L-Arginine Signalling Potential in the Brain: The Peripheral Gets Central

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Abstract: L-arginine is a basic amino acid that has versatile metabolic roles, being involved in the generation of a wide range of biologically active intermediates such as nitric oxide (NO), polyamines, creatine and L-amino acids [1]. Because the levels of L-arginine reflect a metabolic crossroads, the mechanisms of its synthesis and degradation in peripheral tissues are very well described. However, there is an increasing amount of data also implicating this amino acid as a mediator of central nervous system activities, and those are not yet fully understood. Here we shall summarize the tissue–specific pathways controlling L-arginine intracellular and blood levels, and also the emerging evidence pointing to a role of this metabolite in the central regulation of diverse physiological processes, as blood pressure control and inflammatory response. As a conclusion we shall discuss the advantages of targeting L-arginine metabolism over other NO donors, as a general strategy to correct NO deficiency related diseases, and discuss a few new patents recently deposited which take into account the rational perspective outlined in the present review.

Keywords: L-arginine, NO, vasodilation, polyamines, ASS, ASL, arginase, urea cycle, ADMA, Proline-Rich Peptides, L-citrulline, CNS, ROS, nitrosative stress and patents.

INTRODUCTION

As a rule, L-arginine is a semi-essential amino acid, it can be directly obtained from the breakdown of dietary proteins, but it is also de novo synthesized from L-citrulline in the kidney. This process involves the enzymes arginino-succinate sintase (ASS, EC 6.3.4.5) and argininosuccinate lyase (ASL, EC 4.3.2.1) that convert L-citrulline into L-arginine and fumarate in the presence L-aspartate and adenosine triphosphate (ATP). As the kidneys lack some of the urea cycle enzymes, the L-arginine synthesized thereof cannot be processed further being liberated in the circulation.

The contribution of kidney L-arginine synthesis to the systemic levels of that amino acid was determined by the local injection of a radioactive precursor L-[ureido-14C] L-citrulline followed by the detection of labeled proteins not only in peripheral tissues i.e. liver, muscle and kidneys, but also in the brain [2]. It can be assumed that if the L-arginine produced in the kidneys can be found in brain proteins, these amino acids could also be available as substrates for metabolic enzymes or as ligands for membrane receptors in the central nervous system Fig. (1).

The liver expresses the enzymes responsible for L-arginine anabolism i.e. ASS and ASL, and catabolism i.e. arginase (EC 3.5.3.1). The connectivity of the regulatory network comprising these enzymes is such that there is no net production of L-arginine, but a cycle, known as the urea cycle. Its main role is the detoxification of the organism

from ammonia by generating L-ornithine and urea from L- arginine. The urea cycle can eliminate urea in the urine and use the L-ornithine to trigger a new detoxification round [3].

In the kidneys, L-arginine is metabolized by the enzymes involved in creatine synthesis as the arginine-glycine amidinotransferase (GAT), which produces guanidinoacetic acid to be methylated in the liver by guanidinoacetate N-methyltransferase (GAMT, E.C. 2.1.1.2), eventually giving raise to S-adenosylhomocysteine and creatine Fig. (1). The creatine, a key component of the energetic metabolism in the muscles and in the brain is secreted by the liver into the blood flow and absorbed by tissues and organs through specific transporters [5].

Nevertheless, the main source of creatine accumulation in vivo does not seem to be its de novo synthesis in the liver, but its direct ingestion by diet [6]. In this case, the presence of ingested creatine down regulates GAT activity in the kidneys [7], reducing guanidinoacetic acid liberation and ultimately raising the levels of L-arginine vailability to alternative metabolic pathways.

FREE AND PROTEIN-L-ARGININE AND NO PRODUCTION

L-arginine is the unique substrate of a class of enzymes called nitric oxide synthases (NOS) involved in nitric oxide (NO) synthesis. This gas is a ubiquitous signaling molecule with established roles in cardiovascular, immune and neuronal control. It seems to be directly involved in arterial tension control since it regulates the local and systemic resistance of vascular walls, as well as the sodium balance [8]. The NO produced by endothelial cells reaches the neighboring smooth muscle cells Fig. (2) where it activates two kinds of potassium channels and thereby induces the relaxation of the smooth muscle and brings about vascular dilation [9, 10].

