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Transport of BMAA into Neurons and Astrocytes by System x_c^-

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Transport of BMAA into Neurons and Astrocytes by System x_c-.

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Abstract

The study of the mechanism of β- N-methylamino- l-alanine (BMAA) neurotoxicity originally focused on its effects at the N-methyl- d-aspartate (NMDA) receptor. In recent years, it has become clear that its mechanism of action is more complicated. First, there are certain cell types, such as motor neurons and cholinergic neurons, where the dominate mechanism of toxicity is through action at AMPA receptors. Second, even in cortical neurons where the primary mechanism of toxicity appears to be activation of NMDA receptors, there are other mechanisms involved. We found that along with NMDA receptors, activation of mGLuR5 receptors and effects on the cystine/glutamate antiporter (system x_c-) were involved in the toxicity. The effects on system x_c- are of particular interest. System x_c- mediates the transport of cystine into the cell in exchange for releasing glutamate into the extracellular fluid. By releasing glutamate, system x_c- can potentially cause excitotoxicity. However,

through providing cystine to the cell, it regulates the levels of cellular glutathione (GSH), the main endogenous intracellular antioxidant, and in this way may protect cells against oxidative stress. We have previously published that BMAA inhibits cystine uptake leading to GSH depletion and had indirect evidence that BMAA is transported into the cells by system x_c^-. We now present direct evidence that BMAA is transported into both astrocytes and neurons through system x_c^-. The fact that BMAA is transported by system x_c^- also provides a mechanism for BMAA to enter brain cells potentially leading to misincorporation into proteins and protein misfolding.

Keywords

BMAA; System xc-; Glutathione; Glutamate

Introduction

The mechanism of β-N-methylamino-l-alanine (BMAA) toxicity can be divided into two general categories: effects at the level of the cell membrane and effects through misincorporation into proteins. Actions at the cell membrane originally focused on excitotoxicity induced by activation of N-methyl-d-aspartate (NMDA) receptors (Ross et al. [32] ; Weiss et al. [39]). However, more recent reports have cast doubt on that effect being the main mechanism of toxicity. The two most sensitive neuronal populations to BMAA toxicity are motor neurons and cholinergic neurons. The BMAA-induced death in both cases is largely blocked by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists, but not by N-methyl-d-aspartate (NMDA) antagonists (Rao et al. [30] ; Liu et al. [17]). Interestingly, both motor neurons and cholinergic neurons express large numbers of calcium-permeable AMPA receptor channels (Yin et al. [40] ; Carriedo et al. [4]). Additionally, even in conditions where NMDA receptor antagonists are protective, the protection is not complete (Ross et al. [32] ; Weiss et al. [39]). Consistent with this, we found that the non-competitive NMDA receptor antagonist MK-801 blocked about 50% of BMAA toxicity. We found that two other distinct mechanisms were also involved in the toxicity. The mGluR5 receptor antagonist 6-methyl-2-[phenylethynyl]-pyridine (MPEP), and the free radical scavenger, trolox, provided additional protection against BMAA toxicity beyond that provided by MK-801. Furthermore, the combination of these agents provided significantly greater protection than either alone, suggesting that they were acting through distinct mechanisms (Lobner et al. [19]). One possibility for BMAA-induced oxidative stress is competition by BMAA with cystine at system x_c^- leading to decreased cystine uptake. We found that BMAA toxicity did involve action on system x_c^-, causing decreased cystine uptake leading to depletion of cellular glutathione and increased oxidative stress (Liu et al. [16]). These results indicate a complex set of mechanisms for BMAA toxicity. Action on system x_c^- appears to play a central role, both through causing oxidative stress and through releasing glutamate, which can act on various glutamate receptors to induce excitotoxicity.

