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Dossier: Free amino acids in human health and pathologies

II. Glutamine and glutamate

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Abstract

Glutamine and glutamate with proline, histidine, arginine and ornithine, comprise 25% of the dietary amino acid intake and constitute the “glutamate family” of amino acids, which are disposed of through conversion to glutamate. Although glutamine has been classified as a nonessential amino acid, in major trauma, major surgery, sepsis, bone marrow transplantation, intense chemotherapy and radiotherapy, when its consumption exceeds its synthesis, it becomes a conditionally essential amino acid. In mammals the physiological levels of glutamine is 650 μmol/l and it is one of the most important substrate for ammoniagenesis in the gut and in the kidney due to its important role in the regulation of acid–base homeostasis. In cells, glutamine is a key link between carbon metabolism of carbohydrates and proteins and plays an important role in the growth of fibroblasts, lymphocytes and enterocytes. It improves nitrogen balance and preserves the concentration of glutamine in skeletal muscle. Deamidation of glutamine via glutaminase produces glutamate a precursor of gamma-amino butyric acid, a neurotransmission inhibitor. L-Glutamic acid is a ubiquitous amino acid present in many foods either in free form or in peptides and proteins. Animal protein may contain from 11 to 22% and plants protein as much as 40% glutamate by weight. The sodium salt of glutamic acid is added to several foods to enhance flavor. L-Glutamate is the most abundant free amino acid in brain and it is the major excitatory neurotransmitter of the vertebrate central nervous system. Most free L-glutamic acid in brain is derived from local synthesis from L-glutamine and Kreb’s cycle intermediates. It clearly plays an important role in neuronal differentiation, migration and survival in the developing brain via facilitated Ca^{++} transport. Glutamate also plays a critical role in synaptic maintenance and plasticity. It contributes to learning and memory through use-dependent changes in synaptic efficacy and plays a role in the formation and function of the cytoskeleton. Glutamine via glutamate is converted to α-ketoglutarate, an integral component of the citric acid cycle. It is a component of the antioxidant glutathione and of the polyglutamated folic acid. The cyclization of glutamate produces proline, an amino acid important for synthesis of collagen and connective tissue. Our aim here is to review on some amino acids with high functional priority such as glutamine and to define their effective activity in human health and pathologies.

Keywords: Glutamine; Glutamate

1. Introduction

Glutamine is the most abundant amino acid in the plasma [1] and its importance for optimal growth of cells in culture has been known since the 1950s [2]. It exhibits extremely rapid cellular turnover rates and serves as an essential metabolic precursor in nucleotide, glucose and amino sugar biosynthesis, glutathione homeostasis, protein synthesis, and a source of oxidative energy (Fig. 1). As compared to asparagine, glutamine is more lipophilic and less dipolar. Glutamine and glutamate with proline, histidine, arginine and ornithine, comprise 25% of the dietary amino acid intake and constitute the “glutamate family” of amino acids, which are disposed of through conversion to glutamate (Fig. 2). In mammalian cells, glutamine is a key link between carbon metabolism of carbohydrates and proteins and plays an important role in the growth of fibroblasts, lymphocytes and enterocytes [3,4]. It improves nitrogen balance and preserves the concentration of glutamine in skeletal muscle [5]. When the plasma glutamine is insufficient to satisfy the demand, glutamine synthesis occurs from skeletal muscle

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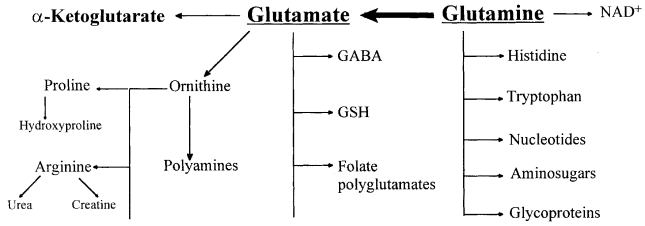
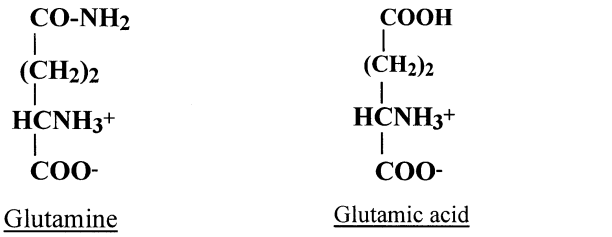