In the brain, the locally produced NO can act as a neuronal modulator [11], being involved in the control of cardiovascular functions by reducing the sympathetic activity of the central nervous system [12]. NO production is also related to cognitive and nociceptive functions controlled by sympathetic plasticity. In the peripheral nervous system it regulates non-cholinergic and non-adrenergic smooth muscle relaxation Fig. (2), which have direct consequences in processes like erection, peristaltic movements and gastric dilation.

L-arginine in proteins can undergo post-translational modification by protein arginine methyltransferases (PRMT) that catalyzes the methylation of terminal nitrogen atoms of guanidinium side chains within L-arginine residues of proteins. The methylated L-arginine, such as the asymmetric dimethylarginine (ADMA), can be released from catabolism of the L-arginine-modified proteins. Synthesis of NO can be selectively inhibited by guanidino-substituted analogs of L-arginine, which act as competitive inhibitors at the active site of the enzyme. One such analog is ADMA, a compound that has been found in human plasma and urine. In contrast to ADMA, its isomer the symmetric dimethylarginine (SDMA) does not inhibit NOS. The methyl groups contained within the dimethylarginine molecules are derived from S-adeno-sylmethionine, an intermediate in the homocysteine/methio-nine pathway. There is experimental evidence that homocysteine may affect endothelium-dependent vascular

function by increasing the formation of ADMA. Both Fig. (1). L-arginine levels in the plasma reflect the integration of the catabolic (dashed lines) and anabolic (solid lines) activities of the displayed enzymes and receptors, some of which are organ-specific. ADMA and SDMA are eliminated from the body by renal excretion. In addition, the metabolism of ADMA, but not of SDMA, occurs via hydrolytic degradation to L-citrulline and dimethylamine by the enzyme dimethylarginine dime-thylaminohydrolase (DDAH) [13].

Recent prospective studies suggest that endothelial dysfunction correlates with an increased risk of future cardiovascular events. In line with these observations, a number of groups found evidence that ADMA is a novel cardiovascular risk factor [14, 15].

L-ARGININE AS A NEUROMODULATOR

Another potential metabolic destiny of L-arginine is its decarboxylation to agmatine, which can be an intermediate in polyamines biosynthesis [16] essential for many life forms, or which can be a direct ligand of □2-adrenergic receptors and imidazoline receptors in mammals [17]. Its ability to bind to □2-adrenergic receptors and imidazoline receptors, as well as its function on various isoforms of NOS has been implicated in many potential benefit effects. For example, agmatine exerts anticonvulsant effect. Pentylenetetrazole (PTZ) is believed to induce convulsant effect. Polyamines can be found in increased concentrations following acute or chronic PTZ administration [18]. Agmatine have been implicated increases the threshold of PTZ-induced seizure in a dose-dependent manner by modulation □2-adrenergic receptors and nitric oxide [19]. Other potential application of agmatine considering it as agonist □2-adrenergic receptors would be as a morphine potentiating agent, to utilize the rewarding effects of opioids in some clinical situations in significantly lower doses [20]. Agmatine is also involved in blocking the tolerance to opioid analgesia, as it potentiates the antinoceptive effect of morphine and inhibits morphine and ethanol-induced withdrawal syndromes [19].

Besides this indirect neuromodulatory action as an agmatine precursor, the L-arginine can also stimulate growth hormone secretion by the pituitary [21]. Exogenous administration of L-arginine results in a twofold rise in growth hormone levels, insulin and glucagon plasma concentrations [22] and increases in dopamine efflux in rat striatum [23]. Moreover it has been recently demonstrated that the L-arginine anomers can induce transient NO produc-tion via imidazoline and □2-adrenergic receptors, possibly acting as agonists of such receptors [24, 25]. However, this L-arginine action has also a NO-independent component, since the dopamine efflux in rat striatum was not prevented by NOS inhibition [23].

