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REVIEW ARTICLE

UNDERSTANDING SAFETY OF GLUTAMATE IN FOOD AND BRAIN

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Abstract : Glutamate is ubiquitous in nature and is present in all living organisms. It is the principal excitatory neurotransmitter in central nervous system. Glutamate is being used as food additive for enhancing flavour for over last 1200 years imparting a unique taste known as “umami” in Japanese. It is being marketed for about last 100 years. The taste of umami is now recognized as the fifth basic taste. Many of the foods used in cooking for enhancing flavour contain high amount of glutamate. Breast milk has the highest concentration of glutamate amongst all amino acids. Glutamate in high doses as gavage or parenteral injection have been reported to produce neurodegeneration in infant rodents. The neurodegeneration was not produced when gluamate was given with food. The Joint FAO/WHO Expert Committee on Food Additives, based on enumerable scientific evidence, has declared that, “glutamate as an additive in food” is not an health hazard to human being.

Glutamate is used as signaling molecule not only in neuronal but also in non-neuronal tissues. Excessive accumulation of glutamate in the synaptic cleft has been associated with excitotoxicty and glutamate is implicated in number of neurological disorders. Excessive accumulation could be attributed to increase release, failure of transport system for uptake mechanism, neuronal injury due to hypoxia-ischemia, trauma and associated metabolic failures. The role blood brain barrier, vesicular glutamate and sodium dependent excitatory amino acid transporters in glutamate homeostasis are emphasized in the review.

Key words : glutamate, monosodium glutamate, excitotoxicity, umami, neurotoxicity

INTRODUCTION

Glutamate continues to excite us. One will hardly find its name as a classical neurotransmitter in a textbook of Physiology 30 years before. It is merely one decade that we recognize the taste of glutamate as one of the basic tastes termed as “umami”. The recognition of glutamate as an excitatory neurotransmitter in mammalian nervous system as well as umami as the fifth basic taste has an interesting historical background. Historically, glutamate was first isolated as glutamic acid from acid

hydrolysate of wheat gluten, by the German scientist Ritthausen in 1866 and thus named it as “glutamic acid”.

The history of glutamate in food is older than the history of science of nutrition. Practice of adding large seaweed (*Laminaria japonica*) to soup stocks has been in use in Japan for last 12 centuries. This seaweed markedly increases the taste of the soup. But what was unknown that it contained high amount of glutamate. It was not until 1908 that the link between the seaweed and glutamate was discovered. The brown crystals left behind after evaporation of a large amount of kombu broth, was scientifically identified as glutamate by Prof Ikeda of Tokyo University (1). He termed this unique flavour as “umami” (2).

Glutamate was first proposed as a synaptic transmitter by Hayashi in 1954 based on its convulsive properties (3). In 1960 Curtis and colleagues showed that L-glutamate depolarized and excited central neurons (4). Glutamate was not accepted as a neurotransmitter for quite sometime because of the technical limitations of experiments at that time. “Glutamate as a CNS neurotransmitter” was accepted in 1979 despite lack of evidence about the locations of the specific synapses, where glutamate is released (5). Around 1970s two reports appeared on the role of glutamate on excitotoxicity that gave new directions to glutamate research (6, 7).

There is no doubt today that glutamate is the principal excitatory neurotransmitter in the central nervous system (CNS). It is now recognized as the fifth basic taste along with its receptors. (8–14). Paradoxically glutamate has excitotoxic properties, which has been associated with the pathogenesis of number neurological disorders (15, 16). It is also widely used as a food additive through out the world. Keeping this in mind the present review focuses on the safety of glutamate in food and brain. The first part of the review deals with: understanding safety of glutamate in food and the second part deals with understanding safety of glutamate in the brain.

UNDERSTANDING SAFTEY OF GLUTAMTE IN FOOD

Understanding the safety of glutamate in food needs knowledge of its natural occurrence, oral intake, absorption and metabolism, taste perception and safety evaluation as a food additive.

Glutamate in natural food

Glutamate is one of the most abundant amino acids in nature. Since glutamate is a building block of protein and free glutamate exists in organs and tissues, it is found naturally in virtually all foods such as milk, vegetables, seafood, poultry, meats, traditional seasonings like fish sauce and soy sauce, and many other foods (17, 18). While protein bound glutamate dose not have any taste, free glutamate plays an important role in food as a tastant. Glutamate was isolated as the taste essence of traditional Japanese soup stock prepared from dried kelp of *Laminaria japonica* (1). Subsequently in 1909, monosodium glutamate (MSG), sodium salt of glutamate, was first marketed in Japan as a seasoning agent. In fact, glutamate has long been used around the world to enhance

the palatability of foods before the discovery of its taste. Foods rich in free glutamate, such as tomatoes, cheese and mushrooms have been used in cooking for their flavour favoring qualities. Glutamate also has been a component of traditional seasonings such as fish and soy sauces. More than 1200 years ago, in ancient Rome, fish sauce called “Garum” was used. Fish and soy sauces have been used in South Eastern Asian countries, China and Japan for more than several centuries.

