S., Ninomiya, H., Miwa, S., Masaki, T., 1997. Cloning and characterization of a brain-specific amino acid transporter. J. Biol. Chem. 272, 8717–8722.

Hosokawa, H., Ninomiya, H., Sawamura, T., Sugimoto, Y., Ichikawa, A., Fujiwara, K., Masaki, T., 1999. Neuron-specific expression of cationic amino acid transporter 3 in the adult rat brain. Brain Res. 838, 158–165.

Hu, J., Akama, K.T., Krafft, G.A., Chromy, B.A., Van Eldik, L.J., 1998. Amyloid-β peptide activates cultured astrocytes: morphological alterations, cytokine induction and nitric oxide release. Brain Res. 785, 195–206.

Huang, Z., Huang, P.L., Panahian, N., Dalkara, T., Fishman, M.C., Moskowitz, M.A., 1994. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. Science 265, 1883–1885.

Iadecola, C., 1997. Bright and dark sides of nitric oxide in ischemic brain injury. Trends Neurosci. 20, 132–139.

Ignarro, L.J., Cirino, G., Casini, A., Napoli, C., 1999. Nitric oxide as a signalling molecule in the vascular system: an overview. J. Cardiovasc. Pharmacol. 34, 879–886.

Isayama, H., Nakamura, H., Kanemaru, H., Kobayashi, K., Emson, P.C., Kawabuchi, M., Tashiro, N., 1997. Distribution and co-localization of nitric oxide synthase and argininosuccinate synthetase in the cat hypothalamus. Arch. Histol. Cytol. 60, 477–492.

Ito, K., Groudine, M., 1997. A new member of the cationic amino acid transporter family is preferentially expressed in adult mouse brain. J. Biol. Chem. 272, 26780–26786.

Ito, A., Tsao, P.S., Adimoolam, S., Kimoto, M., Ogawa, T., Cooke, J.P., 1999. Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethyl-aminohydrolase. Circulation 99, 3092–3095.

Iwase, K., Iyama, K.-i., Akagi, K., Yano, S., Fukunaga, K., Miyamoto, E., Mori, M., Takiguchi, M., 1998. Precise distribution of neuronal nitric oxide synthase mRNA in the rat brain revealed by non-radioisotopic in situ hybridisation. Mol. Brain Res. 53, 1–12.

Jackson, M.J., Zielke, H.R., Zielke, C.L., 1996. Induction of astrocyte argininosuccinate synthetase and argininosuccinate lyase by dibutyryl cyclic AMP and dexamethasone. Neurochem. Res. 21, 1161–1165.

Jin, J.-S., D'Alecy, L.G., 1996. Central and peripheral effects of asymmetric dimethylarginine, an endogenous nitric oxide synthetase inhibitor. J. Cardiovasc. Pharmacol. 28, 439–446.

Jones, M.E., Anderson, D., Anderson, C., Hodes, S., 1961. Citrulline synthesis in rat tissues. Arch. Biochem. Biophys. 95, 499–507.

Jude, E.B., Boulton, A.J., Ferguson, M.W., Appleton, I., 1999. The role of nitric oxide synthase isoforms and arginase in the pathogenesis of diabetic foot ulcers: possible modulatory effects by transforming growth factor β1. Diabetologia 42, 748–757.

Kakuda, D.K., Finley, K.D., Maruyama, M., MacLeod, C.L., 1998. Stress differentially induces cationic amino acid transporter gene expression. Biochim. Biophys. Acta 1414, 75–84.

Kakuda, D.K., Sweet, M.J., MacLeod, C.L., Hume, D.A., Markovich, D., 1999. CAT2-mediated L-arginine transport and nitric oxide production in activated macrophages. Biochem. J. 340, 549–553.

Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E., Endou, H., 1998. Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). J. Biol. Chem. 273, 23629–23632.

Keilhoff, G., Seidel, B., Wolf, G., 1998. Absence of nitric oxide synthase in rat oligodendrocytes: a light and electron microscopic study. Acta Histochem. 100, 409–417.

Khan, N.A., Quemener, V., Seiler, N., Moulinoux, J.-P., 1990. Mechansims of spermidine uptake in cultured mammalian cells and its inhibition by some polyamine analogues. Pathobiology 58, 172–178.

Kharazia, V.N., Petrusz, P., Usunoff, K., Weinberg, R.J., Rustioni, A., 1997. Arginine and NADPH-diaphorase in the rat ventroposterior thalamic nucleus. Brain Res. 744, 151–155.

Kim, J.W., Closs, E.I., Albritton, L.M., Cunningham, J.M., 1991. Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. Nature 352, 725–728.

Kimoto, M., Tsuji, H., Ogawa, T., Sasaoka, K., 1993. Detection of N^G,N^G-dimethylarginine dimethylaminohydrolase in the nitric-oxide generating systems of rat using monoclonal antibody. Arch. Biochem. Biophys. 300, 657–662.