The other main mechanism of BMAA-induced toxicity is through misincorporation into protein. BMAA has been shown to be incorporated into protein in cultures of human fibroblasts (MRC-5 cells), neuroblastoma (SH-SY5Y cells), and umbilical vein endothelial cells (HUVECs) The incorporation is inhibited by l-serine, suggesting that the BMAA is misincorporated in protein in exchange for l-serine. Additionally, BMAA treatment induces autofluorescence indicative of protein aggregation and apoptosis that was blocked by l-serine (Dunlop et al. [10]). BMAA has also been shown to be incorporated into protein in a cell-free system (Glover et al. [12]).

An interesting point regarding the different general mechanisms of BMAA toxicity is that system x_c^- can play a role in both. Our previous studies suggest that BMAA is toxic by competing with cystine at system x_c^-, leading to both glutathione depletion and excitotoxicity. In addition, if BMAA is transported by system x_c^-, it could provide a method for BMAA to enter cells where it could be misincorporated into protein. In the current study, we directly assess the mechanisms by which BMAA enters astrocytes and neurons by measuring the uptake of radiolabeled BMAA.

Methods

Materials

Timed pregnant Swiss Webster mice were obtained from Charles River Laboratories (Wilmington, DE). Serum was from Atlanta Biologicals (Lawrenceville, GA). ^{14}C-BMAA was from PerkinElmer (Waltham, MA). All other chemicals were obtained from Sigma (St. Louis, MO).

Cortical Cell Cultures

Cortical cell cultures containing glial and neuronal cells were prepared from fetal (15-16-day gestation) mice as previously described (Lobner [18]). Dissociated cortical cells were plated on 24-well plates coated with poly-d-lysine and laminin in Eagles' Minimal Essential Medium (MEM, Earle's salts, supplied glutamine-free) supplemented with 5% heat-inactivated horse serum, 5% fetal bovine serum, 2 mM glutamine, and glucose (total 21 mM). Neuron-enriched cultures were prepared exactly as previously described with the addition of 10 µM cytosine arabinoside 48 h after plating to inhibit glial replication. In these cultures, <1% of cells are astrocytes (Dugan et al. [9] ; Rush et al. [33]). Astrocyte-enriched glial cultures were prepared as described for mixed cultures except they were from cortical tissue taken from post-natal day 1-3 mice (Choi et al. [5] ; Schwartz and Wilson [36] ; Rush et al. [33]). Cultures were maintained in humidified 5% CO_2 incubators at 37 °C. All experiments were performed on cultures DIV 13-15; at that age, neuronal cultures are defined as being mature in that they are sensitive to excitotoxicity and astrocyte cultures have formed a confluent layer of astrocytes. Culture media are not changed between plating and the experimental procedures. Mice were handled in accordance with a protocol approved by our institutional animal care committee and in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

^{14}C-BMAA Uptake

To assess ^{14}C-BMAA uptake, cultures were washed three times with a HEPES-buffered saline solution and then exposed to ^{14}C-BMAA (0.025 µCi/mL) for the indicated period of time in the HEPES-buffered saline solution. Following ^{14}C-BMAA exposure, all cultures were washed three times with HEPES-buffered saline solution and dissolved in 250 µL warm sodium dodecyl sulfate (0.5%). Following a 30-min period, the media were mixed by pipetting up and down several times and a 200 µL aliquot was removed and added to scintillation fluid for counting. For inhibition studies, values were normalized to ^{14}C-BMAA uptake in untreated controls on the same experimental plate.

Statistical Analysis

Differences between test groups were examined for statistical significance by means of one-way ANOVA followed by the Bonferroni correction post hoc test, with $p < 0.05$ being considered significant. N values represent individual wells on 24-well plates; data represent two independent experiments.

Results

We directly measured BMAA uptake by exposing cultures to ^{14}C-BMAA and assessed the uptake of radioactivity using scintillation counting. To determine the best time point to measure ^{14}C-BMAA uptake, we exposed mixed neuronal and astrocyte cultures to ^{14}C-BMAA for different periods of time. Uptake of ^{14}C-BMAA increased rapidly over the first 2 h before plateauing (Fig. 1). Therefore, we choose a 2-h period of uptake for the inhibitor studies. We tested the effects of the system x_c^- inhibitor sulfasalazine (SSZ) and the excitatory amino acid transporter (EAAT) inhibitor threo-β-benzyloxyaspartic acid (TBOA) on both astrocyte-enriched and pure neuronal cultures. Inhibition of system x_c^-, but not EAATs, significantly decreased ^{14}C-BMAA uptake in astrocyte cultures (Fig. 2a), while inhibition of either system x_c^- or EAATs significantly decreased the uptake in neuronal cultures (Fig. 2b).^{14}C-BMAA uptake into mixed neuronal and astrocyte cultures was measured for the indicated duration.

