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Review

Arginine metabolism in mammals

Luc Cynober,*†‡ Jacques Le Boucher,* and Marie-Paule Vasson‡

**INSERM U, GRENEMH, Paris, France; †Biochemistry Lab A, Saint-Antoine Hospital, Paris, France; ‡Human Nutrition Research Centre and Biochemistry, Molecular Biology and Nutrition Laboratory Pharmacy School, Clermont-Ferrand, France*

Arginine (ARG), a semi-essential amino acid, is taken up by cells using the y^+ transport system. ARG synthesis occurs from citrulline mainly in the liver and in the kidney. ARG is metabolized either in ornithine and urea mainly in the liver and the intestine or in citrulline and nitric oxide ($NO^\bullet$) in a large number of cell types. Ornithine derived from arginine can be metabolized in citrulline (in the context of the urea cycle), in glutamate or in polyamines. Arginine taken up by the intestine is transformed into citrulline which is poorly taken up by the liver but mainly by the kidney. In the kidney, citrulline is transformed into arginine and subsequently released for peripheral tissues. Intestinal transformation of arginine into citrulline plays a key-role in the metabolic adaptation to high/low protein diets. In the liver, arginine metabolism plays a pivotal role in the urea cycle, the rate of which is conditioned not only to metabolize extra-nitrogen, but also to maintain the acid-base homeostasis. Immune cells exhibit the ability to synthesize both polyamines and $NO^\bullet$ which are potent immunomodulators. The modulation and balance between these two pathways remain to be elucidated. In the context of clinical nutrition, the use of ARG supplemented diets may be advocated while keeping in mind that in severe injury with organ failure such regimens could be detrimental. (J. Nutr. Biochem. 6:402–403, 1995.)

Keywords: arginine; ornithine; nitric oxide; polyamines; urea cycle; immune cells

Introduction

Arginine was first isolated in crystalline form and named in 1886 by Schulze and Steiger, and its presence in animal protein was shown in 1895 by Hedin.[1]

For many years, arginine was considered solely an intermediate metabolite in ureagenesis, in the context of the process of nitrogen detoxification. However, more recent work suggests that the urea cycle has other functions, e.g., control of pH homeostasis[2] or involving the NO synthase pathway.[3] This has wide implications which have not yet been completely evaluated.

Initial studies performed in rats by Rose[4] indicated that arginine was a nonessential amino acid. Subsequent studies conducted in dogs,[5] cats,[6] or man[7] led to a reappraisal. Arginine is now considered an essential amino acid in carnivores such as cats and a conditionally indispensable amino acid in omnivores, i.e., an amino acid that becomes indispensable when de novo capacities of synthesis are insufficient to cover increased needs occurring for example during growth[5,8] or in response to trauma.[7]

Cellular uptake of arginine

The transport of the cationic amino acids (arginine, lysine, and ornithine) is mediated by system y^+ (formerly called Ly^+) which is Na^+ independent.[9] Recent studies indicate that system y^+ should not be considered an active transport but rather a facilitated diffusion system dependent on the membrane potential.[10] The arginine transporter is a MM 67 kD protein forming 6 transmembrane domains[11] now called murine cationic amino acid transporter-1 (MCAT-1).[12] There is a steric constraint for arginine binding to the transporter since methylation of the α-amino group completely eliminates arginine reactivity with system y^+. Similarly, the affinity of D-arginine is about 20 times less than that of L-arginine.[13] On the contrary, L-homo-arginine is specifically transported by system y^+ with a high affinity, making L-homo-arginine a useful probe to study system y^+.

System y^+ was originally observed in Ehrlich cells by Christensen in 1964[14] and further identified in various cell types including fibroblasts,[12,15] endothelial cells,[12] enterocytes,[16] and macrophages.[17] An interesting point is that system y^+ is barely detectable or undetectable in isolated

Address reprint requests to Dr. L. Cynober at the Laboratoire de Biochimie A, Hôpital Saint-Antoine, 184, rue du Fbg Saint-Antoine, 75571 Paris Cedex 12, France.
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hepatocytes in the basal state[9,12] (making the transport of arginine into these cells the rate-limiting step for arginine metabolism) but operates in hepatoma cells.[18] In these latter cells, the kinetic constants for arginine uptake are K_m = 145 ± 7 μM and V_{max} = 3.3 ± 0.1 nmol/mg of protein/min.[9] In fibroblasts, affinity is higher (K_m = 40 ± 5 μM) but transport maximal velocity is lower (V_{max} = 0.75 ± 0.05 nmol/mg of protein/min).

System y^+ is pH insensitive,[9,13] unlike other systems of amino acid transport such as systems A and ASC. System y^+ is subject to trans-stimulation,[9,13] i.e., intracellular accumulation of arginine stimulates its membrane transport. Hepatocytes from glucagon-treated rats exhibit an increased system y^+-like activity. However, isolated hepatocytes (from untreated rats) appear almost insensitive to glucagon.[19] Of greatest interest is the recent work of Pacitti et al.,[20] which indicates that hepatic membrane vesicles from tumor necrosis factor-α (TNFα)-pretreated rats exhibit a time (2 to 4 hr) and dose (50–150 μg/kg of body weight)-dependent stimulation of system y^+. In addition, kinetic analysis revealed that accelerated arginine transport was caused by a 78% increase in V_{max} without modification of transport affinity. Similar results were obtained subsequently by measuring arginine uptake in hepatocytes from lipopolysaccharide (LPS)-treated rats.[21] It is possible that this stimulation is the result of the induction of another distinct newly discovered cationic amino acid transporter MCAT-2B as in activated macrophages.[17]

In addition to system y^+, at high concentrations (over 1 mmol/L) the influx of arginine may either be mediated by a second low-affinity system, with K_m values over 20 mM, or occur by an apparently nonsaturable physical diffusion[13] which can represent up to 45% of total uptake.[20] Recently, other systems for arginine transport have been described, called $B^{0,+}$ and $b^{0,+}$, respectively, Na^+-dependent and Na^+-independent.[16] These systems are detectable in fibroblasts, endothelial cells, and Fao hepatoma cells and may play a role during early development.