Kimoto, M., Whitley, G.S.t.J., Tsuji, H., Ogawa, T., 1995. Detection of N^G,N^G-dimethylarginine dimethylaminohydrolase in human tissues using a monoclonal antibody. J. Biochem. 117, 237–238.

Knecht, E., Hernandez, J., Wallace, R., Grisolia, S., 1979. Immunoferritin location of carbamoyl phosphate synthetase in rat liver. J. Histochem. Cytochem. 27, 975–981.

Koh, J.-Y., Choi, D.W., 1988. Vulnerability of cultured cortical neurons to damage by excitotoxins: differential susceptibility of neurons containing NADPH-diaphorase. J. Neurosci. 8, 2153–2163.

Krebs, H.A., Henseleit, K., 1932. Untersuchungen über die Harnstoffbildung im Tierkörper. Hoppe-Seyler's Z. Physiol. Chem. 210, 33–66.

Kröncke, K.-D., Fehsel, K., Kolb-Bachofen, V., 1995. Inducible nitric oxide synthase and its product nitric oxide, a small molecule with complex biological activities. Biol. Chem. Hoppe-Seyler 376, 327–343.

Kugler, P., Drenckhahn, D., 1996. Astrocytes and Bergmann glia as an important site of nitric oxide synthase I. Glia 16, 165–173.

Kurz, S., Harrison, D.G., 1997. Insulin and the arginine paradox. J. Clin. Invest. 99, 369–370.

Lafon-Cazal, M., Pietri, S., Culcasi, M., Bockaert, J., 1993. NMDA-dependent super-oxide production and neurotoxicity. Nature 364, 535–537.

Laube, G., Veh, R.W., 1997. Astrocytes, not neurons, show most prominent staining for spermidine/spermine-like immunoreactivity in adult rat brain. Glia 19, 171–179.

Leiper, J.M., Santa Maria, J., Chubb, A., MacAllister, R.J., Charles, I.G., Whitley, G.S.t.J., Vallance, P., 1999. Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. Biochem. J. 343, 209–214.

Li, L., Wiesinger, H., 1996. Transport of polyamines in glial cells. Biol. Chem. 377, S182.

Li, G., Regunathan, S., Barrow, C.J., Eshragi, J., Cooper, R., Reis, D.J., 1994. Agmatine: an endogenous clonidine-displacing substance in the brain. Science 263, 966–969.

Lin, Y.C., Chow, C.W., Yuen, P.H., Wong, P.K.Y., 1997. Establishment and characterization of conditionally immortalized astrocytes to study their interaction with ts1, a neuropathogenic mutant of Moloney murine leukemia virus. J. Neurovirol. 3, 28–37.

Lipton, S.A., Choi, Y.B., Pan, Z.-H., Lei, S.Z., Chen, H.S., Sucher, N.J., Loscalzo, J., Singel, D.J., Stamler, J.S., 1993. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 364, 626–632.

Liu, B., Neufeld, A.H., 2000. Expression of nitric oxide synthase-2 (NOS-2) in reactive astrocytes of the human glaucomatous optic nerve head. Glia 30, 178–186.

Lopes, M.C., Cardoso, S.A., Schousboe, A., Carvalho, A.P., 1994. Amino acids differentially inhibit the L-[^{3}H]arginine transport and nitric oxide synthase in rat brain synaptosomes. Neurosci. Lett. 181, 1–4.

Louis, C.A., Mody, V., Henry, W.L., Jr, Reichner, J.S., Albina, J.E., 1999. Regulation of arginase isoforms I and II by Il-4 in cultured murine macrophages. Am. J. Physiol. 276, R237–R242.

Luss, H., Li, R.-K., Shapiro, R.A., Tzeng, E., McGowan, F.X., Yoneyama, T., Hatakeyama, K., Geller, D.A., Mickle, D.A.G., Simmons, R.L., Billiar, T.R., 1997. Dedifferentiated human ventricular cardiac myocytes express inducible nitric oxide synthase mRNA but not protein in response to Il-1, TNF, IFNγ, and LPS. J. Mol. Cell. Cardiol. 29, 1153–1165.

MacAllister, R.J., Vallance, P., 1998. Endogenous inhibitors of nitric oxide synthesis: how important are they? Exp. Nephrol. 6, 195–199.