Results are in disintegrations per minute (DPM) and are expressed as mean ± SEM (n = 8)^{14}C-BMAA uptake into astrocyte-enriched cultures was partially blocked by the system x_c^- inhibitor sulfasalazine (SSZ) but not by the EAAT inhibitor TBOA (a), while uptake into pure neuronal cultures was partially blocked by both sulfasalazine and TBOA (b). Cultures were exposed to ^{14}C-BMAA for 2 h. Results are expressed as mean + SEM (n = 8) after normalizing to untreated control uptake and expressed as % control. Asterisk indicates significantly different from control

Discussion

This is the first report directly assessing the mechanisms by which BMAA is transported into astrocytes or neurons. Previous studies have addressed questions regarding transport of BMAA into the brain and the accumulation of BMAA in the brain. The transport of BMAA across the blood-brain barrier has been shown to be mediated by the large neutral amino acid carrier (Smith et al. [38]). Subsequent studies showed that the accumulation of radiolabeled BMAA in the brain is primarily in the protein-bound fraction and that injection of radiolabeled BMAA into pregnant mice leads to the accumulation of the BMAA in the brains of fetal mice (Karlsson et al. [15]). Since the central nervous system uses amino acids such as glutamate and glycine as neurotransmitters, the release and uptake of amino acids within the brain must be tightly controlled. Two important systems for the regulation of amino acids in the CNS are system x_c^- and EAATs. Therefore, it is not surprising that both of these systems play a role in BMAA uptake. Of particular interest is the role of system x_c^- in BMAA transport because of the complex role that it plays in regulating neuronal death.

System x_c^- is a sodium-independent, chloride-dependent amino acid transporter system localized in the plasma membrane. First characterized in human fibroblast cell cultures, system x_c^- is an antiporter that mediates the uptake of cystine into cells in exchange for exporting glutamate from the cell in a 1:1 ratio (Bannai and Kitamura [3] ; Bannai [2]). Inhibition of system x_c^- mediated cystine uptake can lead to GSH depletion, oxidative stress, and cell death (Miyamoto et al. [23] ; Murphy et al. [25] , [26] ; Sagara et al. [34] ; Ratan et al. [31]). In contrast, upregulation of system x_c^- can protect cells against oxidative stress-induced cell death. For example, insulin-like growth factor 1 (IGF-1) and transforming growth factor-β (TGF-β) upregulate system x_c^- activity and protect dental pulp cells against oxidative stress induced by dental materials (Pauly et al. [27]). Interleukin-1β (IL-1β) upregulates system x_c^- activity, which protects astrocytes from oxidative stress induced by $FeSO_4$ or tert-butyl hydroperoxide (tBOOH) exposure (He et al. [13]). The other main function of system x_c^- is the regulation of extracellular glutamate concentrations. In contrast to the possible neuroprotective mechanism of system x_c^- against oxidative stress, it has been shown that glutamate release via system x_c^- can cause excitotoxicity and lead to neuronal death (Piani and Fontana [28] ; Qin et al. [29] ; Domercq et al. [8] ; Fogal et al. [11] ; Jackman et al. [14]).

Oxidative stress and excitotoxicity are two of the key mechanisms involved in cell death in neurodegenerative diseases (Coyle and Puttfarcken [6] ; Simonian and Coyle [37] ; Doble [7]). The dual nature of system x_c^- suggests that it may provide antioxidant protection during times of increased oxidative stress; however, by releasing glutamate into the extrasynaptic space, it also has the potential to contribute to neuronal death through excitotoxicity. Therefore, the role of system x_c^- in neurodegenerative disease is likely complex and the data suggest that this is in fact the case.

