Peroxy nitrite Stimulates L-Arginine Transport System y+ in Glial Cells

A POTENTIAL MECHANISM FOR REPLENISHING NEURONAL L-ARGININE*

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We have reported previously that peroxynitrite stimulates L-arginine release from astrocytes, but the mechanism responsible for such an effect remains elusive. To explore this issue, we studied the regulation of $^{[3H]}$L-arginine transport by either exogenous or endogenous peroxynitrite in glial cells. A 2-fold peroxynitrite-mediated stimulation of L-arginine release in C6 cells was found to be Na+-independent, was prevented by 5 mM L-arginine and, although only in the presence of Na+ (9–13). Within the brain, free L-arginine is predominantly located in astrocytes (14), which produce large amounts of ‘NO upon iNOS (15) and L-arginine transporter (7) induction. However, unlike in astrocytes, L-arginine content in neurons is very low and is limiting for nNOS activity (16, 17). Consistent with this, glial-derived L-arginine has been shown to be increased upon activation of ionotropic glutamate (non-N-methyl-D-aspartate) receptors (18) and peroxynitrite (19), suggesting a neuronal-astrocytic signaling transduction pathway focused to provide NOS substrate for the neurons. However, direct demonstration of such a pathway and the elucidation of the precise transport system involved remain elusive.

Plasma membrane L-arginine transport is brought about by two families of cationic amino acid transport proteins: Cat (cationic amino acid transporter) and Bat (broad scope amino acid transporter). The Cat family of transporters comprises two families of cationic amino acid transporters (7, 27). The Cat family of constitutive transporter proteins is found in systems $\text{b}^\text{0}^+\text{b}^-\text{, }B_0^+\text{,}$ and $y^+L$ (y$^+$Lat1 and y$^+$Lat2), which are mainly expressed in kidney and intestine, except for y$^+$Lat2, which is expressed in astrocytes (28).

We have reported previously that the neurotoxic ‘NO derivative, peroxynitrite anion (ONOO$^-$), specifically stimulates L-arginine release from astrocytes (19). Although the ONOO$^-$-mediated stimulatory effect was inhibited by L-lysine, hence suggesting the involvement of system y$^+$ (19), an in-depth study focused on elucidating the precise mechanism responsible for L-arginine transport activation has not yet been carried out. In view of the potential critical role of glial cells as neuronal L-arginine suppliers for ‘NO biosynthesis (8, 18, 29), we were prompted to investigate the mechanism through which ONOO$^-$ modulates L-arginine transport across the glial cell plasma membrane as well as the potential relevance of such modulation for neuronal L-arginine uptake.
Peroxynitrite Stimulates System Y+ in Glia

EXPERIMENTAL PROCEDURES

Materials—Peroxynitrite was synthesized and quantified spectro-photometrically \( (\epsilon_{\text{max}} = 1,670 \, \text{m}^{-1} \cdot \text{cm}^{-1}) \) as described previously (30).

Alkaline stock solutions, with an approximate ONOO− concentration of 0.3–0.4 M, were stable at −70 °C for at least 3–4 months. Dulbecco’s modified Eagle’s medium (DMEM), lipopolysaccharide (LPS), amino acids, and N-ethylmaleimide were obtained from Sigma. Fetal calf serum (FCS) was purchased from Roche Diagnostics. L-[2,3,4,5-3H]-Arginine and \( \mu \text{M} \)-labeled 

...prewarmed (−2°C) or Na+−free Hanks’ buffer containing 10 mM l-arginine at 37 °C for 30 min. Uptake experiments were performed in fresh Na+−free Hanks’ buffer containing 0.25 mM CuCl2 l-[2,3,4,5-3H]-arginine (50 μM). In experiments focused on inhibition, l-arginine transportation, and uptake, the l-arginine transporter, AMT (50 μM), or DETANO alone (0.1 mM) was added to the astrocytes. Blanks were performed as described above and, results were expressed as pmol of l-arginine taken up/min/mg of protein. Controls were carried out using degraded ONOO−. In some experiments, neurons were depleted of l-arginine by incubating these cells in DMEM containing arginase (2 units/ml; Sigma) for 24 h as described previously (35) before the co-incubation with astrocytes.