MacAllister, R.J., Parry, H., Kimoto, M., Ogawa, T., Russell, R.J., Hodson, H., Whitley, G.S.t.J., Vallance, P., 1996. Regulation of

nitric oxide synthesis by dimethylarginine dimethylaminohydrolase. Br. J. Pharmacol. 119, 1533–1540.
Malandro, M.S., Kilberg, M.S., 1996. Molecular biology of mammalian amino acid transporters. Annu. Rev. Biochem. 65, 305–336.
Mayer, B., Hemmens, B., 1997. Biosynthesis and action of nitric oxide in mammalian cells. Trends Biochem. Sci. 22, 477–481.
McDonald, K.K., Zharikov, S., Block, E.R., Kilberg, M.S., 1997. A caveolar complex between the cationic amino acid transporter 1 and endothelial nitric-oxide synthase may explain the 'arginine paradox'. J. Biol. Chem. 272, 31213–31216.
Meda, L., Cassatella, M.A., Szendrai, G.I., Otvos, L., Jr, Baron, P., Villalba, M., Ferrari, D., Rossi, F., 1995. Activation of microglial cells by β-amyloid protein and interferon-γ. Nature 374, 647–650.
Merrill, J.E., Murphy, S.P., Mitrovic, B., Mackenzie-Graham, A., Dopp, J.C., Ding, M., Griscavage, J., Ignarro, L.J., Lowenstein, C.J., 1997. Inducible nitric oxide synthase and nitric oxide production by oligodendocytes. J. Neurosci. Res. 48, 372–384.
Minc-Golomb, D., Yadid, G., Tsarfaty, I., Resau, J.H., Schwartz, J.P., 1996. In vivo expression of inducible nitric oxide in cerebellar neurons. J. Neurochem. 66, 1504–1509.
Minghetti, L., Levi, G., 1998. Microglia as effector cells in brain damage and repair: focus on prostanoids and nitric oxide. Prog. Neurobiol. 54, 99–125.
Mitchell, J.A., Hecker, M., Änggard, E.E., Vane, J.R., 1990. Cultured endothelial cells maintain their L-arginine level despite continuous release of EDRF. Eur. J. Pharmacol. 182, 573–576.
Miyanaka, K., Gotoh, T., Nagasaki, A., Takeya, M., Ozaki, M., Iwase, K., Takiguchi, M., Iyama, K.I., Tomita, K., Mori, M., 1998. Immunohistochemical localization of arginase II and other enzymes of arginine metabolism in rat kidney and liver. Histochem. J. 30, 741–751.
Molderings, G.J., Menzel, S., Gothert, M., 1999. Imidazoline derivatives and agmatine induce histamine release from the rat stomach. Naunyn-Schmiedeberg's Arch. Pharmacol. 360, 711–714.
Moro, M.A., De Alba, J., Leza, J.C., Lorenzo, P., Fernandez, A.P., Bentura, M.L., Bosca, L., Rodrigo, J., Lizasoain, I., 1998. Neuronal expression of inducible nitric oxide synthase after oxygen and glucose deprivation in rat forebrain slices. Eur. J. Neurosci. 10, 445–456.
Morris, S.M., Jr, 1992. Regulation of enzymes of urea and arginine synthesis. Annu. Rev. Nutr. 12, 81–101.
Morris, S.M., Jr, 1999. Arginine synthesis, metabolism, and transport: regulators of nitric oxide synthesis. In: Laskin, J.D., Laskin, D.L. (Eds.), Cellular and Molecular Biology of Nitric Oxide. Marcel Dekker, New York, pp. 57–85.
Morris, S.M., Jr, Kepka-Lenhart, D., Chen, L.-C., 1998. Differential regulation of arginases and inducible nitric oxide synthases in murine macrophage cells. Am. J. Physiol. 275, E740–E747.
Murphy, S., 2000. Production of nitric oxide by glial cells: regulation and potential roles in the CNS. Glia 29, 1–14.
Murphy, S., Grzybicki, D., 1996. Glial NO: normal and pathological roles. Neuroscientist 2, 90–99.
Murphy, S., Simmons, M.S., Agullo, L., Garcia, A., Feinstein, D.L., Galea, E., Reis, D.L., Minc-Golomb, D., Schwartz, J.P., 1993. Synthesis of nitric oxide in CNS glial cells. Trends Neurosci. 16, 323–328.
Nagasaki, A., Gotoh, T., Takeya, M., Yu, Y., Takiguchi, M., Matsuzaki, H., Taktsuki, K., Mori, M., 1996. Coinduction of nitric oxide synthase, argininosuccinate synthetase, and argininosuccinate lyase in lipopolysaccharide-treated rats. J. Biol. Chem. 271, 2658–2662.