Fig. 1. Metabolic products derived from glutamine.

and liver. Decreased glutamine availability for macrophages and lymphocytes correlated with low plasma glutamine and citrulline levels [6]. Reduced arginine levels observed after trauma, can be restored to physiological levels using glutamine supplementation whereas, the physiological levels of glutamine (650 μmol/l) are only partly restored [7]. Glutamine is one of the most important substrate for ammoniagenesis in the gut and the kidney due to its important role in the regulation of acid–base homeostasis [8]. It decomposes readily to yield ammonia and glutamate or via intramolecular catalysis to pyroglutamate. The transamination and deamidation of glutamine is involved in ammonia transfer between various tissues. Deamidation of glutamine via glutaminase produces glutamate a precursor



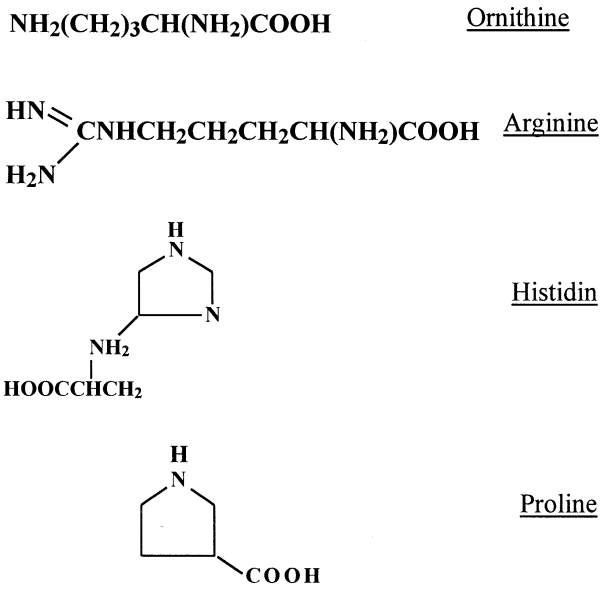


Fig. 2. Glutamate family of amino acids.

of gamma-amino butyric acid, a neurotransmission inhibitor. The transfer of the amide nitrogen from glutamine via the amido transferase reaction is involved in the biosynthesis of purines and pyrrimidines and in the production of hexosamines (Fig. 3). Glutamine via glutamate is converted to α-ketoglutarate, an integral component of the citric acid cycle (Fig. 4). It is a component of the antioxidant glutathione and of the polyglutamated folic acid. The cyclization of glutamate produces proline, an amino acid important for synthesis of collagen and connective tissue. However, too much glutamine in a protein is of pathological importance and a number of neurodegenerative diseases have been found to be due to a CAG expansion that causes expansion of glutamine repeats in affected proteins (CAA and CAG codons are responsible for the insertion of glutamine from its transfer RNA with its anti-codon triplet into the genetically determined position of the coded polypeptide chain). This leads to abnormal protein folding [9] and neuronal diseases [10]. Proteins containing repeats > 41 glutamine residues form toxic neuronal nuclear aggregates in affected cells [11,12]. Although glutamine has been classified as a nonessential amino acid, in major trauma [13], major surgery [14,15], sepsis [16], bone marrow transplantation [17-19] intense chemotherapy and radiotherapy [20-22], when its consumption exceeds its synthesis, it becomes a conditionally essential amino acid [23].

2. Glutamine transport systems

In general uptake of cytoplasmic amino acids at levels above their transmembrane equilibrium utilizes the potential energy present in the transmembrane Na^+ electrochemical gradient. Thus, amino acid transporters fall into two categories: Na^+-dependent and Na^+-independent [24].

2.1. Na^+-dependent transporters

2.1.1. System ASC

Includes Systems ASC (substrate for alanine, serine and cysteine) [25] and B° (described in mouse blastocytes) that share identical zwitterionic substrate selectivities (°) in a Na^+-dependent manner. Described in intestinal and kidney epithelia [26], ASC and B° can be distinguished by their threonine selectivity. The 553 amino acid mammalian glutamine transporter gene (*ASCT2*) [27] was isolated from a mouse testis cDNA. In rat, the *ASCT2* gene was isolated from an astroglia-enriched brain cDNA library [28,29]. The human *hATB°* gene (human amino acid transporter B°) was isolated from human choriocarcinoma and colon carcinoma cell lines and was found to possesses properties nearly identical to ASCT2 [30].