Concerning amyotrophic lateral sclerosis (ALS), system x_c^- has been shown to be upregulated in two different mouse SOD1 mutant models of ALS (Albano et al. [1] ; Mesci et al. [22]). Interestingly, deletion of xCT in the SOD1-G37R mouse led to an earlier onset of symptoms followed by a prolonged symptomatic stage; at end stage of the disease, there were more surviving motor neurons (Mesci et al. [22]).

To date, there are only a couple of in vivo studies that have assessed system x_c^- in Alzheimer's disease. In 18-month-old AßPP23 mice, expression of xCT, as assessed by western blot, was increased in the cortex. At this same time point, GLT-1 expression was decreased and there were increased levels of extracellular glutamate (Schallier et al. [35]). Transgenic mice expressing human APP and wild-type mice injected with $A\beta_{1\text{-}40}$ had increased xCT mRNA levels in microglia in amyloid plaques (Qin et al. [29]).

Studies regarding the effects of system x_c^- in Parkinson's disease models have provided mixed results. In a hemi-Parkinson rat model, injection of 6-hydroxydopamine (6-OHDA) caused an ipsilateral increase in xCT protein in the striatum (Massie et al. [20]) and dopaminergic neurons in the substantia nigra pars compacta were protected from 6-OHDA injection in $xCT^{-}/^{-}$ mice compared to wild-type mice (Massie et al. [21]). These results suggest that system x_c^- may contribute to the neurodegeneration seen in PD. However, the anti-epileptic drug levetiracetam increased xCT expression in striatal astrocytes and significantly decreased dopaminergic cell loss in mice injected with 6-OHDA (Miyazaki et al. [24]).

The mixed results regarding the beneficial or damaging effects of system x_c^- in neurodegenerative diseases are not surprising considering its dual effects. However, two factors are important concerning the role of system x_c^- in the toxicity of BMAA. First, in each of the diseases described earlier, there is an upregulation of system x_c^-. Whether the upregulation is beneficial or damaging under conditions where BMAA is not present appears to vary. Second, BMAA negates the beneficial effects of system x_c^- by blocking cystine uptake but promotes the damaging effect by driving glutamate release. Therefore, in conditions of upregulation of system x_c^-, the presence of BMAA is likely to be even more damaging than normal.

Another important effect that we observed was that blocking EAATs also partially blocked BMAA uptake into neurons. This result is of interest in two ways. First, it provides another mechanism for BMAA to enter neurons. Second, it suggests the possibility that BMAA may competitively inhibit glutamate uptake. If BMAA both drives glutamate release by acting on system x_c^- and inhibits glutamate uptake by EAATs, this could lead to high extracellular glutamate concentrations and excitotoxicity. Therefore, there are a number of reasons to believe that BMAA acting at the level of amino acid transporters on astrocytes and neurons plays a role in its potential involvement in neurodegenerative diseases.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with ethical standards of the institution or practice at which the studies were conducted.

References

1 Albano R, Liu X, Lobner D, Regulation of system xc- in the SOD1-G93A mouse model of ALS, Exp Neurol, 2013, 250, 69, 73, 10.1016/j.expneurol.2013.09.008

2 Bannai S, Exchange of cystine and glutamate across plasma membrane of human fibroblasts, J Biol Chem, 1986, 261, 2256, 2263

3 Bannai S, Kitamura E, Transport interaction of L-cystine and L-glutamate in human diploid fibroblasts in culture, J Biol Chem, 1980, 255, 2372, 2376