Western Blotting—Cells were scraped off the plastic dishes with lysis buffer (12.5 mM Na2HPO4, 116 mM NaCl, 0.5 mM EDTA, 1% (by volume) Triton X-100, 0.1% (w/v) sodium dodecylsulfate, 100 μM N-a-tosyl-l-lysine chloromethylketone, 100 μM phenylmethylsulfonyl fluoride, 1 mM phenanthrolin, 10 μM β-galactosidase and 1 mM benzamidine. Blanks were performed as described above and, results were expressed as pmol of l-arginine taken up/min/mg of protein. Controls were carried out using degraded ONOO−. In some experiments, neurons were depleted of l-arginine by incubating these cells in DMEM containing arginase (2 units/ml; Sigma) for 24 h as described previously (35) before the co-incubation with astrocytes.

Northern Blotting—Northern blotting analysis was carried out in total RNA samples isolated from the cells by the guanidium isothiocyanate method as described previously (7). The samples were electrophoresed (22 μg of RNA/line) on a 1% (w/v) agarose-formaldehyde gel. After transfer to a GeneScreen Plus membrane (PerkinElmer Life Sciences) and cross-linking with ultraviolet irradiation (UV Stratagene, Model 2400, Genetic Research Instruments, Essex, UK), membranes were hybridized for 18 h at 65 °C in the presence of the appropriate random-primed \( [\alpha-32P] dCTP \)-labeled cDNA probes and exposed to Kodak XAR-5 film. As cDNA probes, we used either a 0.9-kb

...l-arginine released obtained in each treatment as compared with controls (degraded ONOO−-treated cells), which were arbitrarily given a value of 100%.

l-Arginine Uptake Experiments—Uptake experiments were carried out as described previously (5, 6). Briefly, 24 h after reseeding, the culture medium was removed, and cells were washed once with pre-warmed (−2°C) or Na+−free Hanks’ buffer containing 10 mM l-arginine at 37 °C for 30 min. For trans-stimulation studies, cells were preincubated in Na+−free Hanks’ buffer containing 10 mM l-arginine at 37 °C for 30 min. Uptake experiments were performed in fresh Na+−free Hanks’ buffer containing 0.25 mM CuCl2 l-[2,3,4,5-3H]-arginine (50 μM). In experiments focused on inhibition, l-arginine transportation, and uptake, the l-arginine transporter, AMT (50 μM), or DETANO alone (0.1 mM) was added to the astrocytes. Blanks were performed as described above and, results were expressed as pmol of l-arginine taken up/min/mg of protein. Controls were carried out using degraded ONOO−. In some experiments, neurons were depleted of l-arginine by incubating these cells in DMEM containing arginase (2 units/ml; Sigma) for 24 h as described previously (35) before the co-incubation with astrocytes.

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iNOS cDNA fragment (a generous gift of Dr. Elena Galea, University of Illinois, Chicago, IL) or a 0.9-kb Cat1 cDNA fragment (generously provided by Dr. Manuel Palacin, University of Barcelona, Barcelona, Spain).

Immunocytochemistry—Immunocytochemistry was carried out on astrocytes grown on glass coverslips, which were fixed for 30 min in PBS containing 4% paraformaldehyde, rinsed with PBS, and permeabilized for 10 min with methanol at −20 °C. Cells were then incubated at room temperature in PBS containing blocking serum (10% normal goat serum) plus anti-3-nitrotyrosine antibody for 3 h. After washing with PBS, cells were incubated in PBS containing the secondary antibody (anti-mouse-IgG-fluorescein isothiocyanate) for 2 h. Finally, coverslips were washed and developed using the SlowFade® light antifade kit (Molecular Probes, Eugene, OR) for fluorescence microphotographs.

Statistical Analysis—Data are expressed as mean ± S.E. values for the number of culture preparations indicated in the figure legends. Statistical significance was evaluated by one-way analysis of variance followed by the least significant difference multiple range test. p < 0.05 was considered significant.