Mother’s milk the first food for babies, and is the only food when they are just born. It has to give them the entire nutrient they need. It was reported that glutamate is the most abundant amino acid in mother’s milk in all the species analyzed (19). The total glutamate content (free and protein bound) in human milk is 161.5 mg/dl to 230.0 mg/dl (20, 21). However, human breast milk contains rather high amount of free glutamate; ten times as high as cow’s milk (21). Interestingly, this high level of free glutamate is found only in the milk of humans and higher primates such as chimpanzees, and the milk of other species has much lower free glutamate levels. The reason for this difference remains unclear, but the amount of glutamate is enough to give a taste, so that human infants may experience ‘umami’ as one of the first tastes after birth. Recently, Singh *et al* reported that glutamate is the dominant free amino acid in the milk of Indian mothers (22). Many typical Indian foods also contain glutamate as shown in Tables I and II (our unpublished data). Food samples purchased from a local market, were analysed for amino acid content according to methods described earlier (22). Among these foods, cauliflower, tomato, gourd, most of the Indian breads (Nan, chapatti, and parantha) and basmati rice contained relatively high amount of glutamate. These glutamate levels are comparable or higher than that of published data. Interestingly, glutamate content of basmati rice was found far higher than that of ordinary rice, which suggests correlation between glutamate content and its deliciousness.

TABLE I: Free Amino Acid content (mg/100 g) in various vegetables in India.

A.A	*Cauliflower*	*Cabbage*	*Carrot*	*Ladies finger*	*Drumstick*	*Tomato*	*Bitter gourd*	*Gourd*
Asp	10.82	0.24	7.08	3.85	11.85	18.29	3.29	82.31
Glu	781.91	24.28	258.19	99.75	42.73	487.38	104.40	896.82
Ser	0.96	0.00	0.00	1.75	1.65	2.79	1.65	69.29
His	704.60	734.67	0.00	654.45	0.00	25.78	620.18	1803.42
Gln	7.32	0.59	99.56	31.06	13.81	21.76	29.41	12.03
Gly	55.63	0.56	62.57	4.50	82.14	38.56	4.26	0.69
Thr	0.89	0.26	4.18	0.02	0.00	0.00	3.32	91.00
Arg	0.05	0.00	32.58	5.32	105.63	0.00	4.88	112.40
Ala	0.00	28.62	1.60	0.00	0.76	1.39	0.00	4.06
Tyr	0.36	0.00	0.00	0.57	15.22	7.85	0.54	0.93
Val	4.30	0.00	78.66	11.39	230.83	1.84	5.22	8.20
Met	36.01	0.00	1.45	9.28	1.94	2.57	7.69	27.15
Phe	0.00	0.00	0.00	5.58	0.00	11.38	7.30	43.72
Ile	7.44	0.00	3.53	0.97	0.00	1.54	2.30	0.50
Leu	2.32	0.00	4.24	30.03	37.95	2.36	1.12	7.07

AA: Amino Acids.

TABLE II: Free Amino Acid content (mg/100 g) in various vegetables and foods in India.

A.A	*Green Chilly*	*Coriander leave*	*Curd*	*Moong dal*	*Nan*	*Chapatti*	*Parantha*	*Basmati rice*	*Ordinary rice*
Asp	6.16	58.03	65.88	223.54	0.18	0.19	0.15	0.23	7.79
Glu	302.92	136.55	189.23	330.67	511.16	515.54	423.11	359.53	10.10
Ser	0.60	0.00	0.48	91.09	35.86	1.41	29.66	0.00	3.68
His	0.00	24.26	0.00	246.40	14.09	6.83	11.65	0.00	36.00
Gln	237.91	369.98	30.29	5.15	132.81	165.43	140.81	4.50	5.49
Gly	155.51	143.10	69.59	3.70	35.64	24.63	44.65	0.16	2.79
Thr	19.37	27.11	1.28	96.34	0.21	16.13	0.15	5.54	5.56
Arg	41.74	68.46	112.52	4.99	63.19	59.05	70.19	4.24	3.09
Ala	38.16	80.12	61.84	20.52	32.38	3.89	26.40	7.82	0.58
Tyr	23.52	162.55	71.21	21.41	56.38	20.50	59.57	4.42	1.24
Val	17.31	6.99	108.79	776.14	83.79	181.38	129.99	22.34	29.80
Met	58.61	240.30	0.00	1.37	0.94	39.09	0.73	2.34	0.39
Phe	56.61	0.00	10.53	114.67	35.76	2.06	48.07	7.25	5.41
Ile	47.82	0.00	132.50	236.98	33.86	7.14	29.72	3.98	1.90
Leu	15.48	77.17	0.74	6.36	0.13	8.49	0.04	0.13	0.34

AA: Amino Acids.