An important question is the mode of intestinal absorption of arginine. Arginine is mainly absorbed in the ileum and the jejunum with saturable and nonsaturable components; by comparison absorption in the colon is very low.[22] The intestinal absorption of arginine on the brush border membrane side involves transport systems $B^{0,+}$ and $b^{0,+}$ shared with lysine, ornithine, and cysteine[23,24] and system y^+.[24] At the basolateral membrane side, arginine is transported by system y^+.[24] It is noteworthy that some dipolar amino acids, especially leucine, stimulate absorption of basic amino acids across the intestinal epithelium.[24]

Enzymes involved in arginine metabolic pathway

Arginine synthesis

In mammals, arginine is synthesized in only one way. This pathway involves argininosuccinate synthase (EC 6.3.4.5) and argininosuccinate lyase (EC 4.3.2.1) transforming citrulline into arginine via arginosuccinate. These enzymes are found predominantly in the liver, but the very high hepatic content of arginase, which splits arginine into ornithine and urea (see below), prevents the release of any arginine from the liver into the circulation even after an ornithine load as demonstrated in an isolated perfused rat liver model.[25] As described later, the major source of endogenously synthesized arginine is derived from citrulline taken up by the kidney. Citrulline itself is mainly derived from glutamine and glutamate metabolism in the intestine, especially in the jejunum, which possesses all the enzymes required.[26]

Arginine catabolism

There are two direct degradative pathways. The first is mediated by arginase, releasing ornithine and urea. The second is catalyzed by nitric oxide synthase. In this latter pathway, citrulline and $NO^\bullet$ are released in an equimolar fashion.

Arginase. This reaction, catalyzing the formation of urea and the generation of ornithine, is strongly exergonic with a change in free energy of −12.3 kcal/mol, making the reaction irreversible.[27]

Arginase (L-arginine ureahydrolase, EC 3.5.3.1) has a molecular mass ranging from 107 to 118 kD, depending on the species[27] and has a trimeric structure; each identical subunit has a molecular mass of 20 to 40 kD[27,28] and can bind two Mn^{2+} ions.[28] There are three isoenzymes[29] with different cellular localization (*Table 1*).

Ornithine, lysine, and branched-chain amino acids are competitive inhibitors of arginase. However, this enzyme has such a high V_{max} that even when inhibited it will never limit flux through the ornithine cycle.[27]

Arginase is found primarily in the liver and, to a lesser extent, in various other organs and tissues (*Table 1*). According to the local enzymatic equipment, the generated ornithine is then metabolized to polyamines through ornithine decarboxylase (ODC, EC 4.1.1.17),[30] to glutamate (a reaction mediated by ornithine aminotransferase, EC 2.6.1.3), and/or to citrulline (ornithine carbamoyl transferase; EC 2.1.3.3). The various pathways derived from the arginase reaction are summarized in *Figure 1*.

Fluxes are also directed according to the physiopathological situations. For example, during inflammation there are large amounts of arginine and arginase in the wound fluid (see below), which favors the formation of ornithine,

Table 1 Tissue arginase activity

Tissue	Arginase activity (arbitrary unit*)	Isoenzyme type
Liver	100	III
Intestine	5.1	I, II
Pancreas	4.0	I, II
Kidney	1.8–2.2	I, II
Fibroblasts	0.5	
Brain	0.10–0.14	I
Lung	0.21	I
Muscle	0.015–0.10	I
Spleen	0.04–0.07	I

Adapted from Refs. 7, 29, and 136.
*In order to normalize values from various publications, arginase activity in liver is considered arbitrarily to be 100 (absolute values: 1,669 to 2,545 μmol urea/min/g).

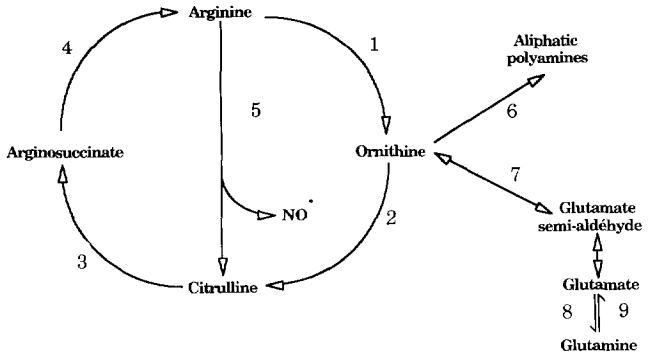

Figure 1 A general view of L-arginine-connected pathways. (1) arginase, (2) ornithine carbamoyl transferase, (3) argininosuccinate synthetase, (4) argininosuccinase (5) NO synthase, (6) ornithine decarboxylase, (7) ornithine transaminase, (8) glutamine synthase, and (9) glutaminase.

further metabolized to glutamate and then to proline. The latter is incorporated into collagen.[31] This probably explains the role of arginine in promoting wound healing.[32]