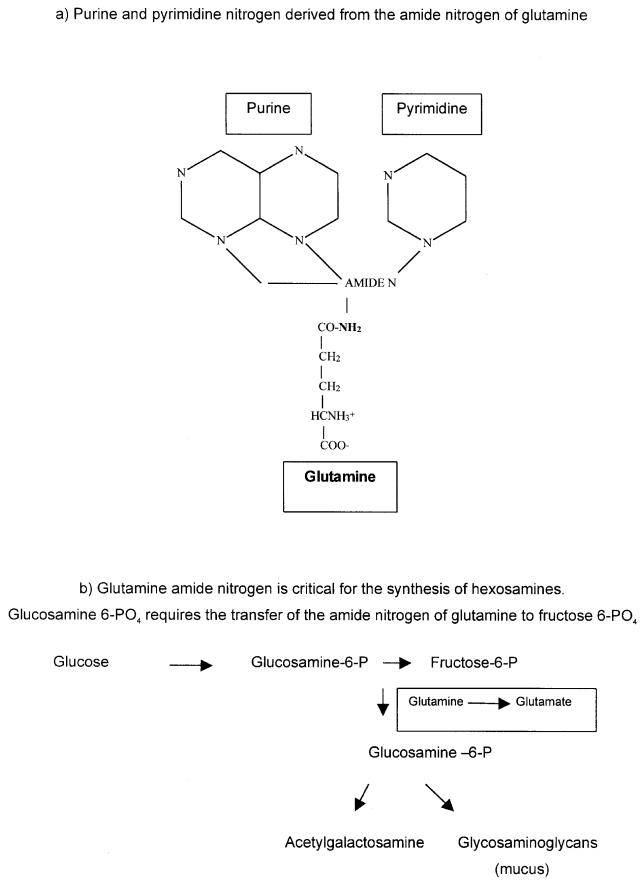


Fig. 3. Interplay between glutamine, nucleosides and hexosamines. (a) Purine and pyrimidine nitrogen derived from the amide nitrogen of glutamine. (b) Glutamine amide nitrogen is critical for the synthesis of hexosamines. Glucosamine 6-PO_4 requires the transfer of the amide nitrogen of glutamine to fructose 6-PO_4.

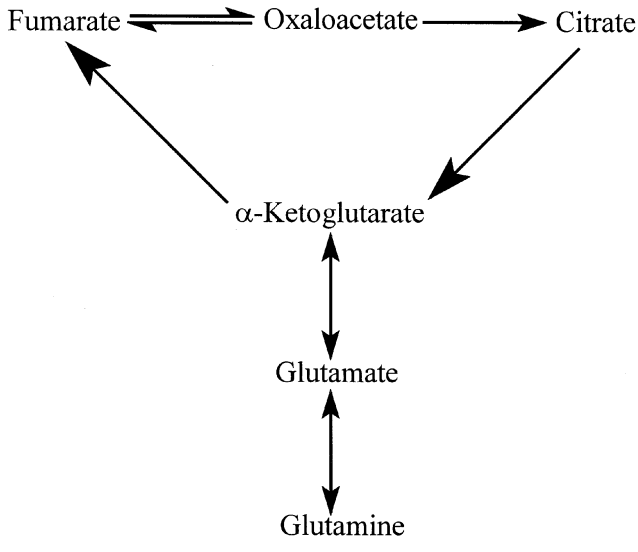


Fig. 4. Interconversion of α-ketoglutarate and glutaate.

2.1.2. System N (SN1)

System N (SN1) has been found in rat hepatocytes [31], skeletal muscle [32], neurons [33] and in human [34]. SN1 has a narrow substrate specificity (only amino acids containing nitrogen in their side chains such as glutamine, histidine and asparagine). The *hSN1* gene located on chromosome 3 contains 16 exons and 15 introns and has been isolated from rat brain (*SN1*), kidney (*mNAT*) and from a human hepatoblastoma cell line (HepG2) (*hSN1*) cDNA libraries [35-37].

2.1.3. System $B^{o,+}$

A specific role for this transport system has been reported for glutamine uptake in mammalian cells [38]. $B^{o,+}$ transports both zwitterionic and cationic amino acids in a Na^+-dependent manner [39]. The cDNA was initially isolated from human mammary gland (*hATB*$^{o,+}$) and found to code for a protein of 642 amino acids [40].

2.1.4. System A

In contrast to other zwitterionic amino acid transporters, system A tolerates *N*-methylated amino acid substrates [41]. It seems to play more of a role in human than in rodent liver [42] and a new subtype of amino acid transport system A has been identified [43].