Nakagomi, S., Kiryu-Seo, S., Kimoto, M., Emson, P.C., Kiyama, H., 1999. Dimethylarginine dimethylaminohydrolase (DDAH) as a nerve-injury-associated molecule: mRNA localization in the rat brain and its coincident up-regulation with neuronal NO synthase (nNOS) in axotomized motorneurons. Eur. J. Neurosci. 11, 2160–2166.
Nakamura, H., 1997. NADPH-diaphorase and cytosolic urea cycle enzymes in the rat spinal cord. J. Comp. Neurol. 385, 616–626.
Nakamura, H., Saheki, T., Nakagawa, S., 1990. Differential cellular localization of enzymes of L-arginine metabolism in the rat brain. Brain Res. 530, 108–112.
Nakamura, H., Saheki, T., Ichiki, H., Nakata, K., Nakagawa, S., 1991a. Immunocytochemical localization of argininosuccinate synthetase in the rat brain. J. Comp. Neurol. 312, 652–679.
Nakamura, H., Yada, T., Saheki, T., Noda, T., Nakagawa, S., 1991b. L-Argininosuccinate modulates L-glutamate response in acutely isolated cerebellar neurons of immature rats. Brain Res. 539, 312–315.
Nakamura, H., Itoh, K., Kawabuchi, M., 1999. NADPH-diaphorase and cytosolic urea cycle enzymes in the rat accessory olfactory bulb. J. Chem. Neuroanat. 17, 109–117.
Nathan, C., Xie, Q.-W., 1994. Regulation of biosynthesis of nitric oxide. J. Biol. Chem. 269, 13725–13728.
Nicholson, B., Sawamura, T., Masaki, T., MacLeod, C.L., 1998. Increased CAT3-mediated cationic amino acid transport functionally compensates in CAT1 knockout cell lines. J. Biol. Chem. 273, 14663–14666.
Nirenberg, M.J., Tate, S.S., Moskovitz, R., Udenfried, S., Pickel, V.M., 1995. Immunocytochemical localization of the renal neutral and basic amino acid transporter in rat adrenal gland, brainstem, and spinal cord. J. Comp. Neurol. 356, 505–522.
Nussler, A.K., Billiar, T.R., Liu, Z.-Z., Morris, S.M., Jr, 1994. Coinduction of nitric oxide synthase and argininosuccinate synthetase in a murine macrophage cell line. Implications for regulation of nitric oxide production. J. Biol. Chem. 269, 1257–1261.
Nussler, A.K., Liu, Z.-Z., Hatakeyama, K., Geller, D.A., Billiar, T.R., Morris, S.M., Jr, 1996. A cohort of supporting metabolic enzymes is coinduced with nitric oxide synthase in human tumor cell lines. Cancer Lett. 103, 79–84.
Olmos, G., DeGregorio-Rocasolano, N., Paz-Regalado, M., Gasull, T., Assumpcio-Boronat, M., Trullas, R., Villarroel, A., Lerma, J., Garcia-Sevilla, J.A., 1999. Protection by imidazol(ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. Br. J. Pharmacol. 127, 1317–1326.
O'Sullivan, D., Brosnan, J.T., Brosnan, M.E., 1998. Hepatic zonation of the catabolism of arginine and ornithine in the perfused rat liver. Biochem. J. 330, 627–632.
Ozaki, M., Gotoh, T., Nagasaki, A., Miyanaka, K., Takeya, M., Fujiyama, S., Tomita, K., Mori, M., 1999. Expression of arginase II and related enzymes in the small intestine and kidney. J. Biochem. 125, 586–593.
Palaiologos, G., Hertz, L., Schousboe, A., 1988. Evidence that aspartate aminotransferase activity and ketodicarboxylate carrier function are essential for biosynthesis of transmitter glutamate. J. Neurochem. 51, 317–320.
Palmer, J.P., Walter, R.M., Ensinck, J.W., 1975. Arginine-stimulated acute phase of insulin and glucagon secretion. I. In normal man. Diabetes 24, 735–740.
Pasqualotto, B.A., Hope, B.T., Vincent, S.R., 1991. Citrulline in the rat brain: immunohistochemistry and coexistence with NADPH-diaphorase. Neurosci. Lett. 128, 155–160.
Perry, T.L., 1982. Cerebral amino acid pools. In: Lajtha, A. (Ed.), Handbook of Neurochemistry, vol. I, 2nd edn. Plenum Press, New York, pp. 151–180.
Pickel, V.M., Pohorille, A., Chan, J., Tate, S.S., Nirenberg, M.J., 1999. Regional and subcellular distribution of a neutral and basic amino acid transporter in forebrain neurons containing nitric oxide synthase. J. Comp. Neurol. 404, 459–472.
Posch, K., Schmidt, K., Graier, W.F., 1999. Selective stimulation of L-arginine uptake contributes to shear stress-induced formation of nitric oxide. Life Sci. 64, 663–670.