RESULTS

Peroxynitrite Stimulates L-Arginine Release in an Na+-independent Fashion—To elucidate the mechanism involved in ONOO−-mediated l-arginine release (19), we first studied the Na+ dependence on this effect in C6 glial cells. Cells were loaded with l-[3H]arginine and incubated either in the absence (Na+-free, choline chloride-containing Hanks’ buffer) or in the presence (Hanks’ buffer) of Na+ at 37 °C for 25 min and then exposed to 100 μM ONOO− to degradable ONOO−. After 5 min, l-arginine release was determined as reported previously (19). As shown in Fig. 1, ONOO− increased l-arginine release by about 2-fold both in the presence (Fig. 1A) and in the absence (Fig. 1B) of Na+. Supplementation of excess (5 mM) l-arginine, l-alanine, or l-leucine partially or fully prevented ONOO−-stimulated l-arginine release in the presence of Na+ (Fig. 1A). However, only l-arginine, but not l-alanine or l-leucine supplementation, was able to prevent ONOO−-mediated l-arginine release in the absence of Na+ (Fig. 1B).

Trans-stimulation of ONOO−-mediated Activation of l-arginine Uptake and Inhibition by the System y′-specific Inhibitor N-Ethylmaleimide—To further investigate the transport system involved in the ONOO−-mediated activation of l-arginine release in glial cells, we tested whether the release was trans-stimulated by t-arginine. To simplify the experimental design, l-arginine uptake instead of efflux was measured in the absence of Na+ (Fig. 1B). Polyethylene glycol (molar ratio 2:1) was radiolabeled with 1 Ci/ml L-[2,3,4,5-3H]arginine (50 μM) either Hanks’ buffer (A) or Na+-free Hanks’ buffer (B) (NaCl was replaced by 132.4 mM choline chloride) at 37 °C for 30 min. After extensive washing, ONOO− (100 μM) was added, and l-[3H]arginine release was measured 5 min later. Where indicated, all incubations were performed in the presence of 5 mM L-arginine, l-alanine, or l-leucine. For trans-stimulation experiments (C), cells were preincubated in Na+-free Hanks’ buffer containing either 50 μM or 10 mM L-arginine at 37 °C for 30 min. For N-ethylmaleimide experiments (D), all incubations were carried out in the presence of this inhibitor at the indicated concentrations. Uptake experiments were performed in Na+-free Hanks’ buffer containing 25 μM CuCl2 l-[2,3,4,5-3H]arginine (50 μM). Peroxynitrite (100 μM) was added, and cells were incubated at 37 °C for 5 min. l-[3H]Arginine retained by the cells was measured in the cell lysates as described under “Experimental Procedures.” Uptake blanks were obtained from cells briefly (−2 s) exposed to l-[3H]arginine medium on ice (−0−4 °C), whose radioactivity was subtracted from sample values. Results are expressed as percentages of l-arginine release/uptake as compared with that released/taken up by control cells (i.e. degradable ONOO−-treated cells), which were arbitrarily given a value of 100%. Control release values were 352 ± 23 and 324 ± 23 cpm/103 cells with and without Na+, respectively. Control uptake values were 79 ± 12 pmol/min/mg of protein. In all cases, data are mean ± S.E. values from three separate experiments. *p < 0.05 as compared with the control group. **p < 0.05 as compared with the None (A and B). 50 μM l-arginine (C), or 0 mM N-ethylmaleimide (D) groups, respectively.

Cells—The functional protein modification by ONOO−-mediated nitration of 3-nitrotyrosine residues (3-nitrotyrosination) is now widely documented (39, 40). In view of the rapid effect brought about by ONOO− on l-arginine transport activity, we wondered whether Cat1 3-nitrotyrosination might be taking place under our own conditions. A 5-min incubation of intact C6 or astrocytic cells in the presence of 100 μM ONOO− revealed intense protein 3-nitrotyrosination, as judged by Western blotting using monoclonal anti-3-nitrotyrosine antibody in both cell types (Fig. 3). Interestingly, among other 3-nitrotyrosinated bands, in both C6 cells and astrocytes, a protein was also found to be anti-Cat1-immunopositive, as judged by parallel Western blotting using an antibody raised against Cat1 protein (Fig. 3). Ectopic immunoprecipitation using anti-Cat1 and Western blotting with anti-3-nitrotyrosine or immunoprecipitation with anti-3-nitrotyrosine and Western blotting with anti-Cat1 were not successful, probably due to the very low level of nitrated Cat1 protein.