An important question that remains elusive is: how precisely the levels of neuroactive L-arginine are controlled by the organism. In the following section we attempt to formulate a hypothesis that could explain these controls, not by producing new data but by the analysis of previously existing results.

CONCENTRATION MATTERS

It seems that the metabolic route taken by either ingested or kidney synthesized arginine depends on concentration thresholds [26]. At micro molar concentrations, L-arginine was shown to trigger growth hormone secretion by the pituitary [21], inducing vasodilatation. The fact that this effect had been partially blocked by the co-injection of somastatin (a hormone secretion inhibitor) and restored by later administration of recombinant growth hormone suggests that the vascular action of L-arginine is to some extent an endocrinous process not necessarily mediated by NO production [27] Fig. (2). Calver and collaborators [28] have also shown that a local administration of either L-arginine or D-arginine (which is not a NOS substrate) has a dilatory action on the hand dorsal vein treated in their protocols [28]. Only at higher concentrations, in the mili molar range, it has been demonstrated that exogenous L-arginine can induce significant NO production in various cell types. 2-Adrenergic receptor antagonists inhibit this NO production. Various sub-types of these receptors are expressed in the central nervous system and the direct administration of specific inhibitors to the brain causes a dramatic increase in arterial pressure and a rise of norepinephrine in the plasma [29].

Apparently the L-arginine obtained in the diet is a poor inducer of NO production. Most likely due to limited absorption and to the action of arginases, which are factors reducing the availability of L-arginine as a NOS substrate, as a matter of fact altered arginase activities were shown to correlate with endothelial dysfunction [30-34]. Moreover, it is well established that intracellular concentrations of L-arginine are at mM ranges, a concentration far above the NOS Km, which would suffice to saturate the enzyme in a way that exogenous L-arginine should not induce further NO production. Nevertheless, the fact that in some cases the ministration of exogenous L-arginine does induce NO release in vivo, a phenomenon known as the L-arginine paradox, suggests that there might be separate intracellular pools of L-arginine directed to different pathways [35].

NO/L-ARGININE BUFFER

NO is a major trigger of various signal transduction pathways, it normally stimulates the enzyme guanylate cyclase inducing cyclic GMP (cGMP) release, and depending on the cell type and physiological context this second messenger can be a modulator of processes as diverse as synaptic plasticity, muscular relaxation and neurosecretion. Therefore, NO has neuromodulatory actions in the central and peripheral nervous systems, both mediated cGMP.

Nevertheless, if excessive levels of NO are produced this can be deleterious to the organism; cells in pro-oxidative state use NO to produce toxic metabolites known as RNOS (reactive nitrogen oxide species), which are reactive species. The process of NO and RNOS accumulation has been recently referred to as “nitrosative stress” [36] and it has been implicated in the etiology of degenerative diseases. For all these reasons, keeping NO production at a safe level, so that a deleterious threshold would not be reached, is of particular interest.

In this sense, keeping a pool of L-arginine directed to NO production by the regeneration of this amino acid can be a suitable strategy. The L-citrulline produced when the L-arginine is converted into NO by NOS can be recycled into L-arginine again by ASS and ASL activities [37]. The rate-limiting step in this cycle known as citrulline-

NO cycle, is [38]. Therefore, molecules that modulate ASS activity are good candidates to increase NO production within physiologically acceptable levels. As previously discussed, the overall concentration of L-arginine are subject to a whole set of metabolic controls with many folds feed-back loops that assure physiological cycles e.g. the urea cycle, the citrulline-NO cycle. For this reason, the NO/L-arginine ratio tends to be kept constant, allowing just transient increases in either species that are instantly corrected to physiological levels, what happens with the OH-/H+ ratio in a buffer solution.