4 Carriedo SG, Yin HZ, Lamberta R, Weiss JH, In vitro kainate injury to large, SMI-32(+) spinal neurons is Ca2+ dependent, Neuroreport, 1995, 6, 945, 948, 10.1097/00001756-199504190-00030
5 Choi DW, Maulucci-Gedde M, Kriegstein AR, Glutamate neurotoxicity in cortical cell culture, J Neurosci, 1987, 7, 357, 368
6 Coyle JT, Puttfarcken P, Oxidative stress, glutamate, and neurodegenerative disorders, Science, 1993, 262, 689, 695, 10.1126/science.7901908
7 Doble A, The role of excitotoxicity in neurodegenerative disease: implications for therapy, Pharmacol Ther, 1999, 81, 163, 221, 10.1016/S0163-7258(98)00042-4
8 Domercq M, Sanchez-Gomez MV, Sherwin C, Etxebarria E, Fern R, Matute C, System xc- and glutamate transporter inhibition mediates microglial toxicity to oligodendrocytes, J Immunol, 2007, 178, 6549, 6556, 10.4049/jimmunol.178.10.6549
9 Dugan LL, Bruno VMG, Amagasu SM, Giffard RG, Glia modulate the response of murine cortical neurons to excitotoxicity: glia exacerbate AMPA neurotoxicity, J Neurosci, 1995, 15, 4545, 4555
10 Dunlop RA, Cox PA, Banack SA, Rodgers KJ, The non-protein amino acid BMAA is misincorporated into human proteins in place of L-serine causing protein misfolding and aggregation, PLoS One, 2013, 8, e75376, 10.1371/journal.pone.00753763783393
11 Fogal B, Li J, Lobner D, McCullough LD, Hewett SJ, System x(c)- activity and astrocytes are necessary for interleukin-1 beta-mediated hypoxic neuronal injury, J Neurosci, 2007, 27, 10094, 10105, 10.1523/JNEUROSCI.2459-07.2007
12 Glover WB, Mash DC, Murch SJ, The natural non-protein amino acid N-b-methylamino-L-alanine (BMAA) is incorporated into protein during synthesis, Amino Acids, 2014, 46, 2553, 2559, 10.1007/s00726-014-1812-1
13 He Y, Jackman NA, Thorn TL, Vought VE, Hewett SJ, Interleukin-1β protects astrocytes against oxidant-induced injury via an NF-κB-dependent upregulation of glutathione synthesis, Glia, 2015, 63, 1568, 1580, 10.1002/glia.228284506211
14 Jackman NA, Uliasz TF, Hewett JA, Hewett SJ, Regulation of system x(c)(−) activity and expression in astrocytes by interleukin-1β: implications for hypoxic neuronal injury, Glia, 2010, 58, 1806, 1815, 10.1002/glia.210504451603
15 Karlsson O, Lindquist NG, Brittebo EB, Roman E, Selective brain uptake and behavioral effects of the cyanobacterial toxin BMAA (beta-N-methylamino-L-alanine) following neonatal administration to rodents, Toxicol Sci, 2009, 109, 286, 295, 10.1093/toxsci/kfp062
16 Liu X, Rush T, Zapata J, Lobner D, Beta-N-methylamino-l-alanine induces oxidative stress and glutamate release through action on system xc(−), Exp Neurol, 2009, 217, 429, 433, 10.1016/j.expneurol.2009.04.002
17 Liu XQ, Rush T, Ciske J, Lobner D, Selective death of cholinergic neurons induced by beta-methylamino-L-alanine, Neuroreport, 2010, 21, 55, 58, 10.1097/WNR.0b013e328333dfd5
18 Lobner D, Comparison of the LDH and MTT assays for quantifying cell death: validity for neuronal apoptosis?, J Neurosci Methods, 2000, 96, 147, 152, 10.1016/S0165-0270(99)00193-4
19 Lobner D, Piana PM, Salous AK, Peoples RW, Beta-N-methylamino-L-alanine enhances neurotoxicity through multiple mechanisms, Neurobiol Dis, 2007, 25, 360, 366, 10.1016/j.nbd.2006.10.002
20 Massie A, Schallier A, Mertens B, Time-dependent changes in striatal xCT protein expression in hemi-Parkinson rats, Neuroreport, 2008, 19, 1589, 1592, 10.1097/WNR.0b013e328312181c
21 Massie A, Schallier A, Kim SW, Dopaminergic neurons of system x(c), −-deficient mice are highly protected against 6-hydroxydopamine-induced toxicity, FASEB J, 2011, 25, 1359, 1369, 10.1096/fj.10-177212
22 Mesci P, Zaïdi S, Lobsiger CS, System xC- is a mediator of microglial function and its deletion slows symptoms in amyotrophic lateral sclerosis mice, Brain, 2015, 138, 53, 68, 10.1093/brain/awu312
23 Miyamoto M, Murphy TH, Schnaar RL, Coyle JT, Antioxidants protect against glutamate-induced cytotoxicity in a neuronal cell line, J Pharmacol Exp Ther, 1989, 250, 1132, 1140
24 Miyazaki I, Murakami S, Torigoe N, Kitamura Y, Asanuma M, Neuroprotective effects of levetiracetam target xCT in astrocytes in parkinsonian mice, J Neurochem, 2016, 136, 194, 204, 10.1111/jnc.13405