Endogenous Peroxynitrite, but Not Nitric Oxide Oxidation, Stimulates l-Arginine Transport Activity in Astrocytes—In

![Fig. 1](image-url)

**Fig. 1.** Peroxynitrite stimulates a trans-stimulable, Na+-independent l-arginine transport activity. C6 glial cells were preloaded with l-[3H]arginine (50 μM) either Hanks’ buffer (A) or Na+-free Hanks’ buffer (B) (NaCl was replaced by 132.4 mM choline chloride) at 37 °C for 30 min. After extensive washing, ONOO− (100 μM) was added, and l-[3H]arginine release was measured 5 min later. Where indicated, all incubations were performed in the presence of 5 mM l-arginine, l-alanine, or l-leucine. For trans-stimulation experiments (C), cells were preincubated in Na+-free Hanks’ buffer containing either 50 μM or 10 mM l-arginine at 37 °C for 30 min. For N-ethylmaleimide experiments (D), all incubations were carried out in the presence of this inhibitor at the indicated concentrations. Uptake experiments were performed in Na+-free Hanks’ buffer containing 25 μM CuCl2 l-[2,3,4,5-3H]arginine (50 μM). Peroxynitrite (100 μM) was added, and cells were incubated at 37 °C for 5 min. l-[3H]Arginine retained by the cells was measured in the cell lysates as described under “Experimental Procedures.” Uptake blanks were obtained from cells briefly (−2 s) exposed to l-[3H]arginine medium on ice (−0−4 °C), whose radioactivity was subtracted from sample values. Results are expressed as percentages of l-arginine release/uptake as compared with that released/taken up by control cells (i.e. degradable ONOO−-treated cells), which were arbitrarily given a value of 100%. Control release values were 352 ± 23 and 324 ± 23 cpm/103 cells with and without Na+, respectively. Control uptake values were 79 ± 12 pmol/min/mg of protein. In all cases, data are mean ± S.E. values from three separate experiments. *p < 0.05 as compared with the control group. **p < 0.05 as compared with the None (A and B). 50 μM l-arginine (C), or 0 mM N-ethylmaleimide (D) groups, respectively.
view of the evidence showing L-arginine transport activity stimulation by exogenous ONOO− and to elucidate the possible physiological relevance of this phenomenon, we were prompted to investigate the possible role of endogenous ONOO− formation on L-arginine transport activity. Accordingly, we used astrocytes in primary culture incubated with LPS (1 μg/ml), an endotoxin that is well known to induce iNOS in these cells (9, 10) and to stimulate iNOS-dependent ONOO− formation (41). We have corroborated iNOS induction (Fig. 4A) as well as functional iNOS activity by measuring LPS-mediated, AMT-(50 μM) inhibitable nitrite released to the culture medium (results not shown) (42). Furthermore, endogenous ONOO− formation was also confirmed by immunocytochemical evidence for LPS-mediated 3-nitrotyrosine formation in these cells (Fig. 5B). Since ONOO− synthesis requires ‘NO reaction with O2− (43), to prevent ONOO− formation, ‘NO synthesis was inhibited in the LPS-treated cells by the iNOS-specific inhibitor AMT, thus preventing cellular 3-nitrotyrosination (Fig. 5C). Both negative control (i.e. degraded ONOO−) and positive control (authentic ONOO−) 3-nitrotyrosinated cells were carried out in parallel to confirm the specificity of 3-nitrotyrosination (Fig. 5, D and E). Immunoprecipitation of LPS-treated astrocytes with anti-3-nitrotyrosine followed by Western blotting failed to detect any Cat1-nitrated protein (not shown), a result that could be due to the low level of Cat1 nitration in this system.

As shown in Fig. 4C, LPS-treated cells showed enhanced L-arginine uptake activity in an L-arginine concentration-dependent fashion. At 50 μM extracellular L-arginine concentration, LPS-treated cells showed a 2.8-fold enhancement in the rate of L-arginine uptake (Fig. 4A) and a 1.5-fold increase in the rate of L-arginine release (Fig. 4B). Inhibition of iNOS activity with AMT (50 μM) significantly, but not fully, prevented LPS-mediated activation of L-arginine transport activity (Fig. 4, A and B). To elucidate the possible role for ‘NO on L-arginine transport activity, we used DETA-NO (0.1 mM), a compound widely used as a chemical source of ‘NO (44). DETA-NO (0.1 mM) was shown to continuously release 0.28 μM ‘NO for about 24 h in Hanks’ buffer at 37 °C as measured by an ‘NO-sensitive electrode. This steady-state ‘NO concentration is closely similar to that observed in LPS-treated astrocytes (15). Incubation of astrocytes with DETA-NO (0.1 mM) during 24 h did not change the rates of L-arginine uptake or release (Fig. 4, A and B). A degraded solution of DETA-NO was used as control, showing no alteration in these parameters when compared with untreated astrocytes (not shown).