Nitric oxide synthase. Nitric oxide synthase (NOS) is also called arginine deiminase (EC 1.14.13.39). This is a family of dimeric enzymes with molecular weights ranging from 125 to 155 kD.[23,33,34] NOS has been identified and characterized in numerous cells (*Table 2*). The substrate is arginine and the products are citrulline and $NO^{\bullet}$ as a result of the oxidation directed toward one of the chemically equivalent guanidino nitrogens of L-arginine.[17] $NO^{\bullet}$ is then further oxidized to nitrite and nitrate. In macrophages the simultaneous generation of $NO^{\bullet}$ and O_2^- results in the formation of peroxynitrite ($ONOO^-$), which in turn is protonated in peroxynitrous acid (ONOOH). This latter acid can decompose to hydroxyl radical ($^{\bullet}OH$) and nitrogen dioxide ($^{\bullet}NO_2$) or undergo intramolecular rearrangement to give NO_3^- and H^+.[38] The exact metabolic route is not well understood. It seems that this reaction operates in two steps, both catalyzed by NOS, with Nω-hydroxyl-L-arginine as a stable reaction intermediate.[39] Only L-arginine and, to a minor extent, L-homoarginine are substrates for NOS.[39] A number of cofactors are required for $NO^{\bullet}$ synthesis including $NADPH_2$ and Ca^{2+}. Among them, tetrahydrobiopterin appears to be rate-limiting.[35] Arginine could also be limiting in the reaction, which would explain why cellular arginine uptake is increased in macrophages when NOS is activated.[17]

While the pathway itself appears to be identical in the different cells, its regulation is quite different, allowing three isoenzymes to be distinguished, representing three distinct gene products, which have been isolated and purified.[35] Two, which are typically present in endothelial cells and in neuronal tissue, are constitutive (and therefore called cNOS). The endothelial cNOS is mostly membrane bound via a myristylation site,[17] whereas the neuronal cNOS is located in the cytosol of central and peripheral neurons.[35] These enzymes synthesize small amounts of $NO^{\bullet}$ in response to the appropriate stimuli, e.g., acetylcholine (endothelial cells) or glutamate (cerebral cells).[40]

The last isoform, found in a wide variety of cells including macrophages, is not present at the basal state but is inducible (and therefore called iNOS) by various microbes, microbial products, including LPS, and inflammatory cytokines, especially interferon γ (IFNγ).[17,41] The LPS activating effect is largely (but not exclusively) mediated through LPS-induced TNFα and interleukin-1 (IL-1) secretion.[42] In some cases, there is a strong synergy among these stimulating agents. For example, LPS, IFNγ, IL-1, and TNFα act synergically on hepatocytes[43,44] leading to an increase in NOS mRNA, peaking 6–8 hr after stimulation with a further decline by 25% at 24 hr.[44] Also, IFNγ strongly potentiates IL-1 and TNFα actions on nitric oxide production by brain vascular endothelial cells.[45] On the contrary, glucocorticoids, TGFβ, IL-4, IL-8, IGF-I, and thrombin inhibit iNOS synthesis.[23,33,35,37,44] IL-10 has been found to inhibit iNOS as well as to stimulate IFNγ-induced iNOS expression.[46]

The role of intracellular calcium in the induction of NOS remains unclear. While it has been reported that the calcium ionophore A23187 could mimic IFNγ action in macrophages, others have demonstrated that the induction of NOS in bone marrow–derived macrophages is independent of changes in intracellular calcium levels.[38] Expression of iNOS requires a lag time and is dependent upon protein synthesis.[39,46]

Common points and differences between cNOS and iNOS are summarized in *Table 3*. For a long time it was thought that cNOS was calmodulin-dependent whereas iNOS was not. More recent studies[33] indicate, however, that calmodulin is tightly bound to iNOS in macrophages.

However, recent studies suggest that the simple distinction between cNOS and iNOS is not sufficient to take into account differences between isoforms. For example, human hepatocyte iNOS has only an 80% amino acid sequence homology to macrophage iNOS[47] whereas, for the cNOS isoforms, homology of over 90% is found between humans and other species.[17] Furthermore, there are species differ-

Table 2 Cells expressing NO synthase activity (NOS) (c) constitutive, (i) inducible

cNOS	iNOS
Endocardial cells	Astrocytes
Endothelial cells	Bone marrow cells
Retina	Brain glial cells
Skeletal myocytes	Cardiac myocytes
Neurons	
Platelets	Chrondrocytes
	Endothelial cells
	Fibroblasts
	Hepatocytes
	Keratinocytes
	Macrophages (including Kupffer)
	Mesengial cells
	Monocytes
	Neutrophils
	Osteoclasts
	Retinal pigment epithelium
	Renal tubular epithelium
	Smooth muscle cells
	Splenocytes

Adapted from Refs. 23, 35–37.

ences with cell-to-cell differences. For instance, the quantity of $NO^{\bullet}$ produced by human monocytes, if any, is considerably less than seen in murine cells,[17,46] whereas cultured human hepatocytes exhibit the same strong $NO^{\bullet}$ synthesis as seen in rat hepatocytes.[35] The fact that human monocytes express no detectable iNOS mRNA in response to LPS, TNFα, or IL-1, through southern analysis on human genomic DNA, reveals a specific human iNOS gene and suggests that the iNOS gene may have become inoperative during evolution.[46]

Other pathways. Arginine is an amidine group donor in transamidination reactions, for example with glycine in the biosynthesis of the creatine precursor, guanidine acetic acid.[23] In nerve tissue, arginine can also form γ-guanidinobutyric acid, the role of which is not known.[7]

Organ specificities in arginine metabolism

Liver

During catabolism of amino acids, approximately 1 mol of NH_4^+ is formed per day when the daily protein consumption is 100 g.[27] In mammals, the major metabolic pathway responsible for the removal of ammonia is the synthesis of nontoxic urea in the liver. This is performed in the classical urea cycle proposed 60 years ago by Krebs and Henseleit.