2.1.5. System y+L

This transport activity was described in the plasma membrane of human erythrocytes. It mediates the uptake of cationic amino acids with high affinity in a Na^+-independent manner, but require Na^+ to transport zwitterionic amino acids such as glutamine [44].

2.2. Na^+-independent transporters

2.2.1. System L

The contribution of system L (for leucine preferred transport activity) to mediate glutamine transport is minimal as compared to total uptake [45-47].

2.2.2. System $b^{o,+}$

System $b^{o,+}$ is a relatively weak glutamine transport mediator and in contrast to $B^{o,+}$, system $b^{+,+}$ mediates the transport of both cationic and zwitterionic amino acids [48].

3. Glutamine metabolism

In a cellular milieu, glutamine is transferred from extracellular medium to the cytoplasm and may be transported to mitochondria via a transport system specific for L-glutamine and asparagine. This system is inhibited by

thiol reagents and by L-glutamate-γ-hydroxamate a glutamine analog [49-51].

In healthy animals, the small intestine is the principal site of dietary glutamine uptake, whereas skeletal muscle and lung can be major export sites. In sepsis, the liver becomes the major organ of glutamine uptake. Following endotoxin administration [52] or starvation [53], uptake can increase by up to10-fold. In septic states, increased glutamine in the liver is mediated by TNF-alpha, glucocorticoids and prostaglandins [54]. In skeletal muscle, glutamine synthetase (GS) expression and activity increased greatly whereas no change in glutaminase activity was observed during sepsis. Despite an increase in GS expression and activity the rate of release exceeds that of synthesis resulting in intracellular glutamine pool depletion [55]. If skeletal muscle is the major glutamine exporter, the high flow of blood through the pulmonary circulation and the presence of GS make the lungs a site for glutamine metabolism [56]. Here also the GS gene expression is induced during sepsis [57]. Lymphocytes and macrophages are also great glutamine consumers while in an inflammatory state; glutamine utilization is 10-fold greater in proliferating lymphocytes than in resting cells [58]. In the bowel, decreased glutamine utilization during sepsis occurs in the mucosal cells rather than in lymphatic tissue [59] and reduced intestinal uptake of circulating glutamine has been observed during Interleukin-1 (IL-1) treatment [60]. The maintenance of blood acid–base balance is essential for survival. Thus increased renal ammoniagenesis and gluconeogenesis from plasma glutamine constitute an adaptative response that restores in part the acid–base balance during metabolic acidosis [61]. Renal catabolism of glutamine is acutely activated in response to the onset of metabolic acidosis and is primarily due to increased glutamine plasma and cellular decrease of glutamate and alpha-ketoglutarate (α-KG). During normal acid–base balance, approximately two thirds of the ammonium ions produced from glutamine are trapped in the tubular lumen and excreted in acidified urine. Acute activation of the Na^+/H^+ exchanger, acidifies the fluid in the tubular lumen and facilitates the trapping and excretion of ammonium ions. During chronic acidosis increased renal catabolism is sustained to increase expression of glutaminase and glutamate dehydrogenase enzymes and to increase the transporters of mitochondrial glutamine. Changes in renal glutamine metabolism after endotoxin administration suggest that during sepsis, early renal failure and altered glutamine metabolism may impair the kidney's ability to maintain acid/base homeostasis [62]. In human it has been shown that insulin, glucagons and epinephrine affect glutamine metabolism [63].

The gluconeogenesis from glutamine occurs principally in the kidney, whereas alanine conversion is essentially limited to the liver [64]. Renal gluconeogenesis contributes to 20–25% of whole body glucose production [65]. Overall glutamine gluconeogenesis is responsible for about 5% of systemic glucose appearance, and renal production of glucose from glutamine accounts for nearly 75% of all glucose derived from glutamine. Thus, it seems that glutamine is a significant contributor to whole body glucose homeostasis, while glutamate is not.