Glutamate perception

Free glutamate imparts umami taste in foods and umami is often described as ‘meaty’, ‘broth like’ or ‘savory’ (23, 24) Typical umami taste compound is MSG. Inosine 5-monophosphate (IMP) and guanosine 5-monophosphate (GMP) also elicit umami taste, which were found from dried bonito (also material for traditional Japanese soup stock) and shiitake mushroom respectively. The umami taste is significantly enhanced when MSG is presented in combination with IMP or GMP exerting the synergic effect of umami. The results of several studies indicated that the umami taste could not be reproduced by mixing the other basic tastes and is a unique taste quality (25, 26). Now, the umami taste is recognized as one of the basic tastes scientifically (8).

Recently receptors for umami have been found. These are membrane-bound G-proteins coupled receptors (GPCR) as well as sweet and bitter taste receptors. In 2000, Chaudhari *et al* reported for the first time a candidate umami receptor might be structurally related to metabotropic glutamate receptors 4 (mGluR4) (9). The structure of taste-mGluR4 expressed in taste buds was an N-terminal truncated form of the brain type mGluR4. This receptor was expressed in Chinese hamster ovary (CHO) cells and responded to MSG in an appropriate concentration range for umami taste. On the other hand, in 2002, Nelson *et al.* reported that T1R3 responds to MSG and a wide range of amino acids in combination with T1R1, while it responds to sweet substances in combination with T1R2 in mice (10) Li *et al* also reported that the T1R1/T1R3 heterodimer were expressed in taste cells and respond to MSG (11). Human T1R1/T1R3 heterodimer receptors expressed in HEK293 cells responded to MSG and responses to MSG were enhanced by adding low concentrations of IMP. This result suggests that the heterodimer receptors are responsible for the synergic effect of umami. Subsequently, in 2003, Zhao *et al.* reported that mutant mice lacking T1R1/T1R3

heterodimer receptor showed a total absence of behavioral or neural responses to MSG (12). However, Damak et al. reported that nerve and behavioral responses to glutamate in T1R3-knockout mice were reduced but still appreciable (13) Maruyama et al. also showed that although umami responses were diminished in T1R3-knockout mice, many cells responded to umami-taste stimulation (14). In 2006, san-Gabriel *et al.* also reported that a variant of brain-mGluR1 with a short N-terminal extracellular domain was expressed in circumvallate taste cells (27).

Currently, at least three candidate umami receptors, taste-mGluR4, T1R1/T1R3 and taste-mGluR1 have been found and are likely involved in umami taste perception. Although transduction mechanisms for umami taste have not been clarified yet, the recent investigation with knockout mice has demonstrated that a taste-specific G protein, Ga gustducin, phospholipase C (PLC)-b2 and a transient receptor potential channel, TRPM5, are essential molecules for umami taste as well as sweet and bitter tastes (28, 29). These findings strongly supported the idea that umami is a basic taste. Very recently, San-Gabriel *et al.* reported that mGluR1 exists in glandular stomach and is likely involved in the gastric phase regulation of protein digestion (30). Several physiological studies on glutamate suggest that umami taste triggers a variety of physiological responses, such as secretion of saliva and pancreatic enzymes, and changes of metabolic parameters, to prepare the body to receive and metabolize proteins (31). Umami taste and glutamate could be the signal of protein intake for the body.

Oral intake, absorption and metabolism of glutamate

Glutamate is a component of organs and tissues as a building block of protein. A 60 kg adult body contains 1.4 kg of glutamate on average (32). It has a key role in the metabolism of major nutrients and is important for the reconstruction of body protein and the metabolism of energy. The dietary glutamate is absorbed from the intestinal tract, and more than a half of it is utilized as major energy source for the intestines, and others are converted to different amino acids such as alanine, proline and arginine in the intestinal wall (33). These amino acids are delivered first to the liver to maintain amino acid balance in the blood and then to the various organs and tissues of the body where it serves in the reconstruction of body protein and energy. Dietary glutamate is also specific precursor for the biosynthesis of glutathione. In case of protein bound glutamate, it is degraded in the gastrointestinal tract into small peptides or free glutamate, and they are absorbed from the intestinal tract in a similar way to free glutamate.

Glutamate is the most abundant amino acid in mother’s milk. The daily intake of free glutamate in a breast fed infant is about 36.0 mg/kg body weight while the daily intake of protein bound glutamate is approximately 357.0 mg/kg body weight (34). Human infants ingest more glutamate than human adults on a body weight basis and they have the clear ability to metabolize large amounts of glutamate (35).

Typical protein intake in Western countries by a 70 kg man is about 100 g per

day and approximately 20% of protein is glutamate. Roughly estimated, the average daily intake of protein bound glutamate is about 15 g and free glutamate is about 1 g. In contrast, average daily intake of added MSG ranges between 0.5 – 3.0 g a day, depending upon local dietary customs and cuisine. The intake of glutamate from added MSG is much less than that consumed from foods.