Peroxynitrite-mediated L-Arginine Release from Astrocytes Is Taken up by Co-cultured Neurons—To further elucidate the potential physiological relevance of ONOO−-mediated activation of L-arginine transport in glial cells, we investigated whether, upon an ONOO− stimulus, astrocytic-released L-arginine could be taken up by neighboring neurons. Accordingly, [3H]arginine-preloaded insert-seeded astrocytes were co-incubated with neurons, and either degraded or active ONOO− (0.1 mM) was added. After 5 min, astrocyte-containing inserts were removed, and the radioactivity present in neuronal lysates was measured. As shown in Fig. 6, ONOO− treatment significantly increased L-[3H]arginine uptake by neurons. However, L-[3H]arginine uptake was unmodified by ONOO− treatment to neurons cultured alone (results not shown). Furthermore, the effect of ONOO− was enhanced when neurons were L-arginine-depleted prior to the co-culture with astrocytes (Fig. 6).

**DISCUSSION**

The studies carried out by Grima et al. (8, 18) first reported glutamate-stimulated L-arginine release from glial cells, possibly by acting on non-N-methyl-D-aspartate receptors. These authors suggested that this would be a neuronal-astrocytic signaling transduction pathway whose job would be to provide L-arginine for NOS activity within neighboring neurons (8, 18). In fact, sustained ‘NO production in astrocytes (7) as well as in macrophages (45) has been shown to be dependent of an efficient L-arginine (Cat2) transporter activity. Our later work in cultured astroglial cells showed that exogenously added peroxynitrite anion stimulated L-arginine release in a dose-dependent (from 50–1000 μM) and selective way (19), a protein carrier-mediated effect that was not mimicked by ‘NO nor by other oxygen-derived free radicals such as H2O2 or O2. Given the potential pathophysiological relevance of this possible neuronal-astroglial intercellular communication, the first aim of the present work was to elucidate the molecular mechanism leading to ONOO−-mediated activation of L-arginine transport in glial cells.

Despite the apparent complexity of the cationic amino acid
transport systems found in the brain (i.e. the Cat and Bat proteins), these can be identified on the basis of their interactions with inorganic ions, particularly with Na⁺ (22). Thus, to elucidate the Na⁺ dependence in the ONOO⁻-mediated stimulation of L-arginine release, we incubated C6 glial cells either in the presence or in the absence of Na⁺, and the effect of ONOO⁻ (100 μM) was evaluated. Our results showed that there was a 2-fold stimulation of L-arginine release, which was found to be independent of extracellular Na⁺. Since several Na⁺-independent cationic amino acid transporter systems can operate in glial cells (25, 28), we next investigated the specificity of the ONOO⁻-sensitive transport activity. In particular, system y⁺ for cationic amino acids appears to be a major Na⁺-independent carrier system in glial cells, although (and only in the presence of Na⁺) it does show a weak interaction with neutral amino acids (6, 7). Our results revealed that only in the presence of Na⁺ was ONOO⁻-mediated L-arginine stimulation abolished by both excess cationic (L-arginine and L-lysine) (19) and neutral (L-alanine and L-leucine) amino acids. By contrast, in the absence of Na⁺, only L-arginine, but not L-alanine or L-leucine, was able to abolish ONOO⁻-mediated L-arginine release. These results are consistent with the notion that system y⁺ could be a potential target of ONOO⁻.

The possibility that system y⁺ might be involved in the ONOO⁻-mediated stimulation of L-arginine transport was further corroborated by two sets of data, i.e. 1) the specific system y⁺ inhibitor, N-ethylmaleimide (38), dose dependently (k, of ~40 μM) abolished the ONOO⁻-mediated stimulation of L-arginine uptake; and 2) preloading of cells with L-arginine (10 mM) stimulated ONOO⁻-mediated L-arginine uptake. Since system y⁺ has been shown previously to be subject to transnitrosylation and tyrosine nitration as well as lipid peroxidation (39), it does show a weak interaction with neutral amino acids (6, 7).