Pou, S., Pou, W.S., Bredt, D.S., Snyder, S.H., Rosen, G.M., 1992. Generation of superoxide by purified brain nitric oxide synthase. J. Biol. Chem. 267, 24173–24176.

Pow, D.V., 1994. Immunocytochemical evidence for a glial localisation of arginine, and a neuronal localization of citrulline in the rat neurohypophysis: implications for nitrergic transmission. Neurosci. Lett. 181, 141–144.

Pow, D.V., Crook, D.K., 1997. Immunocytochemical analysis of the transport of arginine analogues into nitrergic neurons and other cells in the retina and pituitary. Cell Tissue Res. 290, 501–514.

Prado, R., Watson, B.D., Wester, P., 1993. Effects of nitric oxide synthase inhibition on cerebral blood flow following bilateral carotid artery occlusion and recirculation in the rat. J. Cerebr. Blood Flow Metab. 13, 720–723.

Rabier, D., Kamoun, P., 1995. Metabolism of citrulline in man. Amino Acids 9, 299–316.

Raijman, L., 1974. Citrulline synthesis in rat tissues and liver content of carbamoyl phosphate and ornithine. Biochem. J. 138, 225–232.

Rao, V.L.R., Butterworth, R.F., 1996. L-[^{3}H]Nitroarginine and L-[^{3}H]arginine uptake into rat cerebellar synaptosomes: kinetics and pharmacology. J. Neurochem. 67, 1275–1281.

Rao, V.L.R., Audet, R.M., Butterworth, R.F., 1997. Portocaval shunting and hyperammonemia stimulate the uptake of L-[^{3}H]arginine but not of L-[^{3}H]nitroarginine into rat brain synaptosomes. J. Neurochem. 68, 337–343.

Ratnakumari, L., Qureshi, I.A., Butterworth, R.F., Marescau, B., De Deyn, P.P., 1996. Arginine-related guanidino compounds and nitric oxide synthase in the brain of ornithine transcarbamylase deficient spf mutant mouse: effect of metabolic arginine deficiency. Neurosci. Lett. 215, 153–156.

Ratner, S., 1973. Enzymes of arginine and urea synthesis. Adv. Enzymol. Relat. Areas Mol. Biol. 39, 1–90.

Ratner, S., Morell, H., Carvalho, E., 1960. Enzymes of arginine metabolism in brain. Arch. Biochem. Biophys. 91, 280–289.

Redmond, H.P., Daly, J.M., 1993. Arginine. In: Klurfeld, D.M. (Ed.), Human Nutrition — a Comprehensive Treatise, vol. 8. Plenum Press, New York, pp. 157–166.

Regunathan, S., Reis, D.J., 2000. Characterization of arginine decaboxylase in rat brain and liver: distinction from ornithine decarboxylase. J. Neurochem. 74, 2201–2208.

Regunathan, S., Feinstein, D.L., Raasch, W., Reis, D.J., 1995. Agmatine (decarboxylated arginine) is synthesized and stored in astrocytes. NeuroReport 6, 1897–1900.

Reutens, D.C., McHugh, M.D., Toussaint, P.-J., Evans, A.C., Gjedde, A., Meyer, E., Stewart, D.J., 1997. L-Arginine infusion increases basal but not activated cerebral blood flow in humans. J. Cerebr. Blood Flow Metab. 17, 309–315.

Rose, W.C., 1937. The nutritive significance of the amino acids and certain related compounds. Science 86, 298–300.

Rossi, F., Bianchini, E., 1996. Synergistic induction of nitric oxide by β-amyloid and cytokines in astrocytes. Biochem. Biophys. Res. Commun. 225, 474–478.

Rothnagel, J.A., Rogers, G.E., 1984. Citrulline in proteins from enzymatic deimination of arginine residues. Methods Enzymol. 107, 624–631.

Saadoun, S., Garcia, A., 1999. Endothelin stimulates nitric oxide-dependent cyclic GMP formation in rat cerebellar astroglia. Neuroreport 10, 33–36.

Sadasivudu, B., Indira, H.R., 1974. Distribution of enzymes involved in the disposal of arginine and ornithine in different regions of the brain. Brain Res. 79, 326–329.

Sadasivudu, B., Rao, T.I., 1976. Studies on functional and metabolic role of urea cycle intermediates in brain. J. Neurochem. 27, 785–794.

Sakai, N., Kaufmann, S., Milstien, S., 1995. Parallel induction of nitric oxide and tetrahydrobiopterin synthesis by cytokines in rat glial cells. J. Neurochem. 65, 895–902.

Salimuddin, Nagasaki, A., Gotoh, T., Isobe, H., Mori, M., 1999. Regulation of the genes for arginase isoforms and related enzymes in mouse macrophages by lipopolysaccharide, Am. J. Physiol. 277, E110–7.

Sastre, M., Regunathan, S., Reis, D.J., 1997. Uptake of agmatine into rat brain synaptosomes: possible role of cation channels. J. Neurochem. 69, 2421–2426.

Satriano, J., Kelly, C.J., Blantz, R.C., 1999. An emerging role for agmatine. Kidney Int. 56, 1252–1253.

Sattler, R., Xiong, Z., Lu, W.-Y., Hafner, M., MacDonald, J.F., Tymianski, M., 1999. Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. Science 284, 1845–1848.

Schmidlin, A., Wiesinger, H., 1994. Transport of L-arginine in cultured glial cells. Glia 11, 262–268.