Blood brain barrier for glutamate

Glutamate is the most abundant amino acid in the brain. It is synthesized at rates in proportion to the metabolic demand (36). Plasma glutamate concentrations may fluctuate during the day due to dietary intake, metabolism and protein turn over. The assumptions are that if these changes are transferred directly to the brain interstitial space, there can be disrupting effect on the brain level. Interestingly brain levels are much higher than plasma levels (37). The presence of blood brain barrier (BBB) prevents exogenous glutamate from entering the brain to a large extent.

Maintenance of low concentration of glutamate in the extracellular fluid (1-3 µmol /L) (whereas the intracellular glutamate is 4000–12000 times more, ~12000 µmol/g), energy dependent transport is required (38). To date 5 isoforms of Na^+ dependent excitatory amino acid transporters (EAAT) have been described., which exists in various neuronal cells of the brain (39). Three members of the EAAT family have been shown to be expressed (EAAT 1, 2, and 3) in the abluminal membrane of the blood brain barrier (40).

The glutamate concentration inside the endothelial cells increase by two main mechanisms: glutamate transport into the cells from the ECF and conversion of glutamine to glutamate by glutaminase. When intracellular glutamate level rises above the plasma concentration net transport of glutamate into the blood occurs across the luminal surface through the facilitative carrier (41). The absence of facilitative carriers on the abluminal membrane prevents passive movement of glutamate into the brain ECF. The presence of EAAT on the abluminal membrane provides a mechanism to increase intracellular glutamate concentration and therefore the removal of glutamate from the endothelial cells. Therefore the blood brain barrier mechanism provides protection against the development of neurotoxicity by preventing the accumulation of glutamate.

Safety evaluation of glutamate as food ingredient

MSG, sodium salt of glutamate, is widely used as a flavor enhancer all over the world and also one of the most studied food ingredient, in history. After marketed in Japan in 1909, MSG had been used as a food ingredient in many countries for half century, into the late 1960's. Although there was not much safety data, MSG was generally regarded as a safe substance, similar to GRAS (Generally Recognized As Safe) status in the United States (42), in part because glutamate is one of the most abundant amino acids found in nature and a component of all protein. It was also thought that intake of glutamate from added MSG was much less than that normally ingested from foods. The range oral intake varies from 0.4 g/person

in Italy to 3 g/person in Taiwan (43). Despite the safe use of glutamate in food in human, there are some apprehension because of excitotoxic property of glutamate, the important literature related to the issue has been addressed here.

Lucas and Newhouse first observed the toxic effect of glutamate as degeneration in the inner layers of retina in 1957 when feeding the newborn mice with MSG (44). Later in 1969 Olney reported that the injection or force feeding of very large doses (0.4–0.5 g/kg bw of MSG) to new born mice (2–9 or 10–12 days old) produced brain damage particularly in the arcuate nucleus (AN) of the hypothalamus (6, 7). The term excitotoxiocity was coined by him. He also assessed that the neuronal death was restricted to postsynaptic neuron and glutamate agonists were as neurotoxic as their efficiency to activate glutamate receptors. This property of glutamic acid has been widely used a tool to produce brain lesion in vitro and vivo. In vitro it is not only glutamate but also NMDA agonists (quinolinic acid or ibotenic acid and AMPA/kainite agonists (kainic, quisqualic and domoic acids) induce neuronal degeneration (45).

The effect of brain lesion after MSG administration had been studied by Takasaki et al quantitatively (46, 47). In weaning mice (7 day old) the minimum oral dose was 0.7 g/kg, BW MSG in 10% aqueous solution whereas in the adult the dose was 1.2 g//kg, BW. Basal diet containing 5, 10 or 15% when fed to pregnant, lactating and weaning mice *ad libitum*, did not result in any degeneration in the AN in all treated mice. When MSG was given subcutaneously with either 4.0 or 5.0 g/kg BW, it produced lesion in the hypothalamus.. These studies indicate that placenta is virtually impermeable to glutamate (48). These studies also indicate that in order to produce neurotoxic effect in infant mice MSG has to given not only in relatively high concentration but also as a bolus solution. It is also emphasized that when MSG is given to animal as a component of food even at ingested doses exceeding that produce neurodegeneration when given as a single dose by gavage, no neurotoxicty was reported (49, 50).