4. Glutamine and glutamate metabolic response to injury

Surgery, accidental or thermal injury can be characterized by a loss of nitrogen from the body, breakdown of skeletal muscle protein and translocation of the amino acids to visceral organs and the wound site [66,67]. In addition there is increased excretion of creatinine and 3'-methyl-histidine, both substances found mainly in muscle tissue. From the amino acids released from skeletal muscle, alanine and glutamine represent 50–70% of the amino acid nitrogen exported to visceral tissues [68]. Both GLN and ALA support the enhanced gluconeogenesis that can occur in injured patients. The negative nitrogen balance observed after injury is primarily consequence of increased excretion of urea in the urine. However, protein catabolism is a generalized response to trauma and does not reflect the simple loss of protein from injured tissue. In particular the hormonal and inflammatory environment is a major regulator of this catabolic response [69]. Cortisol has a pronounced effect in upregulating GLN synthesis in skeletal muscle [70] and glucagons appear essential in enhancing hepatic uptake of GLN and facilitating ureagenesis [71]. Inflammatory factors such as proinflammatory cytokines, leukotrienes and other factors such as catecholamines contribute to the catabolic response. Nevertheless GLN taken up by the kidney contributes to the ammonia and ammonium ions, which are excreted in the urine. This pathway is a major contributor in neutralizing the large acid load that is generated after injury. After thermal or elective injury GLN concentrations can fall to a low level in lymphocytes. It was suggested that the low level of GLN in lymphocytes may have contributed to impaired immunological function occurring after these injuries. GLN supplementation has been reported to improve immunological function in immunosuppressed patients after elective surgery [72] and burn injury [73]. In patients undergoing elective surgery, GLN supplementation can attenuate the negative post-operative nitrogen balance, diminishing the fall in intracellular concentrations of GLN in the skeletal muscle free amino acid pool and supporting muscle protein synthesis. In addition GLN can enhance immunological responses alterations and reduce bowel permeability.

5. Glutamine and cancer

In malignant cells, transport of glutamine across the plasma membrane occurs at a faster rate than in non-malignant counterparts [74]. Moreover, mitochondrial glutaminase was reported to be more active in tumor than in normal cells [75]. Several glutaminase isoforms from a human colon adenocarcinoma HT-29 cDNA library have been cloned. Among these isoforms, two show high homology with rat kidney-type glutaminase and a third isoform [76] is expressed only in cardiac and skeletal muscle [77]. Glutaminase isoforms from human breast cancer cell line ZR75-1 seems to be liver-type glutaminase with high expression levels in human liver and lower expression in pancreas and brain [75]. Glutaminase expression could be inhibited by antisense mRNA and the transfected cells lost their tumorigenic capacity in vivo [78]. Glutamine-supplemented nutrition has proved to be a valuable treatment regimen in cancer patients undergoing bone marrow transplantation protocols with or without high dose chemotherapy or irradiation [79-82]. These treatments for hematologic or solid tumors commonly cause gastrointestinal complications, including nausea, vomiting, inflammation of the oral and esophageal mucosa (mucositis), abdominal pain, and diarrhea [83]. After high dose chemotherapy for bone marrow transplantation, patients may develop a potentially lethal veno-occlusive disease, which is due to subendothelial swelling and narrowing the central hepatic veins. The veno-occlusive disease seems to be related at least in part to oxygen free radical-mediated liver injury and depletion of glutathione and other antioxidants [84]. Administration of intravenous and oral glutamine in combination with oral vitamin E diminishes symptoms of veno-occlusive disease after bone marrow transplantation [85,86]. Glutamine supplementation could be considered as metabolic support in patients undergoing treatment for cancer. It attenuates glutathione depletion in plasma, liver and gut after chemotherapy and upregulates systemic and tissue immune function during catabolic stress [87,88].

6. From glutamine to glutamate

L-Glutamic acid is an ubiquitous amino acid present in many foods either in free form or in peptides and proteins. Animal protein may contain from 11 to 22% by weight of glutamic acid and plants protein as much as 40% glutamate by weight. It is present in free form at high concentrations in some foods such as tomatoes and a variety of fruits [89]. The sodium salt of glutamic acid is added to several foods to enhance flavor. A large number of pathways are involved in the metabolism of glutamic acid [90,91]. It is transformed to alanine in intestinal mucosal cells and to glucose and lactate in the liver [92]. L-Glutamate is the most abundant free amino acid in brain and it is the major excitatory neurotransmitter of the vertebrate central nervous system. Most free L-glutamic acid in brain is derived from local synthesis from L-glutamine and Kreb's cycle intermediates. It clearly plays an important role in neuronal differentiation, migration and survival in the developing brain via facilitated Ca^{++} transport [93]. Active in about one third of CNS synapses [94], glutamate plays a critical role in synaptic maintenance and plasticity [95] and has been reported to mediate and inhibit post-synaptic potential in dopamine containing neurons [96,97]. It contributes to learning and memory through use-dependent changes in synaptic efficacy and plays a role in the formation and function of the cytoskeleton. The post-translational modification of tubulin by polyglutamylation regulates its interaction with microtubule-associated proteins [98,99]. In addition foly-polyglutamates (intracellular substrates and regulators of one carbon metabolism) synthesis is required for normal folate retention by cells [100].