25 Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT, Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress, Neuron, 1989, 2, 1547, 1558, 10.1016/0896-6273(89)90043-3
26 Murphy TH, Schnaar RL, Coyle JT, Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake, FASEB J, 1990, 4, 1624, 1633
27 Pauly K, Fritz K, Furey A, Lobner D, Insulin-like growth factor 1 and transforming growth factor-β stimulate cystine/glutamate exchange activity in dental pulp cells, J Endod, 2011, 37, 943, 947, 10.1016/j.joen.2011.03.0313122157
28 Piani D, Fontana A, Involvement of the cystine transport system xc- in the macrophage-induced glutamate-dependent cytotoxicity to neurons, J Immunol, 1994, 152, 3578, 3585
29 Qin S, Colin C, Hinners I, Gervais A, Cheret C, Mallat M, System xc- and apolipoprotein E expressed by microglia have opposite effects on the neurotoxicity of amyloid-beta peptide 1-40, J Neurosci, 2006, 26, 3345, 3356, 10.1523/JNEUROSCI.5186-05.2006
30 Rao SD, Banack SA, Cox PA, Weiss JH, BMAA selectively injures motor neurons via AMPA/kainate receptor activation, Exp Neurol, 2006, 201, 244, 252, 10.1016/j.expneurol.2006.04.017
31 Ratan RR, Murphy TH, Baraban JM, Macromolecular synthesis inhibitors prevent oxidative stress-induced apoptosis in embryonic cortical neurons by shunting cysteine from protein synthesis to glutathione, J Neurosci, 1994, 14, 4385, 4392
32 Ross SM, Seelig M, Spencer PS, Specific antagonism of excitotoxic action of ‘uncommon’ amino acids assayed in organotypic mouse cortical cultures, Brain Res, 1987, 425, 120, 127, 10.1016/0006-8993(87)90490-2
33 Rush T, Liu XQ, Hjelmhaug J, Lobner D, Mechanisms of chlorpyrifos and diazinon induced neurotoxicity in cortical culture, Neuroscience, 2010, 166, 899, 906, 10.1016/j.neuroscience.2010.01.025
34 Sagara JI, Miura K, Bannai S, Maintenance of neuronal glutathione by glial cells, J Neurochem, 1993, 61, 1672, 1676, 10.1111/j.1471-4159.1993.tb09802.x
35 Schallier A, Smolders I, Van Dam D, Region- and age-specific changes in glutamate transport in the AβPP23 mouse model for Alzheimer’s disease, J Alzheimers Dis, 2011, 24, 287, 300
36 Schwartz JP, Wilson DJ, Preparation and characterization of type 1 astrocytes cultured from adult rat cortex, cerebellum, and striatum, Glia, 1992, 5, 75, 80, 10.1002/glia.440050111
37 Simonian NA, Coyle JT, Oxidative stress in neurodegenerative diseases, Annu Rev Pharmacol Toxicol, 1996, 36, 83, 106, 10.1146/annurev.pa.36.040196.000503
38 Smith QR, Nagura H, Takada Y, Duncan MW, Facilitated transport of the neurotoxin, beta-N-methylamino-L-alanine, across the blood-brain barrier, J Neurochem, 1992, 58, 1330, 1337, 10.1111/j.1471-4159.1992.tb11346.x
39 Weiss JH, Koh JY, Choi DW, Neurotoxicity of beta-N-methylamino-L-alanine (BMAA) and beta-N-oxalylamino-L-alanine (BOAA) on cultured cortical neurons, Brain Res, 1989, 497, 64, 71, 10.1016/0006-8993(89)90970-0
40 Yin HZ, Turetsky D, Choi DW, Weiss JH, Cortical neurones with Ca2+ permeable AMPA/kainate channels display distinct receptor immunoreactivity and are GABAergic, Neurobiol Dis, 1994, 1, 43, 49, 10.1006/nbdi.1994.0006