Exhaustive description of the urea cycle is outside the scope of this review. We limit our purpose here to data relevant to the regulatory role of arginine. Readers who are interested can refer to the recent comprehensive reviews by Meijer et al.[27] and Meijer.[48]

Ureagenesis is subject to both short-term and long-term regulation. Short-term regulation is assured by N-acetylglutamate, which activates carbamoyl-phosphate synthase I (CPS-I, EC 6.3.4.16). N-acetylglutamate is formed from glutamate by a reaction catalyzed by N-acetylglutamate synthase (EC 2.3.1.1). Arginine is an allosteric activator of this enzyme.[49,50] In this scheme it is evident that the product of the urea cycle, i.e., arginine, is able to further accelerate the cycle through a modulation in cascade (*Figure 2*). However, we must point out that arginine synthesis occurs in cytoplasma whereas N-acetylglutamate synthase is located in mitochondria. Therefore, an activating role for arginine implies a transfer of this amino acid from cytoplasma to mitochondria. Mitochondria accumulate arginine against the gradient of concentration, and mitochondrial levels of arginine are about 20 to 40 times the Ka of arginine, for N-acetylglutamate synthase.[49] Alternatively, it has been suggested[51] that N-acetylglutamate synthase is closely associated with the mitochondrial membrane and that binding of arginine to a specific receptor on the membrane is how arginine activates the enzyme.

Table 3 Common points and differences between cNOS and iNOS

cNOS		iNOS
	Cytosolic NADPH, H^+ dependent inhibited by L-arginine analogues BH4-dependent	
Constitutive		Inducible
Ca^{2+}/calmodulin-dependent		Ca^{2+}/calmodulin tightly bound to the enzyme
pmol $NO^{\bullet}$ released short lasting released		nmol $NO^{\bullet}$ released long-lasting release
unaffected by glucocorticoids		induction inhibited by glucocorticoids

Adapted from Refs. 3 and 56.

Gastrointestinal tract

The enterocyte isolated from pigs at birth or during suckling acts as a producer of arginine with glutamine, citrulline, and ornithine as main sources.[52] This anabolic process may be a way of maintaining arginine homeostasis in the neonate, i.e., when other organs such as the kidneys (see below) are not sufficiently mature to take on this task.[52]

However, as soon as weaning is over, the gut switches and acts as a user of arginine because it expresses arginase (isoenzyme II) and ornithine carbamoyltransferase (OCT).[51] The gut thus releases urea and citrulline.[53,54] When L-[U-^{14}C]arginine, in amounts close to the concentration found in the intestinal lumen 3 hr after a high protein meal (2 μmol/0.3 mL), was perfused into isolated segments of rat jejunum in situ, only 60% of the arginine was absorbed intact, while 33% was hydrolyzed of which 38% was released into the blood as ornithine and the remaining as citrulline, CO_2, and proline.[54] Interestingly, ornithine formed 32% and urea 17% of ^{14}C-labeled metabolites released into the blood. This two-to-one ratio between ornithine and urea is intriguing because an equimolar ornithine/urea release would have been expected if ornithine was the end product of arginine or a lower output of ornithine than urea if ornithine was further metabolized, which is the case. This could be explained by the presence of urease or, more likely, a diffusion of urea in the lumen.

Another intriguing feature of arginine and related amino acid metabolism in the gut is that ornithine is paradoxically not as good a precursor of citrulline[55] as arginine. This could be due to the fact that ornithine translocase (required for processing of ornithine in the mitochondria before metabolization into citrulline) is closely associated with arginase.[27,56] Hence, arginine appears to be a better substrate for the complex arginase-ornithine translocase-OCT than ornithine.[56] In the cytosol of enterocytes, ornithine appears to be driven in substantial amounts toward polyamine formation.[55] The compartmentalization between the polyamine and the citrulline pathways is presumably a means to direct the fluxes of two related amino acids toward two pathways with widely different aims.

As indicated earlier, enterocytes express ODC,[57] leading to a local production of aliphatic polyamines. However, despite the high arginase and ODC activities in enterocytes, most of the arginine metabolized in the gut is released in the form of citrulline into the portal blood stream.

Arginine deiminase has also recently been discovered in

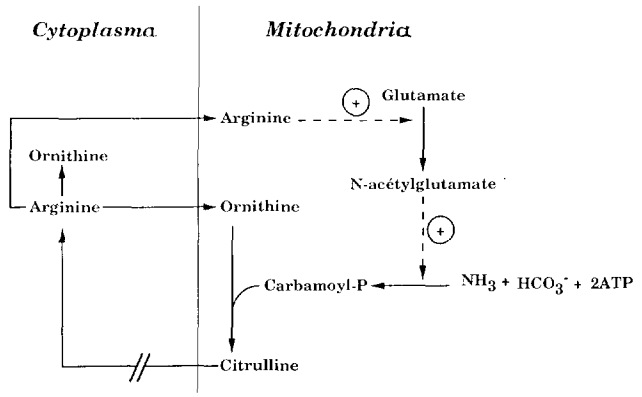

Figure 2 The regulatory role of L-arginine in the urea cycle.

gastric smooth muscle[58] and in the intestine,[59,60] especially in the ileum, which has an extensive capacity for NO• generation.[61] NO• generation could be implicated in gastric emptying.[58] Both endothelial and smooth muscle cells of submucosal blood vessels have the ability to synthesize NO•.[62] NOS activity is found in nonadrenergic noncholinergic myenteric neurons and in their efferents to the circular muscle.[61,63] It is noteworthy that this enzyme activity is very low at birth, increasing strongly postweaning.[64]

Kidney

The kidney is the major organ in citrulline disposal and arginine synthesis.[65,66] The only enzyme related to arginine metabolism present in large amounts in the kidney is argininosuccinate lyase. This enzyme is mostly located in the cortex (cortex, 40.6 ± 7.6; medullar, 5.0 ± 0.9; papilla, 5.9 ± 0.3 μmol/hr/g wet wt).[67] The N donors, required for the synthesis of the guanidine group of arginine, are aspartate, glutamate, or glutamine.[67] The absence or the low activity of arginase in the kidney explains why citrulline, taken up by this organ, is released as arginine. This has been demonstrated in a series of elegant studies by Dhanakoti et al.[67] involving in vivo citrulline perfusion in rats and the measurement of arginine and citrulline arteriovenous kidney differences.