The fact that glutamate at high doses does not induce parallel changes in the brain level does not necessarily convey that discrete areas of the brain are impermeable to circulating glutamate. There are some areas of the brain that do not have a blood brain barrier and do allow rapid l-glutamate uptake from the circulation. As apparent from brain lesion it is suggested that glutamate do penetrate and accumulate in specific brain regions like the AN. However, studies show that oral dose of 4.0 g MSG/kg in adult and 2 g/kg, BW in 4-day mice did not change glutamate level in the AN (51). Similar results have been reported in adult and infant guinea pigs after neurotoxic dose of MSG (52). These data do not support vulnerability of the AN to MSG induced degeneration resulting from glutamate accumulation. It is noteworthy that when MSG was given as a component of food there was no increase in the extracellular increase in hypothalamus (53). Therefore the proposition that selective vulnerability of the AN to MSG induced neurotoxocity in sensitive species results from accumulation of glutamate in this region raises some doubt. The effect might possibly be related to the lesser capacity of intestinal epithelium

and liver to transaminate glutamate or to a lesser expression of glutamate transporters in the hypothalamus (54). Developmental changes in the expression of subunits of NMDA receptor may contribute to the pattern of vulnerability to overload of glutamate at the reported developmental age of the neonatal rodents (55).

Species differences

Mice are the most susceptible, followed by rats and guinea pigs to MSG neurotoxicity (50). In monkeys of different ages (0.02–80 days), no lesions were observed with doses ranging from 0.25 to 4 g/kg, BW and with different routes of administration, except in case of the reports of Olney and Sharpe (1969) and Olney et al (1972), which report lesions with doses ranging from 1 to 4 g/kg given orally and subcutaneously (6, 7). Reynolds et al (1979) described possible causes for this discrepancy, attributing it to the dehydrated state of animals, artifacts induced by experimental procedures and interpretation of results (56).

Numbness, weakness and palpitation as the main symptoms after eating in a Chinese restaurant was reported as a 'Letter to Editor' in 1968 (57). All the symptoms described in subsequent anecdotal reports were subjective and no physiological changes in objective parameters were observed. Many experiments using scientifically rigorous design like double-blind placebo-controlled multiple challenge study has demonstrated that the ingestion of MSG in food is not associated with adverse reactions (58).

Research on the safety and usefulness of MSG has undergone rigorous review by scientific advisory bodies to FAO/WHO, EU and various national governments like US Food and Drug Administration (FDA). At the 3lst meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1987, the evaluation arrived at was "Acceptable Daily Intake (ADI) not specified" (59). The term "ADI not specified" means that the total dietary intake of glutamates arising from their use at the levels necessary to achieve the desired technological effect as food additives, and from their normal naturally occurring levels in food, does not present any hazard to health. In 1991, Scientific Committee for Food of European Community (EC/SCF) also established ADI for glutamate as "Not specified" (60). MSG has been deemed GRAS (Generally Recognized as Safe) without any limitations by the USFDA together with sugar, salt, pepper, vinegar, and baking powder since 1958 (42). In 1995, Federation of American Society for Experimental Biology (FASEB), reviewed scientific data sponsored by FDA, and safety of MSG was reaffirmed (61). In 1997, Symposium on "Safety and Usefulness of Glutamate as a Flavour Enhancer: Current state of the knowledge" was held at Mysore, India, in which the literature on safety of glutamate in food was evaluated by Indian scientists confirming that glutamate is safe in adults as well as in infants (62). The expert panel recently concluded "consensus meeting: monosodium glutamate - an update" at University of Hohenheim, Germany in 2006, has regarded use of the MSG in food harmless for whole population (116).

Summary

There are important species differences and difference with age and routes of administration in the glutamate sensitivity

of CNS. The brain in general is a net exporter of glutamate and the presence of the blood brain barrier prevent exogenous glutamate from acting on the brain. Ingestion of glutamate is not associated with elevation in maternal milk. Glutamate does not pass readily the placental barrier. It is unlikely that plasma glutamate concentration ever rises to excitotoxic levels by dietary ingestion of the flavour enhancers, such as MSG or hydrolyzed vegetable protein. For the same reason, ingested glutamate cannot be an etiological factor in neurogenerative diseases ordinarily, i.e. without some sort of preexisting metabolic abnormalities that render some cells vulnerable to normal levels of glutamate or other endogenous agents. The delayed CNS and other effects of glutamate administration in animals are also irrelevant to oral ingestion of MSG in human infants.

UNDERSTANDING SAFTEY GLUTAMTE IN BRAIN

Glutamate is the principal excitatory neurotransmitter in the CNS. During recent advances in the field it is realized that it is much more than a conventional neurotransmitter. It is not only the predominant excitatory neurotransmitter in the mature neurons but also it can influence immature neural cell proliferation, migration, differentiation and survival processes (63, 64). Extracellular glutamate level has been shown to be high in embryonic CNS (63).

Glutamate and its receptors are essential for the normal functioning of the CNS. However their excessive activation by glutamate is thought to contribute to neuronal damage in many neurological disorders ranging from hypoxic–ischemic and traumatic brain injuries to chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease and ALS (65).

Neither the normal functioning of glutamatergic synapses nor the pathogenesis of above diseases can be properly understood unless we have a clear understanding of the glutamatergic transmission and the excitotoxic events due to excessive stimulation of glutamate receptors. This part of the review deals with presynaptic release of glutamate, its post synaptic action, synaptic termination of glutamate action, excessive release in brain and lastly the excitotoxic events.