Schmidlin, A., Wiesinger, H., 1995. Stimulation of arginine transport and nitric oxide production by lipopolysaccharide is mediated by different signaling pathways in astrocytes. J. Neurochem. 65, 590–594.

Schmidlin, A., Wiesinger, H., 1998. Argininosuccinate synthetase: localization in astrocytes and role in the production of glial nitric oxide. Glia 24, 428–436.

Schmidlin, A., Kalbacher, H., Wiesinger, H., 1997a. Presence of argininosuccinate synthetase in glial cells as revealed by peptide-specific antisera. Biol. Chem. 378, 47–50.

Schmidlin, A., Fischer, S., Ricciardi-Castagnoli, P., Wiesinger, H., 1997b. Role of arginine transport in the production of nitric oxide in cultured neural cells. In: Teelken, A.W., Korf, J. (Eds.), Neurochemistry. Plenum Press, New York, pp. 625–629.

Schmidlin, A., Fischer, S., Wiesinger, H., 2000. Transport of l-citrulline in neural cell cultures. Dev. Neurosci. 22, 393–398.

Schmidt, H.H.W., Warner, T.D., Ishii, K., Sheng, H., Murad, F., 1992. Insulin secretion from pancreatic B cells caused by L-arginine-derived nitric oxide. Science 255, 721–723.

Schmidt, K., Klatt, P., Mayer, B., 1993. Characterization of endothelial cell amino acid transport systems involved in the actions of nitric oxide synthase inhibitors. Mol. Pharmacol. 44, 615–621.

Schmidt, K., Klatt, P., Mayer, B., 1994. Uptake of nitric oxide synthase inhibitors by macrophage RAW 264.7 cells. Biochem. J. 301, 313–316.

Schmidt, K., List, B.M., Klatt, P., Mayer, B., 1995. Characterization of neuronal amino acid transporters: uptake of nitric oxide synthase inhibitors and implication for their biological effects. J. Neurochem. 64, 1469–1475.

Schulz, J.B., Matthews, R.T., Beal, M.F., 1995. Role of nitric oxide in neurodegenerative diseases. Curr. Opin. Neurol. 8, 480–486.

Schuman, E.M., Madison, D.V., 1994. Nitric oxide and synaptic function. Annu. Rev. Neurosci. 17, 153–183.

Segal, M.B., Preston, J.E., Collis, C.S., Zlocovic, B.V., 1990. Kinetics and Na independence of amino acid uptake by blood side of perfused sheep choroid plexus. Am. J. Physiol. 258, F1288–F1294.

Seidel, B., Stanarius, A., Wolf, G., 1997. Differential expression of neuronal and endothelial nitric oxide synthase in blood vessels of the rat brain. Neurosci. Lett. 239, 109–112.

Seiler, N., Daune-Anglard, G., 1993. Endogenous ornithine in search for CNS functions and therapeutic applications. Metab. Brain Dis. 8, 151–179.

Sheng, H., Ignarro, L.J., 1996. Biochemical and immunohistochemical characterization of nitric oxide synthase in the rat retina. Pharmacol. Res. 33, 29–34.

Shih, V.E., 1978. Urea cycle disorders and other congenital hyperammonemic syndromes. In: Stanbury, J.B., Wyngaarden, J.B., Frederickson, D.S. (Eds.), The Metabolic Basis of Inherited Disease. McGraw-Hill, New York, pp. 362–386.

Shuttleworth, C.W.R., Burns, A.J., Ward, S.M., O'Brien, W.E., Sanders, K.M., 1995. Recycling of L-citrulline to sustain nitric

oxide-dependent enteric neurotransmission. Neuroscience 68, 1295–1304.

Shuttleworth, C.W.R., Conlon, S.B., Sanders, K.M., 1997. Regulation of citrulline recycling in nitric oxide-dependent neurotransmission in the murine proximal colon. Br. J. Pharamcol. 120, 707–713.

Simmons, W.W., Closs, E.I., Cunningham, J.M., Smith, T.W., Kelly, R.A., 1996. Cytokines and insulin induce cationic amino acid transporter (CAT) expression in cardiac myocytes. Regulation of L-arginine transport and NO production by CAT-1, CAT-2A, and CAT-2B. J. Biol. Chem. 271, 11694–11702.

Sonoki, T., Nagasaki, A., Gotoh, T., Takiguchi, M., Takeya, M., Matsuzaki, H., Mori, M., 1997. Coinduction of nitric-oxide synthase and arginase I in cultured rat peritoneal macrophages and rat tissues in vivo by lipopolysaccharide. J. Biol. Chem. 272, 3689–3693.

Sperandeo, M.P., Borsani, G., Incerti, B., Zollo, M., Rossi, E., Zuffardi, O., Castaldo, P., Taglialatela, M., Andria, G., Sebastio, G., 1998. The gene encoding a cationic amino acid transporter (SLC7A4) maps to the region deleted in the velocardiofacial syndrome. Genomics 49, 230–236.