Immune cells

Macrophages contain high amounts of arginase, the importance of which is discussed below. Also, these cells are able to produce large amounts of NO• in response to LPS and cytokines (discussed previously). Furthermore, and most interestly, macrophages, in certain conditions of activation, are able to generate arginine from citrulline.[68] This arginine-citrulline (with NO• production)-arginine cycle has been suggested[69] to play a role in maintaining intracellular L-arginine availability for NO• generation under conditions (i.e., inflammation, see below) where extracellular L-arginine concentration is very low, due to high arginase activity in extracellular fluid surrounding the wound.

Arginine interorgan exchanges

Arginine interorgan exchanges have been thoroughly studied by arteriovenous difference measurement techniques in both humans[70] and animals.[66,67] More recently, another approach has allowed the role of various organs in the maintenance of arginine homeostasis to be clearly established. After liver transplantation for ornithine carbamoyltransferase deficiency, plasma citrulline and arginine remain low, whereas after transplantation for argininosuccinate synthase deficiency, plasma citrulline levels remain high and arginine levels low.[71] This means that deficiencies that persist in other organs (respectively the intestine and the kidney) do not allow the normal arginine homeostasis to be fully re-established. A synthetic picture of arginine interorgan exchanges is given in *Figure 3*. Most dietary arginine is taken up either by the intestine and further released as citrulline or by the liver to be metabolized. It is clear that the intestine, and not the liver as thought initially,[72] is the source of citrulline derived from arginine.

Citrulline uptake by the liver is low,[66,73] and this amino acid is primarily metabolized in the kidney; 83% of the citrulline released by the intestine is taken up by the kidney.[66] The importance of the kidney is underlined by the fact that hypercitrullinemia is one of the prime characteristics of the impaired plasma amino acid pattern in renal failure.[70,74,75] In the kidney, citrulline is metabolized to arginine, which is released. Peripheral tissues are thus the major sites for arginine uptake and degradation. Finally, the importance of citrulline as an arginine precursor in vivo is suggested by experiments in dogs[5] or rats[76] fed an arginine-free diet which induces growth failure; consumption of an equimolar intake of citrulline resulted in a normal weight gain. Furthermore, specific inhibition of citrulline synthesis from arginine in the intestine is responsible for severe growth retardation of rat pups.[8]

For a large part, citrulline is also derived from glutamine and glutamate metabolism in the enterocyte.[77,78] In a recent elegant study,[79] it was shown that feeding rats with a glutamine-enriched diet increases the arterial plasma level of citrulline by 30%, the uptake of citrulline by the kidney by 40%, and the production of arginine by this organ by 38%. In humans, de novo arginine synthesis is about 16 μmol/kg/hr, independent of arginine content in the diet.[80]

Significance of arginine metabolism

The urea cycle

Arginine plays a key role in modulating ureagenesis. Primary evidence of this is the fact that of all the urea cycle intermediates, arginine is most taken up by the liver. Ornithine uptake is lower[25] and citrulline uptake is insignificant[66] even at high non-physiological levels.[81] As described earlier, arginine activates N-acetylglutamate synthase catalyzing the synthesis of N-acetyl-L-glutamate, an allosteric obligatory activator of CPS-I in the liver of ureotelic animals. A line of evidence suggests that the modulating effect of arginine is dependent on nutritional status rather than on an increase in local arginine synthesis through argininosuccinate synthase activation[82]: (1) this amino acid has no action on mitochondria from fasted rats, whereas it increases 6.5- to 7.5-fold in rats fed 0% to 60% casein[50]; (2) feeding rats with a high protein diet increases CPS, OCT,[51] N-acetylglutamate synthase activities,[82,83]

and the modulatory effect of arginine.[83] It is therefore likely that arginine acts as a key signal for the activation of ureagenesis during high-protein feeding. We must however mention other studies that deny any regulatory role for arginine; when rats were injected intraperitoneally with 1.5 g of amino acids/kg, glutamate, N-acetylglutamate, and CPS-I activities all peaked 5 to 15 min after injection even when arginine was omitted from the injected mixture.[84]

When considering the net balance of ureagenesis ($2\,NH_4^+ + 2HCO_3^- \rightarrow$ urea + CO_2 + $3H_2O$), it is usual to focus on the fact that urea formation allows the removal of ammonia. However, at least as important is that ureagenesis removes bicarbonates in an equimolar fashion. Accordingly, it is now considered[85,86] that hepatic ureagenesis plays a considerable role in the maintenance of bicarbonate homeostasis. In acidotic conditions the decrease in glutamine consumption by periportal hepatocytes leads to a decreased ureagenesis rate and therefore to a sparing of HCO_3^-.[85–87] The role of arginine in the process of adaptation to acidosis is not known but it is interesting that administration of ornithine α-ketoglutarate counteracts acidosis in starved rats.[88]