Glutamate in the brain is exclusively produced from α-ketoglutarate, an intermediate in the tricarboxylic acid cycle of intermediary metabolism.

Synaptic release of glutamate

Although glutamate is a ubiquitous amino acid and as it is required by all cells for protein synthesis and intermediary metabolism, neuronal cells which release this as a neurotransmitter have evolved specialized mechanism for its regulated release from the presynaptic terminals. The majority of glutamate that is released as a neurotransmitter is derived from glutamine (66). Nearby astrocytes take up the released glutamate at the synaptic cleft by powerful excitatory amino acids transporters (39). In the astrocyte glutamate is converted to glutamine by glutamine synthetase (67). A system of N transporter on the astrocyte and a closely related A transporter on the neuron

mediate the transfer of glutamine back to the neuron (68).

The processing and transport of glutamate within the neuron are highly organized Glutamate, like other neurosecretory substances, is initially synthesized by the endoplasmic reticulum and then transported to the Golgi apparatus for additional processing. After emerging from the Golgi apparatus and wrapped inside a vesicular (bilipid) membrane, glutamate is then transported down the axon via a complex system of microtubules. Mitochondria also accompany these transport molecules, providing the required energy. Upon reaching the axonal tip the vesicle with the enclosed glutamate merges with the presynaptic membrane by the process called exocytosis to release the glutamate into the synaptic space between neurons. The vesicular membrane is then recycled and transported back up the neuronal axon in a retrograde fashion via the microtubular network. The synaptic glutamate is finally freed to interact with specific receptor sites on the postsynaptic membrane of the adjacent neuron to initiate an important cascade of molecular events within that neuron.

Quantal size is a fundamental parameter controlling the strength of synaptic transmission. The transmitter content of synaptic vesicles is one mechanism that can affect the physiological response to the release of a single vesicle. This important process of synaptic vesicle loading is mediated by a transport protein collectively known as vesicular glutamate transporters (VGLUT) (69). These newly discovered transporters provide a new viewpoint of understanding of the glutamatergic neuron system in the brain, which is different from the receptor mechanism.

Glutamatergic neurons are categorized into subgroups depending on which isoforms they contain. VGLUT comprises three isoforms, VGLUT1, 2, and 3, and is a potential marker for the glutamatergic phenotype. The efficacy of gluatamategicc neurotransmission more specifically presynaptic regulation depend on these transporters (70). Their transport properties and distribution in the brain have been studied extensively. VGLUTS have been shown to be present in various regions of the brain. It has also been shown in cat retina and inter-mediolateral horn of the rat spinal cord. (71, 72). The presence of VGLUT and VGLUT in noradrenergic and serotonerigic neurons respectively implicate glutamate cotransmission with monoaminergic transmission (73). The two (VGLUT) presynaptically mark and differentiate two distinct excitatory neuronal populations and thus define a cortical and a subcortical glutamatergic system (VGLUT1 and VGLUT2 positive, respectively). These two systems might be differentially implicated in brain neuropathology. Still, little is known on the modalities of VGLUT1 and VGLUT2 regulations in response to pharmacological or physiological stimuli.

Recent studies indicated that VGLUT is also expressed in non-neuronal cells, and localized with various organelles such as synaptic-like microvesicles in the pineal gland, and hormone-containing secretory granules in endocrine cells, stomach, intestine and testis (74).

It is L-glutamate is stored in these organelles, secreted upon various forms of stimulation, and then acts as a paracrine-like modulator. Thus, VGLUTs highlight a novel framework of glutamatergic signaling revealing its diverse modes of action.

Because glutamatergic neurotransmission begins with vesicular release, compounds that block the uptake of glutamate into the vesicle may reduce excitotoxic events. Several classes of competitive VGLUT inhibitors have emerged.

Post synaptic action of glutamate

There are two main types of glutamate receptors, the ionotropic rceptors that gate channel directly and metabotropic recetors that gate channel indirectly by second messenger system (75–77). The directly coupled ionotropic receptor can be further subdivided into three subtypes: NMDA (*N*-methyl-D-aspartate), AMPA (a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate), and kainate. These subtypes are named after their selective chemical agonists, which resemble glutamate but do not naturally exist in the brain. The AMPA and kainate receptors are some time referred as non-NMDA receptors.

The NMDA receptor-channel contributes to the late component of the excitatory post synaptic potential (EPSP). It has three exceptional properties (76, 78–80). (I) It controls high conductance cation channel that is permeable to Ca^{2+}, Na^{+} and K^{+}. (II) The opening of the channel requires glycine as co agonist. Normally the glycine present in the extracellular space is sufficient to allow the functioning of the NMDA receptor-channel. (III) It possesses the unique property of being both voltage and chemical gated. In the NMDA activated channel, in open state it is blocked by extracelluar Mg^{2+} like a plug. When the membrane is depolarized by the action of non-NMDA receptors, Mg^{2+} is expelled by electrostatic repulsion allowing Ca^{2+} and Na^{+} to enter. When glutamate is present and the cell is depolarized, maximum current flow through the channel. The NMDA receptor has another property that it is inhibited by the hallucinogen phencyclidine and MK801 both of which bind to a site within the open channel (76).