POLYAMINES AS A STORAGE COMPARTMENT

As an alternative to NO production, L-arginine can be deviated to polyamines synthesis Fig. (3). Agmatine, putrescine, spermidine, spermine and phosphoarginine are some examples of L-arginine metabolites that belong to this class of molecules. They have an ubiquitary biodistribution through the whole organism, being restricted to specific regions in the central nervous system [39]. Interestingly, while the L-arginine and agmatine are able to efficiently cross the blood-brain barrier, the remaining polyamines only do to a very limited extent [40]. The polyamines have numerous biological roles, being involved in the control of cell proliferation acting as pro- and anti-apoptotic agents [41], in the control of oxidative status acting as reactive oxygen species (ROS) scavengers [42, 43], and in the control of nervous function acting as neurotransmitters accumulated in synaptic vessels and liberated under neuron depolarization [44]. Therefore, we suggest that even though the accumulation of L-arginine derived polyamines in CNS has clear signaling role, their accumulation elsewhere could act as a functional storage compartment for L-arginine. Because high amounts of L-arginine will only bring about toxic levels of NO production if this amino acid is available as a substrate for NOS.

CURRENT & FUTURE DEVELOPMENTS - NEW PATENTS IN THE LIGHT OF A RATIONAL TARGET IDENTIFICATION

The amino acid L-arginine can act as neuromodulator through direct binding to specific receptors, but can also exert signaling functions through a variety of its metabolites, i.e. the creatine in the liver, the agmatine in CNS and NO in peripheral tissues and in the brain. As we have discussed, the metabolic route taken by this amino acid is highly dependent on concentration thresholds, physical localization and the hard-wired architecture of physiological cycles.

Many patents have been deposited for molecules which are potential NO donors e.g. US2005/010935 - Methods for treating blood disorders with Nitric Oxide donor components [45]. Mostly, they are based on the fact that there are many pathological states related to NO deficiency. The mentioned patent protects the following compounds as potential NO donors: N-hydroxy-L-arginine, isosorbide dinitrate and/or isosorbide mononitrate. As we have previously discussed, a caveat of this kind of approach is the undesired production of RNOS and RNS with deleterious actions in the CNS and also in peripheral tissues. Here we have proposed an alternative approach for controlled NO induction, we believe that the rational identification of the cytosolic L-arginine as a major NO donor whose own levels are subjected to metabolic fine-tuning controls that assure that the amino acid is kept in a homeostatic range, can be an important contribution to the field.

An interesting patent, which is more inline with the reasoning we have exposed, has been deposited in 2001, US2001/002382 - L-arginine in combination with other compounds for treating cardiovascular diseases [46]. It protects L-arginine containing peptides and salts to be used in combination with sterol containing compounds as means to treat and to prevent heart disease e.g. atherosclerosis, restenosis, thrombosis and other conditions such as hypertension and poor circulation.

Our group has recently deposited a patent BR2007/ 000003 - Proline-Rich Peptides, Pharmaceutical Composition, use of one or more peptides and method of treatment [47] that protects peptide sequences originally isolated from Bothrops jararaca snake venom and induce NO production in a controlled fashion in various cell types. These are proline rich peptides composed of 5 to 14 amino acids residues that can induce ASS activity in vitro and in vivo [48], and can also induce rises in [Ca2+]i [49], a required step for neuronal and endothelial NOS activation [50, 51].

All the results contemplated by this patent BR2007/ 000003 are consistent with the use of these Proline-Rich Peptides (PROs) as prototype molecules for the development of new drugs aiming to treat a range of pathological states related to NO production miss function, e.g. lung hypertension, pre-eclampsia, essential hypertension, coagulo-pathies and citrullinemia.

The high selectivity of snake venom components for mammalian proteic targets has been optimized by thousands of years of prey/predator co-evolution and millions of years of natural selection. Assuming, as widely accepted, that the venoms main biological function has originally to do with prey immobilization and digestion, without being deleterious to the snake itself, other venom originated molecules have been and are being used as structural models for drug development [52].

In line with the ongoing trend of toxin-inspired drug development, we believe that PROs are good candidate molecules for the treatment of the various diseases relying on NO deficiency as cause or effect, since they do interfere with L-arginine metabolism, a source of NO synthesis which has its own levels subjected to very precise mechanisms of physiological control, being therefore the most likely target able to regulate NO production without generating undesired reactive by products.