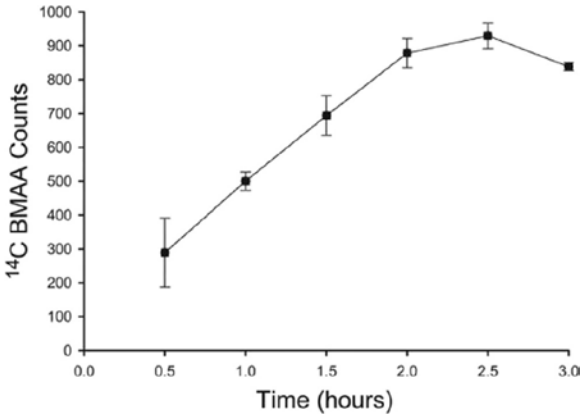

Figure 1. ^{14}C-BMAA uptake into mixed neuronal and astrocyte cultures was measured for the indicated duration. Results are in disintegrations per minute (DPM) and are expressed as mean ± SEM (n = 8)

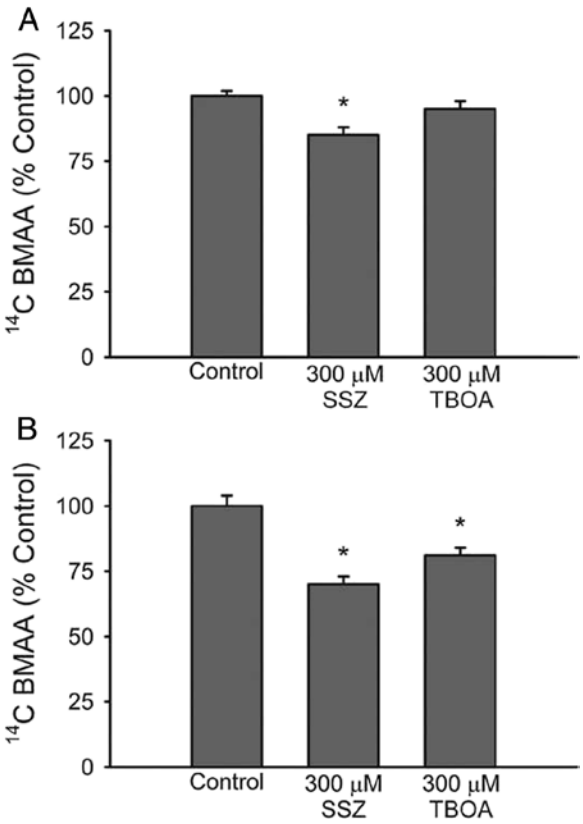

Figure 2 ^{14}C-BMAA uptake into astrocyte-enriched cultures was partially blocked by the system x_c- inhibitor sulfasalazine (SSZ) but not by the EAAT inhibitor TBOA (a), while uptake into pure neuronal cultures was partially blocked by both sulfasalazine and TBOA (b). Cultures were exposed to ^{14}C-BMAA for 2 h. Results are expressed as mean + SEM (n = 8) after normalizing to untreated control uptake and expressed as % control. *Asterisk* indicates significantly different from control