It is well known that ONOO⁻ causes protein sulphydryl nitrosylation and tyrosine nitration as well as lipid peroxidation (39, 40). It could be speculated that ONOO⁻-stimulated L-arginine release would be due to a possible modification in the system y⁺ L-arginine transporter Cat1. Interestingly, ONOO⁻-induced protein nitration in both C6 cells and astrocytes, as judged by the observed enhancement in 3-nitrotyrosine immunoreactivity. Noticeably, and in particular, ONOO⁻-nitrated an anti-Cat1-immunopositive band, strongly suggesting ONOO⁻-mediated Cat1 3-nitrotyrosination. Unfortunately, and possibly due to a low level of nitrated Cat1
To investigate the possible physiological role for ONOO\(^{-}\)-mediated stimulation of L-arginine transport activity, we carried out two sets of experiments, \textit{i.e.} to modulate L-arginine transport activity by endogenously produced ONOO\(^{-}\) in astrocytes and to investigate L-arginine trafficking from astrocytes to neurons upon an ONOO\(^{-}\) stimulus. Thus, our results showed that incubation of astrocytes with LPS caused iNOS activity-dependent 3-nitrotyrosination, suggesting ONOO\(^{-}\) formation in these cells (41). Furthermore, ONOO\(^{-}\)-producing astrocytes showed increased L-arginine transport, consistent with former results obtained by Schmidlin and Wiesinger (35). In this study, inhibition of iNOS activity by AMT partially prevented LPS-mediated increase in transport activity (see also Ref. 35). As indicated by Cat1 mRNA levels, there was no modification in Cat1 expression in LPS-treated cells (see also Ref. 7). In addition, \textit{\textasciitilde}NO cannot be responsible for the AMT-inhibitable LPS-mediated increase in transport activity because exposure of cells to \textit{\textasciitilde}NO under conditions closely similar to those obtained with LPS treatment (\textit{i.e.} using 0.1 mM DETA-\textit{NO} for 24 h) failed to alter L-arginine transport activity. Therefore, since both \textit{\textasciitilde}NO and \textit{\textasciitilde}O\(_2\) are necessary for endogenous ONOO\(^{-}\) synthesis (41), our data are consistent with the iNOS-dependent Cat1 nitration and activation by ONOO\(^{-}\). Whether induction of the other system \(\gamma\), Cat2, by LPS treatment would be responsible for the iNOS activity-independent increase in L-arginine transport is unknown. However, this would be a plausible hypothesis since Stevens \textit{et al.} (7) have shown that \textit{\textasciitilde}NO biosynthesis in astrocytes upon LPS treatment is dependent on induced alternatively spliced Cat2 encoding L-arginine transport in astrocytes.

Finally, we have studied the possible trafficking of L-arginine between astrocytes and neurons. Thus, whereas ONOO\(^{-}\) failed to increase L-arginine uptake in neurons when cultured alone, using a co-culture system L-arginine released from ONOO\(^{-}\)-treated astrocytes was efficiently taken up by neurons. Furthermore, ONOO\(^{-}\)-mediated neuronal uptake of astrocytic-released L-arginine increased when neuronal L-arginine was depleted previously, thus highlighting a potential physiological relevance of this phenomenon under L-arginine-starving conditions.

In conclusion, our results show that both exogenous and endogenous ONOO\(^{-}\) stimulates L-arginine transport in glial cells, possibly as a direct effect on the \(\gamma\) L-arginine transporter system. Thus, when the NOS substrate L-arginine becomes a limiting factor for \textit{\textasciitilde}NO synthesis, ONOO\(^{-}\) formation in neurons (17) would diffuse to astrocytes, thereby representing a possible intercellular signal designed to activate L-arginine release. In fact, here we show, for the first time, that L-arginine released from astrocytes is efficiently taken up by neurons in co-culture. Whether the reported glutamate-mediated increase in L-arginine release (8, 18) is brought about by endogenous ONOO\(^{-}\) production remains obscure, but our previous (19) and present results are indeed compatible with such a hypothesis. This mechanism would replenish cytosolic L-arginine to facilitate physiological \textit{\textasciitilde}NO instead of neurotoxic ONOO\(^{-}\) in neurons. If so, such a neuronal-glial interaction could help to prevent the propagation of neuronal death upon excess glutamate-receptor activation.

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