Significance of arginine metabolism in the intestine

As discussed previously, a major control of the ureagenesis rate is the bioavailability of arginine arriving through the portal vein.[83,89] The ability of the intestine to convert arginine into citrulline is a good way to modulate arginine flux to the liver and therefore the ureagenesis rate since citrulline uptake by the liver is very low. The intestine must be able to adapt arginine production to nutritional intake. The key point of this control is probably the presence of N-acetylglutamate synthase in high amounts in the jejunum and ileum[90] the activity of which decreases when protein intake is high.[73] In the same way, and in contrast to the liver, OCT activity decreases when protein intake increases.[51]

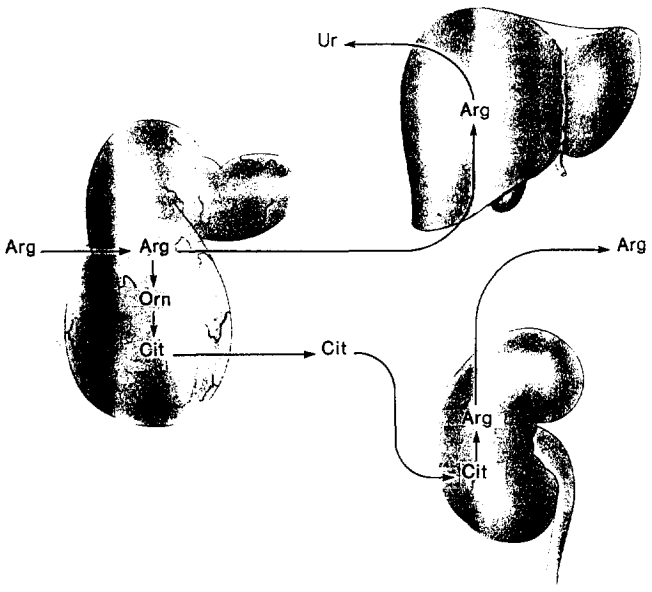

Figure 3 Interorgan arginine exchanges. From L. Cynober and M. Marcollet. *Encycl. Med. Chir.* 10-375-A-10, with permission.

Arginine and immune cell functionality

Much work indicates that arginine modulates immunity in vivo.[23] This is also true in vitro. For example, incubating rat alveolar macrophages with increasing amounts of arginine increases phagocyte activity with a maximum for arginine at 25 mM.[91]

Until the last decade, the effect of arginine was not understood. It is now clear that this effect is mediated through NO• and polyamine synthesis.

The nitric oxide synthesis pathway. The fact that several molecules (NG-monomethyl-L-arginine, L-nitroarginine methylester, etc.) act as specific inhibitors of NOS[40] has allowed rapid progress in the understanding of the functions of the arginine/NO• pathway. NO• research currently involves a wide range of fields[33,40]; this review will only focus on aspects related to biochemical nutrition.

The constitutive NO synthase. For several years, a factor required for the acetylcholine-induced relaxation of vascular smooth muscle was identified as the endothelium-derived relaxing factor (EDRF).[92] Many other endogenous vasoactive substances were also found to act through the release of EDRF and to elicit endothelium-dependent vasodilation: bradykinin, histamine, adenine nucleotides, and thrombine.[40] Some years later, however, it was firmly established that EDRF is in fact NO•.[39]

It is likely that this action of NO• involves the activation of soluble guanylate cyclase, which catalyzes the conversion of GTP to cyclic GMP (cGMP).[93] In turn cGMP induces smooth muscle relaxation through alterations in Ca^{2+} levels or activation of protein kinases.

The mechanism of activation of guanylate cyclase by NO• is not fully understood. It has been suggested[39] that activation results from the binding of NO• to a heme prosthetic group linked to guanylate cyclase. The fact that a heme-deficient mutant of guanylate cyclase has an unaffected basal activity but is completely insensitive to stimulation by NO• supports this hypothesis.[36]

The inducible NO synthase. In macrophages, NO• acts as a cytotoxic agent against tumor cells and bacteria.[33] The molecular targets in the victim cells are Cu-Fe proteins, releasing free Cu^{2++}, and generating O_2 and highly toxic hydroxyl radicals.[40] In addition, as stated above, NO• and O_2^- can combine, leading to peroxynitrite formation. The net effect is massive oxidative injury. Also NO• inhibits several enzymes indispensable for energy production and cell respiration: glyceraldehyde-3-phosphate dehydrogenase (glycolysis), aconitase (Krebs cycle), NADPH-ubiquinone oxidoreductase, and succinate-ubiquinone oxidoreductase (electron transport chain).[35] This action may be dependent upon the S-nitrosylation and ADP-ribosylation of these proteins by nitrosonium (NO^+), which can be formed from NO• by the removal of one electron.[94]

Other mechanisms of action are possible. In hepatocytes, LPS and cytokines induce NO• production and, in parallel, cGMP production.[44] It is also possible that some actions, such as cytostasis of tumor cells, may be supported by intermediary Nω-hydroxy-L-arginine or by unidentified metabolites of this molecule.[95]

Nitric oxide inhibits neutrophil superoxide anion produc-

tion via a direct action on a membrane component of the $NADPH_2$-dependent oxidase.[96] These observations suggest that $NO^{\bullet}$, released in response to cytokines by endothelial cells or macrophages at sites of inflammation, may protect against neutrophil-dependent tissue injury and excess lymphocyte T proliferation.

However, the relationship between cytokines and $NO^{\bullet}$ appears to be a network since $NO^{\bullet}$ itself is able to induce TNFα production by various cells including neutrophils.[97]

Increasing evidence indicates that nitric oxide may play a role in acute and chronic inflammation. Treatment with inhibitors of NOS reduces the degree of inflammation in rats with chronic[98] or acute inflammation whereas L-arginine enhances it.[33] However, in other circumstances inhibition of NOS may have detrimental effects (see below).