7. Glutamate receptors and transporters

Neuronal glutamate is released from vesicles by many stimuli. It is a Ca^{++}-dependent mechanism that can be obtained during cerebral ischemia when the Na^{+} and K^{+} gradient across the membrane is reduced [101,102]. Once released glutamate acts at multiple subtypes of post-synaptic and presynaptic receptors [103]. The synaptic release of glutamate is controlled by presynaptic receptors which include, metabotropic (*mGluR*), cholinergic (nicotinic and muscarinic), adenosine (A1), kappa opioid, gamma-aminobutyric acid (GABA)B, cholecystokinin and neuropeptide Y (Y2) receptors [104].

There are two major groups of glutamate receptors, ionotropic and metabotropic. The ionotropic receptors are tetrameric or pentameric and include the *N*-methyl-D-aspartate (NMDA) and the non NMDA receptors. The NMDA receptors function as a gate keeper for sodium and calcium [105,106]. The presence of NR1 appears invariant whereas the selection of NR2 subunits (A, B, C or D) determines the time constants to open the channels and modifies the effect of various antagonists. *S*-Sulfo-L-cysteine and *trans*-1-aminocyclobutane-1,3-dicarboxylate are agonists of these receptors with glycine needed as coagonist. Each receptor unit appears to have two glycine and two glutamate binding sites [105].

The non NMDA receptors include α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate receptors. They mediate fast excitatory synaptic transmission and are associated with voltage-independent channels that gate a depolarizing current primarily carried by an influx of

Na^+ ions [106]. AMPA agonists include ATPA (α-amino-3-hydroxy-5-*tert*-butyl-4-isoxazole propionate) and aniracetam [107]. Acromelic acid and domoic acid are potent agonists for kainate receptors.

Beyond their properties of opening ion channels, ionotropic receptors have functional properties. These are provided by the capacity of the intracellular carboxy terminal to interact with a variety of intracellular proteins, which includes those involved in signal transduction. Moreover, AMPA receptors activate a tyrosine kinase, Lyn, that in turn activates the mitogen-activated protein kinase pathways [108].

The metabotropic receptors (*mGluR*) are coupled to intracellular second messengers via G proteins [109]. They have little sequence homology with other metabotropic receptors. By molecular cloning, eight subtypes of metabotropic glutamate receptors (mGluR1–mGluR8) have been identified [110]. According to the transduction mechanism they mediate, they are distributed into three groups.

Group I receptors, consist of mGluR1 and mGluR5. This group stimulates phospholipase C, (producing diacylglycerol, which activates protein kinase C, PKC) and inositol-1,4,5-triphosphate (producing phosphoinositide hydrolysis which elicits the release of Ca^{++} from intracellular stores) as second messengers. The most potent agonist for this group is quisqualate.

Group II receptors include mGluR2 and mGluR3. This group inhibits forskolin or G_s-coupled receptors which stimulate cAMP formation [111]. The agonist for this group is the 2,3-dicarboxycyclopropyl-glycol.

Group III contains mGluR4, mGluR6, mGluR7 and mGluR8. It has the same action as group II receptors with a lower inhibition in cAMP formation and with L-amino-4-pyrophosphobutyrate (L-AP4) and L-serine-*O*-phosphate (O-SOP) as agonists [110]. Group II and III are negatively coupled to adenyl cyclase.

Glutamate transporters are distributed among cell types and brain regions. However, excess glutamate released into synapses is taken up by both neuronal and glial cells and transported by Na^+-dependent glycoproteins which have a high affinity for glutamic acid. In glia, GLAST (glutamate and aspartate transporter), are predominant in the cerebellum and hippocampus [112,113] and GLT (glutamate transporter) is found in astrocytes throughout the brain [114] but predominate in rat hippocampus [113]. In neurons EAAC1 (excitatory amino acid transporters) are located primarily in neurons, EAAT4 mainly expressed in cerebellum [115-117]. The neuronal transporters seem to be linked to a Cl^- channel which opens when glutamate binds and it diminishes the synaptic activity by hyperpolarizing the post-synaptic membrane [118]. All these transporters are Na^+-dependent and it was suggested that one molecule of glutamate is coupled to the co-transport of three Na^+ and one H^+ and the counter-transport of one K^+ [101].