The NMDA-receptor mediated channels open and close slowly in response to glutamate and contribute to the late phase of EPSP. When there is a repeated firing by the presynaptic neurons, the EPSP summate depolarizing the membrane by 20 mv or more, the NMDA receptor gives rise o larger current. The late current in NMDA receptor mediated channel is carried by Ca^{2+} (75–77).

Excessive accumulation of intracellular calcium is the key observed process leading to neuronal death or injury, and the NMDA receptors activate channels that allow the influx of extracellular (Ca^{2+} and Na^{+}). Over stimulation of this type of glutamate receptor would then lead to neuronal calcium overload. Some types of AMPA and kainate receptors can contribute to intracellular calcium overload because their coupled membrane ion channels are at least partially permeable to calcium (76).

The stimulation of NMDA receptor is linked to neurotoxicity. Definite evidence came from demonstration of insensitiveness

of non neuronal cell to glutamate toxicity. However transfecting them with a gene containing the NMDA receptor transform them into vulnerable for degeneration (81).

Termination of the action of glutamate

Fundamental to the property of a neurotransmitter, is its timely removal from the synaptic cleft. If the synapse has no such machinery it does not qualify as a neurotransmitter. In case of acetylcholine, it is degraded immediately by the enzyme acetylcholine esterase. No such enzyme is available for termination of glutamate action.

The synaptic uptake of glutamate from extracellular space is accomplished by a family of transporters present on the neuronal and astrocyte surfaces. These EAATs are located on the plasma membrane of the neurons and glias (39), These EAATs rapidly terminate the action of glutamate and maintain extracellular concentration at low level (39, 82–86).

Five type of high affinity (EAAT1-5) have been identified (39). The transporters EAAT1 and EAAT2 in glial cells are responsible for the majority of glutamate uptake (84). They are all sodium dependent and the transmembrane gradient of Na^+ and K^+ provide the driving force for the transport. One molecule of glutamate is coupled to the co transport of three Na^+ and one H^+ and counter transport of one K^{2+} (83).

Excessive accumulation of glutamate

The key event that triggers the entire excitotoxic cascade is the excessive accumulation of glutamate in the synaptic space. This can be achieved by (i) altering the normal cycling of cerebral glutamate to increase the release of glutamate into the extracellular space (ii) decrease glutamate uptake/transport from the synaptic space and (iii) by frank spillage of glutamate from injured neurons.

Augmented glutamate release due to astrocyte activation has been observed in several neurodegenerative disorders (115). Astrocytes react to synaptically released glutamate with elevation of intracellular Ca^+ resulting in the release of glutamate from it. This release of gliotransmitter glutamate from astrocyte is controlled by molecules linked to inflammatory reactions such as the tumour necrosis factor α and prostaglandins in various neurodegenerative disorders.

The linkage between impaired transporter function and excitotoxic concentration of glutamate suggests that transporter malfunction is a plausible mechanism of neurogenerative diseases. The inadequate clearance of excitatory amino acids glutamate may contribute to the neurodegeneration seen in a variety of conditions, A role for glutamate transporters has been postulated in acute conditions such as stroke, CNS ischemia (88–92), and seizure (93, 94), as well as in chronic neurodegenerative diseases such as Alzheimer's disease (95, 96) and ALS (97–100).

Spillage from neuronal injury is another mechanism that markedly elevates the extracellular glutamate levels (101, 102). Normal extracellular glutamate concentration is about 0.6 µmol/L. Substantial neuronal excitotoxic injury occurs with glutamate

concentrations of 2 to 5 µmol/L. Traumatic injury to neurons can produce exposure of the normal intracellular glutamate concentrations of about 10 µmol/L to the extracellular space. Mechanical injury to a single neuron, therefore, can risk all of the neighboring neurons. Several mechanisms are proposed for the abnormal release of glutamate in neuronal injury. Abnormal release of glutamate from its storage sites in neuronal vesicles is at least one factor. A feedback loop is generated as this released glutamate stimulates additional glutamate release. Ischemia also causes energy failure that impairs the reuptake by glutamate transporters. These transporters behave as symporters, which rely on the sodium gradient across cell membranes to move glutamate against its concentration gradients into the cell. The sodium gradient, however, is maintained by an energy-dependent pump that fails in ischemia. Such failure not only affects glutamate transport out of the synaptic space but also causes the transporters to run backward, becoming a source of extracellular glutamate rather than a sink for it. Ischemia deprives the neurons of oxygen and glucose, resulting in energy failure; however, energy failure itself is not particularly toxic to neurons. Neural toxicity occurs with the resultant activation of the cascade of glutamate receptor–dependent mechanisms. Glutamate receptor blockers are being used to minimize the spread of neuronal death beyond the immediate physically disrupted neurons in persons with head or spinal cord injuries.