Stevens, B.R., Vo, C.B., 1998. Membrane transport of neuronal nitric oxide synthase substrate L-arginine is constitutively expressed with CAT1 and 4F2hc, but not CAT2 or rBAT. J. Neurochem. 71, 564–570.

Stevens, B.R., Kakuda, D.K., Yu, K., Waters, M., Vo, C.B., Raizada, M.K., 1996. Induced nitric oxide synthesis is dependent on induced alternatively spliced CAT-2 encoding L-arginine transport in brain astrocytes. J. Biol. Chem. 271, 24017–24022.

Stoll, J., Wadhwani, K.C., Smith, Q.R., 1993. Identification of the cationic amino acid transporter (system y^+) of the rat blood–brain barrier. J. Neurochem. 60, 1956–1959.

Stuhlmiller, D.F., Boje, K.M., 1995. Characterization of L-arginine and aminoguanidine uptake into isolated rat choroid plexus: differences in uptake mechanisms and inhibition by nitric oxide synthase inhibitors. J. Neurochem. 65, 68–74.

Szabo, C., 1996. Physiological and pathophysiological roles of nitric oxide in the central nervous system. Brain Res. Bull. 41, 131–141.

Takiguchi, M., Mori, M., 1995. Transcriptional regulation of genes for ornithine cycle enzymes. Biochem. J. 312, 649–659.

Tam, S.Y., Roth, R.H., 1997. Mesoprefrontal dopaminergic neurons: can tyrosine availability influence their functions? Biochem. Pharmacol. 53, 441–453.

Tan, C.H., Ng, F.H., 1995. High and low affinity transport of L-arginine in rat brain synaptosomes. Experientia 51, 1052–1054.

Tojo, A., Welch, W.J., Bremer, V., Kimoto, M., Kimura, K., Omata, M., Ogawa, T., Vallance, P., Wilcox, C.S., 1997. Colocalization of demethylating enzymes and NOS and functional effects of methylarginines in rat kidney. Kidney Int. 52, 1593–1601.

Tolias, C.M., McNeil, C.J., Kazlauskaite, J., Hillhouse, E.W., 1999. Astrocytes rather than neurons mediate interleukin-1β dependent nitric oxide and superoxide radical release in primary hypothalamic rat cell cultures. Neurosci. Lett. 273, 57–60.

Torrents, D., Estevez, R., Pineda, M., Fernandez, E., Lloberas, J., Shi, Y.-B., Zorzano, A., Palacin, M., 1998. Identification and characterization of a membrane protein (y^+L amino acid transporter-1) that associates with 4F2hc to encode the amino acid transport activity y^+L. A candidate gene for lysinuric protein intolerance. J. Biol. Chem. 273, 32437–32445.

Ueno, S., Sano, A., Kotani, K., Kondoh, K., Kakimoto, Y., 1992. Distribution of free methylarginines in rat tissues and in the bovine brain. J. Neurochem. 59, 2012–2016.

Vadgama, J.V., Evered, D.F., 1992. Characteristics of L-citrulline transport across rat small intestine in vitro. Pediatr. Res. 32, 472–478.

Vargiu, C., Cabella, C., Belliardo, S., Cravanzola, C., Grillo, M.A., Colombatto, S., 1999. Agmatine modulates polyamine content in hepatocytes by inducing spermidine/spermine acetyltransferase. Eur. J. Biochem. 259, 933–938.

Vasta, V., Meacci, E., Farnararo, M., Bruni, P., 1995. Identification of a specific transport system for L-arginine in human platelets. Biochem. Biophys. Res. Commun. 206, 878–884.

Vega-Agapito, V., Almeida, A., Heales, S.J.R., Medina, J.M., Bolanos, J.P., 1999. Peroxynitrite anion stimulates arginine release from cultured rat astrocytes. J. Neurochem. 73, 1446–1452.

Vincent, S.R., Kimura, H., 1992. Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience 46, 755–784.

Waddington, S.N., Tam, F.W.K., Cook, H.T., Cattell, V., 1998. Arginase activity is modulated by IL-4 and OHArg in nephritic glomeruli and mesangial cells. Am. J. Physiol. 274, F473–F480.

Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K., Eppenberger, H.M., 1992. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem. J. 281, 21–40.

Wang, W.W., Jenkinson, C.P., Griscavage, J.M., Kern, R.M., Arabolos, N.S., Byrns, R.E., Cederbaum, S.D., Ignarro, L.J., 1995. Co-induction of arginase and nitric oxide synthase in murine macrophages activated by lipopolysaccharide. Biochem. Biophys. Res. Commun. 210, 1009–1016.

Watford, M., 1989. Channeling in the urea cycle: a metabolon spanning two compartments. Trends Biochem. Sci. 14, 313–314.

Wayte, J., Buckingham, J.C., Cowell, A.-M., 1996. [^{3}H]L-arginine transport and nitric oxide synthase activity in foetal hypothalamic cultures. NeuroReport 8, 267–271.