Plasma membrane glutamate transporters transport D- as well as L-aspartate whereas the vesicular glutamate transporter appears to be selective for L-glutamate. Although glutamate transport inhibitors such as L-trans-pyrrolidine-2,4-dicarboxylic acid have been isolated [119,120], it is not yet clear whether high levels of extracellular glutamic acid (responsible for the hyperexcitation and/or death of neurons) are related to the absence or inhibition of these transporters [121]. Despite these specific active compounds, glutamate transport activity may be modulated in a non-classical fashion. It has been shown that arachidonic acid blocks glutamic acid uptake [122], serotonin modulates its binding to receptors [123] and interleukin-1β and neuropeptide Y increase neuronal glutamic acid release [124,125].

8. Glutamate and taste sensation

Psychometric studies revealed that glutamate not only enhances the perception of sweetness and saltiness and diminishes sourness and bitterness [126,127], but also has its own taste named umami (“savory taste”) which is not shared by glutamine [128,129]. Glutamate with certain 5'-ribonucleotides (inosine-5'-monophosphate or guanosine-5'-monophosphate) are taste active chemicals present in unprocessed or processed plant or animal products used in human foods [130-133]. The transmitter of the taste receptor cell is unknown as are the exact mechanisms by which interaction of a chemical with its receptor on a taste cell leads to altered firing of the primary gustatory afferents [134]. However, two molecular mechanisms appear to be involved in umami taste transduction. One is based on an NMDA-type glutamic acid ion channel receptor. The activation by glutamate of NMDA receptors mediates primary sensory transduction [136]. The other is based on the hyperpolarization caused by mGluR4 metabotropic receptor activation that modulates the strength of transduction through the monosodium glutamate (MSG) receptor or through connections with neurons also sensitive to olfactory stimuli [135-138]. Taste preference may also be located in neurons of the lateral hypothalamic area [136]. In contrast to the selective localization of mGluR4 to taste cells, the NMDA receptor units are expressed by several cell types in the tongue. In mice lacking the mGluR4 gene, the sweet and salty taste preference was unaltered [137].

9. Involvement of glutamate in neurotoxicity

Brain lesions and neuron degeneration caused by subcutaneous injections of glutamate in mice led to the concept of