Intracellular excitotoxic events

The key mediator of glutamate induced excitotoxic neuronal damage is Ca^{2+}, which under physiological conditions govern a multitude of cellular processes including neuronal growth, differentiation and synaptic activities (104). The accumulation of high intracellular calcium levels triggers a cascade of membrane, cytoplasmic, and nuclear events leading to neurotoxicity (105). Elevation of the intracellular calcium, however, appears to be a complex issue, because inducing similar intracellular calcium levels by using a metabolic inhibitor such as cyanide or membrane depolarization with potassium causes less permanent neuronal damage than with glutamate.

Homeostatic mechanisms for maintaining a low Ca^{2+} concentration inside the neuron keep the Ca^{2+} mediated signal spatially and temporally localized. In excitotoxicity excessive release of synaptic glutamate disturbs Ca^{2+} homeostasis (106). Glutamate activates postsynaptic NMDA, AMPA and kainite receptors leading to opening of associated ion channels allowing influx of Ca^{2+} and Na^{+}. Though physiological elevation of intracellular Ca^{2+} are salient features of normal cell functioning, excessive influx along with mobilization of intracellular Ca^{2+} pool can overwhelm C^{2+}a regulatory mechanism leading cell death. (107).

The glutamate-induced elevated calcium levels proceed to over activate a number of enzymes, including protein kinase C, calcium/cadmodulin-dependent protein kinase II, phospholipases, proteases, phosphatases, nitric oxide synthase, endonucleases, and ornithine decarboxylase. Some of these enzymes can also produce positive feedback loops to accelerate the downward spiral toward neuronal death. Activation of

phospholipase A, for example, would generate platelet-activating factor and arachidonic acid and its metabolites. Platelet-activating factor directly contributes to the excitotoxic cascade by increasing glutamate release. Arachidonic acid inhibits reuptake of glutamate from the synaptic space, leading to further activation of glutamate receptors and more arachidonic acid formation. Increased arachidonic acid levels form oxygen free radicals, which activate phospholipase A, leading to more arachidonic acid formation. These enzymes and the generated feedback loops rapidly lead to neuronal self-digestion by protein breakdown, free radical formation, and lipid peroxidation (104–106).

Another important activated enzyme is nitric oxide synthase, which forms nitric oxide (NO). NO performs a variety of normal biological functions but the excessively stimulated NMDA receptors will produce abnormally increased levels of NO and superoxide ions (108–110). These substances may react and form peroxynitrite, which is extremely toxic, resulting in neuronal death (111). NO can damage DNA as well as inhibit mitochondrial respiration, which in turn would create more free radicals and cause additional membrane depolarization (112). The NO initiated neurotoxic cascades are important components of the mechanism of cell death in many neurodegenerative disorders.

The evidence of excitotoxicity in various neurological disorders is mostly indirect and circumstantial. Reasonably direct evidence is seen in case of ALS (16). Apart from defect in EAAT there is recent evidence of increased sensitivity of anterior horn cells to glutamate excitoxicty. In most of the diseases implicated, the excessive glutamate pathology varies from disease to disease. The normal intact brain is remarkably resistant to very high level of glutamate. One of the important factors is the recent finding of different glutamate transporters and their distribution. These EAAT are dependent on NA^+–K^+ pump for maintaining the glutamate homeostais. The NMDA receptor is voltage and ligand dependent. The maintenance of resting membrane potential requires energy. Any impairment in mitochondria function will predispose to NMDA receptors mediated excititoxicity. In this way excitotoxicty could be a final common pathway to neuronal death (113, 114). In a wide variety of neurological disorders glutamate might not be related to the primary cause of the diseases.

Remarkable volume of new information during last 15 years have established the roles for glutamate as: (*a*) an excitatory neurotransmitter in the CNS, (*b*) as a basic taste substance (“umami”), (*c*) a key component of both the nitrogen and energy economies of several organs in the body, including the placenta, liver, gastrointestinal tract and brain. (*d*) a safe food additive, through the careful clarification of the nonphysiologic conditions under which exogenous (and endogenous) glutamate can become neurotoxic in brain.

There is no doubt today that glutamate is the principal excitatory neurotransmitter in the CNS. Its role as a signaling molecule in non-neuronal tissues is fast emerging. It is unfortunate that we term it as endogenous toxin, killer neurotransmitter or a taste to kill. Available knowledge presented in this review does not support glutamate as the

sole culprit in the process of neurotoxicity or neurodegenretion. Glutamate is important and indispensable for the functioning of the CNS and important in food. The physiological control mechanisms of our body keep a check on its excitotoxic properties.

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