Westergaard, N., Beart, P.M., Schousboe, A., 1993. Transport of L-[^{3}H]arginine in cultured neurons: characteristics and inhibition by nitric oxide synthase inhibitors. J. Neurochem. 61, 364–367.

White, M.F., Gazzola, G.C., Christensen, H.N., 1982. Cationic amino acid transport into cultured animal cells. I. Influx into cultured human fibroblasts. J. Biol. Chem. 257, 4443–4449.

Wiencken, A.E., Casagrande, V.A., 1999. Endothelial nitric oxide synthetase (eNOS) in astrocytes: another source of nitric oxide in neocortex. Glia 26, 280–290.

Wiesinger, H., 1995. Glia-specific enzyme systems. In: Kettenmann, H., Ransom, B. (Eds.), Neuroglia. Oxford University Press, Oxford, pp. 488–499.

Williams, K., 1997. Interaction of polyamines with ion channels. Biochem. J. 325, 289–297.

Wilson, R.I., Godecke, A., Brown, R.E., Schrader, J., Haas, H.L., 1997. Mice deficient in endothelial nitric oxide synthase exhibit a selective deficit in hippocampal long-term potentiation. Neuroscience 90, 1157–1165.

Windmueller, H.G., 1982. Glutamine utilization by the small intestine. Adv. Enzymol. Rel. Areas Mol. Biol. 53, 201–237.

Windmueller, H.G., Spaeth, A.E., 1981. Source and fate of circulating citrulline. Am. J. Physiol. 241, E473–E480.

Withers, P.C., 1998. Urea: diverse functions of a 'waste' product. Clin. Exp. Pharmacol. Physiol. 25, 722–727.

Wolf, G., 1997. Nitric oxide and nitric oxide synthase: biology, pathology, localization. Histol. Histopathol. 12, 251–261.

Wood, J.H., 1982. Physiological neurochemistry of cerebrospinal fluid. In: Lajtha, A. (Ed.), Handbook of Neurochemistry, vol. I, 2nd edn. Plenum Press, New York, pp. 415–487.

Wu, G., Morris, S.M., Jr, 1998. Arginine metabolism: nitric oxide and beyond. Biochem. J. 336, 1–17.

Xia, Y., Zweier, J.L., 1997. Superoxide and peroxinitrite generation from inducible nitric oxide synthase in macrophages. Proc. Natl. Acad. Sci. USA 94, 6954–6958.

Xia, Y., Dawson, V.L., Dawson, T.M., Snyder, S.H., Zweier, J.L., 1996. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. Proc. Natl. Acad. Sci. USA 93, 6770–6774.

Xia, Y., Tsai, A.-L., Berka, V., Zweier, J.L., 1998. Superoxide generation from endothelial nitric-oxide synthase. A Ca^{2+}/calmodulin-dependent and tetrahydrobiopterin regulatory process. J. Biol. Chem. 273, 25804–25808.

Yang, X.C., Reis, D.J., 1999. Agmatine selectively blocks the *N*-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. J. Pharmcol. Exp. Ther. 288, 544–549.

Yu, Y., Terada, K., Nagasaki, A., Takiguchi, M., Mori, M., 1995. Preparation of recombinant argininosuccinate synthetase and argininosuccinate lyase: expression of the enzymes in rat tissues. J. Biochem. 117, 952–957.

Yu, J.-G., O'Brien, W.E., Lee, T.J.-F., 1997. Morphological evidence for L-citrulline conversion to L-arginine via the argininosuccinate pathway in porcine cerebral perivascular nerves. J. Cerebr. Blood Flow Metab. 17, 884–893.

Yudkoff, M., Nissim, I., Pleasure, D., 1987. [^{15}N]Aspartate metabolism in cultured astrocytes. Biochem. J. 241, 193–201.

Yudkoff, M., Nissim, I., Nissim, I., Stern, J., Pleasure, D., 1989. Effects of palmitate on astrocyte amino acid content. Neurochem. Res. 14, 367–370.

Zhang, W.Y., Takiguchi, M., Koshiyama, Y., Gotoh, T., Nagasaki, A., Iwase, K., Yamamoto, K., Takshima, H., Negi, A., Mori, M., 1999. Expression of citrulline–nitric oxide cycle in lipopolysaccharide and cytokine-stimulated rat astroglioma C6 cells. Brain Res. 849, 78–84.

Zhao, M.L., Liu, J.S., He, D., Dickson, D.W., Lee, S.C., 1998. Inducible nitric oxide synthase expression is selectivley induced in astrocytes isolated from adult human brain. Brian Res. 813, 402–405.

Zharikov, S.I., Herrera, H., Block, E.R., 1997. Role of membrane potential in hypoxic inhibition of L-arginine uptake by lung epithelial cells. Am. J. Physiol. 272, L78–L84.