The polyamine synthesis pathway. Aliphatic polyamines (putrescine, spermine, spermidine) exhibit several important regulatory properties including effects on cell multiplication and differentiation.[30,99] Polyamines also modulate immune status: the full response of lymphocytes to mitogenic agents requires the expression of ODC.[99] Polyamine synthesis requires an adequate ornithine supply, which can be achieved by arginine catabolism as described in the next section.

Balance between the urea cycle/$NO^{\bullet}$ pathway and the polyamine pathway. As discussed earlier, arginine is a key metabolite in generating polyamines and $NO^{\bullet}$. Since these two mediators have different immunoregulatory properties despite being derived from the same precursor, the specific activation of a given arginine pathway should occur in different tissues and/or at different times to give a coherent response. Nω-hydroxyl-L-arginine, the intermediate in $NO^{\bullet}$ formation from arginine, is a potent inhibitor of arginase in the liver and in macrophages.[100] In addition, arginase and NOS differ in their affinity to the substrate: NO synthases have K_m values of 10 to 100 μM while the K_m values of arginases were found to be above 1 mM.[101] Normal nonactivated mouse macrophages have been found contain[101] arginase activity or not.[102] When mouse macrophages are exposed in vitro to LPS there is prompt appearance (24 hr) of substantial arginase activity in cells and in the supernatant medium[102] in 48 hr with a decrease in arginine level in the incubation medium. This phenomenon is transient: arginase activity decreases by 84% at 48 hr. Both LPS-activated macrophages and their supernatent medium exhibited a cytotoxic effect on tumor cells. Interestingly, when fractionating the supernatant medium on a Sephadex G-200 column it was noticed that a single fraction contained arginase activity and the cytotoxic activity against V79 Chinese hamster lung tumor cells or against various lymphoma and sarcoma cell lines. In addition, this fraction corresponded to an MM 120,000 protein, i.e., the molecular mass of arginase. There is no clear explanation for these results. It is possible that arginase has antitumor effects, depleting arginine in tumor cells.

In fact, it seems that arginine and ornithine may act cooperatively in regulating immune response in inflammatory situations. The proposed scheme[38,103–105] is the following: the activated macrophages synthesize $NO^{\bullet}$ (as evidenced by high levels of nitrite and citrulline in wound fluid) and, at the moment of their lysis,[38] release arginase into the extracellular fluid surrounding the inflammatory site. As a result, arginine is actively transformed to ornithine, which is transported into lymphocytes for polyamine synthesis. Also, generated ornithine can support proline requirements for collagen synthesis in fibroblasts.[38] Therefore, the production of ornithine from arginine may be an intracellular signal. It is also noteworthy that cellular arginine deprivation promotes cellular polyamine uptake.[106] Taken as a whole, it can be suggested that arginine availability is a major determinant in the synthesis of immunomodulators.[103]

Arginine requirements

As stated in the introduction to this review, arginine is now considered a semiessential amino acid but there are considerable differences among species according to enzyme equipment.[51]

Arginine-deficient diets induce hyperammoniema, vomiting, tremors, and hyperglycemia in cats[107] and immature dogs.[7] In adult rats, feeding with an arginine-deficient diet for 13 days leads only to a marked increase (40-fold) in urinary excretion of orotic acid.[108] It is likely that, in rats like in humans, intestinal citrulline synthesis from glutamate and glutamine is sufficient to provide enough arginine to sustain growth.[51] However, repletion of protein-depleted or starved rats requires arginine for optimal growth.[7]

Until recently, nothing was known about arginine flux values in humans. Castillo et al.,[80] perfusing L-[guanidino-^{13}C]-arginine in healthy subjects fed with arginine-rich or -free diets for 6 days, established fluxes at 69 ± 8 and 63 ± 14 μmol/kg/hr, respectively, in the fasted state and 87 ± 12 and 51 ± 7 μmol/kg/hr in the fed state. In a further study,[109] the same authors established that homeostasis of arginine metabolism in healthy adults depends primarily upon the regulation of the rate of arginine degradation, the involvement of the de novo rate of arginine synthesis being minor if significant.

Arginine metabolism in disease

Elevated plasma arginine concentrations in patients with gastrointestinal[110] or breast cancer[111] have been documented. This modification is probably tumor-mediated since plasma arginine concentrations revert promptly to normal after tumor removal.[110] In addition, this variation seems related to malignancy since, for example in breast cancer, plasma arginine is increased in malignant but not in benign disease (respectively 167 ± 17 and 111 ± 10 μmol/L; $P < 0.05$).[111] In the same study, it was demonstrated that the free arginine tumor content was three times higher in malignant than in benign disease. The significance of this finding is not understood as yet, but the fact that arginase has antitumoricidal effects[104] could be relevant.

Another very exciting aspect is the relation between hypercholesterolemia and hypoargininemia as reported in several studies[112,113] except one.[114] In addition, the use of an

arginine-deficient diet in rats induces a marked increase in liver triglycerides and cholesterol.[115]

This relationship in the context of atherosclerosis is not yet well understood but these two factors could act in synergy since hypercholesterolemia impairs endothelial relaxation and since, as described above, NO• derived from arginine is an important regulator of vascular tone. L-arginine perfusion restores the acetylcholine-induced increase in coronary blood flow in patients with hypercholesterolemia.[116] Also important in the same context is the fact that arginine perfusion inhibits platelet aggregation in healthy subjects.[93]

Finally, it has been clearly demonstrated that arginine and lysine compete for cellular uptake.[117] This might explain why plant proteins (relatively rich in arginine compared with lysine) are less atherogenic than animal proteins (which are richer in lysine).[113,118] It has been recently claimed[119] that solutions for parenteral nutrition rich in arginine may be detrimental for lysine availability. However, our personal feeling, based on the fact that lysine is not rate-limiting in protein synthesis in catabolic patients,[118] is that we should consider the reverse: excessive lysine intake could be detrimental for arginine supply required in the context of response to aggression.