glutamate as an “excitotoxic” agent and have raised the question about the possible harm induced by this amino acid as a food additive [139]. Except in rhesus monkeys, brain lesions occur in most animal species [140]. Glutamate can be neurotoxic through its NMDA agonist effect (quinolinic acid or ibotenic acid), AMPA-kainate agonists (kainic, quisqualic and domoic acids) or group I metabotropic receptors. The primary mechanism of neurotoxicity linked to glutamate release is ionic disequilibrium related to excessive entry of Na^+ and Ca^{++} through ligand-gated and voltage sensitive channels [141-143]. Neuronal death results from necrosis [144] or apoptosis with post-translational activation of caspase 3 (cysteine proteases) [145]. The neurotoxic effects, which result from overstimulating glutamate receptors, have been shown by rendering insensitive non-neuronal cells sensitive to glutamate after transfection with a gene containing the NMDA [146]. In human, neurotoxicity linked to altered glutamate receptors has been related to domoic acid intoxication (domoic acid is synthesized by marine diatoms (*Nitschia pungens*) and concentrated in blue mussels (*Mytilus edulis*) [147]. Acute symptoms (within 1–4 h) can be prevented by the administration of an NMDA receptor antagonist [148]. In cerebral ischemia, traumatic brain injury or perinatal asphyxia, acute neurotoxicity and cell death is attributed to glutamate acting on AMPA, NMDA or on mGluR1 receptors. NMDA and AMPA receptor antagonists have been shown to be protective in cerebral ischemia and traumatic brain injury in animal models [149]. Chronic neurodegeneration which includes motor neuron disease (MND) or amyotrophic lateral sclerosis (ALS), Huntington’s, disease, Parkinson’s disease and Alzheimer’s disease depend on endogenous glutamate activating NMDA or AMPA receptors [141-142]. The role of AMPA receptors on ALS was observed following reduction in the expression of GLT-1 in the spinal cord and brain regions and loss of motor neurons [150-152] which can be prevented by AMPA receptor antagonists such as GYKI 52466 [153]. Since defective glutamate transporters and enhanced AMPA receptor activation are involved in ALS, riluzole and antiglutamate drugs have been proposed in its treatment [154]. AMPA receptor antagonists were also reported to protect against the toxic effects of mutations in Cu/Zn superoxide dismutase in cultured neurons [155]. Various pathologies including epilepsy, amnesia, hyperalgesia and schizophrenia were reported to be associated with alterations of glutamate receptors. Ion channel defects (voltage-sensitive calcium, potassium or sodium channels but also sodium/hydrogen exchangers and nicotinic cholinergic receptors) have been identified in animals and humans and have been linked to epileptic syndromes [156-157]. When administered focally or systemically to experimental animals, glutamate can cause convulsion via ligand-gated ion channels (NMDA or non-NMDA receptors) to increase sodium and calcium conductance. Selective agonists including NMDA, AMPA, kainate, ibotenic acid, domoic acid, or other excitatory endogenous compounds such as quinolinate or some sulfur-containing amino acids can also cause convulsions. In addition, activation of group I mGluR enhances neuronal excitability by potentiation of the effects of NMDA and AMPA and depolarization. Thus, the 3,5-dihydroxyphenylglycine, agonist acting on group I receptors can cause convulsions. In general when agonists acting on group I receptors (mGluR1 or mGluR5) are injected into the brain, they produce epileptic activity and focal neurodegeneration probably related to membrane depolarization due to potassium conductance reduction. In contrast, antagonists selective for mGluR1 (AIDA and LY 36785) and for mGluR5 (MPEP and SIB 1893) have anticonvulsant activity [158-162]. Interactions between the activation of glutamatergic receptors and other transmitter systems, ion transport, gene activation and receptor modification place glutamate-mediated transmission glutamate synapses in pivotal positions and as potential targets for drug intervention in some neurological and psychiatric disorders.

All classes of NMDA receptor antagonists (felbamate, remacemide), channel site antagonists, glycine site antagonists, polyamine site antagonists as well as AMPA/kainate (topiramate) antagonists display wide spectrum anticonvulsant properties in both acute and chronic animal epilepsy models [163-165].

Various mutations of glutamatergic receptor subunits by genetic manipulation in transgenic mice have been reported. Transgenic mice with an editing deficient AMPA receptor subunit, GluR2 display early onset epilepsy. Transgenic mice with GLT-1 knockout, display spontaneous epileptic activity [166], and mice treated chronically with antisense probes to EAAC-1 or with antisense probes to GLT-1 or GLAST show reduced transporter levels and increased epileptic activity [167].

In conclusion, glutamate excitotoxicity is probably the final common pathway in a number of nervous system diseases. To assure proper synaptic function and to prevent excitotoxic injury of neurons, glutamate concentrations are normally maintained at low micromolar levels via the activity of Na^+ glutamate transporters expressed by neurons and astrocytes. In the mammalian nervous system, astrocytic glutamate uses the transmembrane electrochemical gradients for Na^+, K^+, and H^+ to maintain the glutamate homeostasis. Disruption of these gradients or membrane depolarization can lead to glutamate release from astrocytes [168-172]. Unlike neurons, glial cells retain the ability to proliferate and uncontrolled cancerous proliferation of glial cells results in gliomas, a primary brain tumor. Glioma cells are impaired in their ability to remove glutamate from the extracellular space. In addition, they release glutamate at concentrations that can induce neurotoxicity [172]. Glioma

cell released glutamate can be sufficient to activate NMDA and AMPA/kainate receptors on hippocampal neurons, thereby inducing delayed Ca^{++}-dependent cell death.

In amnesia, AMPAkines (compounds that potentiate glutamate's action at AMPA receptors) can reduce amnesia symptoms. Moreover, D-cycloserine a partial agonist at the glycine site of the NMDA receptor has been proposed as a therapy in Alzheimer's disease. Hyperalgesia clearly involves NMDA receptors in the spinal cord. In psychosis, it has been suggested that impaired function or inactivation of some NMDA receptors may be a contributory factor in schizophrenia [173]. Thus, potentiation of NMDA receptor function may be a valid therapeutic approach and clinical trials of glycine and D-cycloserine have been undertaken [174]. Standard antipsychotic drugs such as haloperidol and clozapine may be effective partially through NMDA receptor potentiation [175].

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