As described above, LPS induces the synthesis of NOS. There is now a line of evidence to support the idea that iNOS overexpression is responsible for the vascular collapse and shock in sepsis.[38] NOS inhibitors effectively reverse the hypotension following LPS injection in rats,[120] after TNFα infusion in dogs[35] and during septic shock in humans.[121]

In contrast, pulmonary hypertension in severe acute respiratory distress syndrome may be associated with a lack of endogenous NO• production. When NO• is inhaled, vascular relaxation can be obtained, resulting in a decrease in pulmonary artery pressure and improved arterial oxygenation.[122]

Also, the synthesis of NO• from L-arginine has a role in maintaining the macrovascular integrity of the intestinal mucosa following acute endotoxin challenge in the rats.[123] These examples underline the fact that NO• production in response to injury is a positive response that becomes harmful when excessive.

Conclusion: Arginine as an immune metabolic modulator; implications in therapeutic strategy

There are a number of studies[7,23,56] indicating that supplementation of enteral or parenteral diets with arginine improves response to stress. It is likely that the effects of arginine on nitrogen metabolism and immune functions are triggered by distinct mechanisms. The former is not always associated with the latter and in addition the former does not seem dose-related, whereas the effect on immunity is.[7,56] Arginine action on nitrogen metabolism could be linked to its ability to stimulate hGH secretion.[7] Recently, it was shown[124] that dietary enrichment with arginine improves the survival of septic mice (60% versus 20% in the control isonitrogenous group). There is also strong evidence[7] to support the idea that arginine has an antitumoricidal effect. In particular, Reynolds et al.[125] have demonstrated that arginine administration to an immunogenic tumor (C 1300 Neuroblastoma) decreases tumor weight gain, increases survival (by 27% compared with nonsupplemented rats), thymus weight, lymphocyte response to Con A, and lymphocyte IL-2 production. These effects are not seen in TBJ bearing rats (a weakly immunogic tumor). Hence, it can be suggested that the antitumor effect of arginine is linked to its immunoregulatory properties.

The beneficial effect of arginine on immunity is probably the result of intricated factors. NO• production is probably a major one. It has been shown that NO• plays a major role in arginine-mediated thymuline release[126] in the lysis of tumor cells. Blocking NO• synthase increases the hepatic injury[127] and lethality[128] in mice challenged with LPS and administration of high doses of a NO• synthase inhibitor increases the LPS-induced hypotension.[120] However, conversely, high intake of arginine in situations of great stress such as burn[129] or peritonitis[130] has been shown to be detrimental. We[73] and others[98] have spoken about the "Dr. Jekyll and Mr. Hyde" nature of NO• and arginine. As suggested by Miller et al.[98] this ambiguity might lie in the enzymatic source of NO• (constitutive vs. inducible). The protective effect of NO• under acute conditions could reflect local vascular actions, maintaining blood flow in the face of necrosis. However, what is a defensive mechanism could promote injury under chronic conditions when both inducible and constitutive forms of NO synthase are active.[98] Also the type of action (beneficial or detrimental) could be dependent upon the amount, the duration, and the anatomic site of NO• synthesis.[17]

From a practical point of view, it is a puzzling problem to determine whether arginine or ornithine should be used in artificial nutrition since the metabolism of both molecules

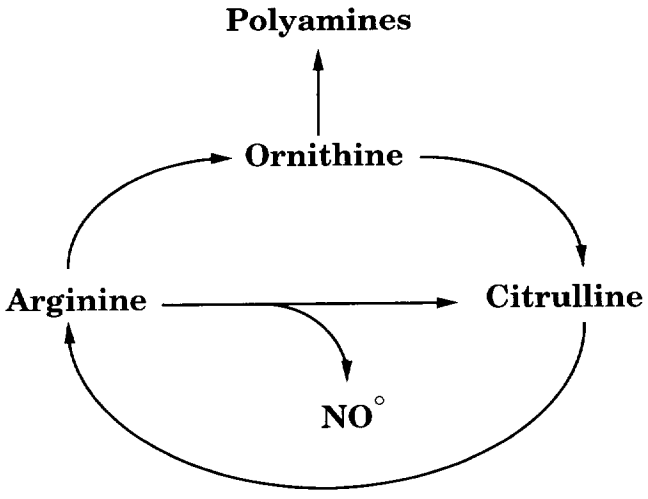

Figure 4 Arginine and ornithine: two interelated amino acids in the generation of immunomodulatory substances.

are inter-related (*Figure 4*) and ornithine displays the same properties as arginine on nutritional status and immunity of traumatized patients[132]; oral administration[131] or perfusion[91] of arginine gives plasma ornithine, and similarly high plasma and tissue levels of arginine are obtained after ornithine as α-ketoglutarate salt administration by the oral[133] or parenteral (L. Guyot et al., unpublished data) route. Conversely, the use of an arginine-deprived diet, which is able to reduce carcinogenesis in mice, also deeply decreases the ornithine pool.[134]

We must be cautious in speculating on this matter because of in vivo recycling; however, we must also keep in mind that when administering arginine we have arginine → ornithine + urea, whereas when administering ornithine, we have ornithine + NH_3 → arginine.

In other words, it appears that arginine is a urea-generating molecule, whereas ornithine is a nitrogen-sparing molecule, as demonstrated in a recent study on burn rats receiving either ornithine α-ketoglutarate or arginine α-ketoglutarate.[135]

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