

Mechanisms of L-Cysteine Neurotoxicity*

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We review here the possible mechanisms of neuronal degeneration caused by L-cysteine, an odd excitotoxin. L-Cysteine lacks the omega carboxyl group required for excitotoxic actions via excitatory amino acid receptors, yet it evokes N-methyl-D-aspartate (NMDA)-like excitotoxic neuronal death and potentiates the Ca^{2+} influx evoked by NMDA. Both actions are prevented by NMDA antagonists. One target for cysteine effects is thus the NMDA receptor. The following mechanisms are discussed now: (1) possible increase in extracellular glutamate via release or inhibition of uptake/degradation, (2) generation of cysteine α -carbamate, a toxic analog of NMDA, (3) generation of toxic oxidized cysteine derivatives, (4) chelation of Zn^{2+} which blocks the NMDA receptor-ionophore, (5) direct interaction with the NMDA receptor redox site(s), (6) generation of free radicals, and (7) formation of S-nitrosocysteine. In addition to these, we describe another new alternative for cytotoxicity: (8) generation of the neurotoxic catecholamine derivative, 5-S-cysteinyl-3,4-dihydroxyphenylacetate (cysdopac).

KEY WORDS: L-Cysteine; neurotoxicity; N-methyl-D-aspartate receptors; free radicals; catecholamines.

INTRODUCTION

The role of L-cysteine in the central nervous system is not wholly understood. It is a rate-limiting precursor for glutathione synthesis in neurons (1–3) and provides inorganic sulfate for detoxification reactions (4). It may thus be involved in neuroprotection (5). It also protects nerve cells by forestalling the entry of heavy metal ions into the brain across the blood-brain barrier (6). In addition to this, L-cysteine may play a role as a neuro-modulator, since it is released from brain slices upon

depolarization in a Ca^{2+} -dependent manner (7,8), it excites neurons (9) and is taken up by both neurons and glial cells (1).

An excess of L-cysteine has proved neurotoxic in vivo in developing animals with a still immature blood-brain barrier (10) and in cultured neurons in vitro (11). L-Cysteine must thus also be considered a potent excitotoxin (9–16), comparable in its potency to other excitatory amino acids. Administration of exogenous L-cysteine even evokes behavioral deficits (17). These excitotoxic actions have been implicated in the pathogenesis of several neurological disorders, e.g., amyotrophic lateral sclerosis, and Parkinson's, Alzheimer's (4) or Hallervorden-Spatz diseases (18) and in hypoxic/ischemic and hypoglycemic brain damage (16,19–21). Neuronal damage produced by L-cysteine may thus be of great clinical importance, but little is known as to the causative mechanism(s) (9). In the present article, we endeavor to review the most plausible mechanisms which may mediate the toxic effects of L-cysteine in the central nervous system.

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Release of Cysteine in Hypoxia and Hypoglycemia.

It is reasonable to think that the toxicity of L-cysteine manifests itself only when there is an excess in the extracellular fluid of the brain, since the compound is an endogenous naturally occurring constituent in all cells. Such is the situation in the case of ischemic brain damage. The total tissue concentrations of L-cysteine approach 700 μM (21). In cerebral ischemia it is released into the extracellular space mainly from glial cells. Li and associates (22) recently reported that the net efflux of L-cysteine, i.e., the difference between release and uptake, is increased in anoxia/aglycemia by a mechanism which involves γ -glutamyltransferase (γ -GT). The increased net efflux of L-cysteine could then be due to the breakdown of glutathione (GSH) by γ -GT rather than to the enhanced release alone. The breakdown of GSH by γ -GT may be massive during sustained ischemia in vivo (22). The decrease in extracellular L-cysteine by acivicin (an inhibitor of γ -GT) during anoxia/aglycemia corresponds quantitatively to the increase in GSH. The same authors also report a massive increase in cysteine sulfinate (CSA) during anoxia/aglycemia, which is likewise attenuated by inhibition of γ -GT. These changes are, at least in part, related to the alterations in extracellular L-cysteine oxidized to CSA, possibly by the free radicals generated (22). During the reperfusion period following ischemia, spontaneous oxidation and overproduction of free radicals is likely to occur. Another possibility is that L-cysteine oxidation to CSA is catalyzed by metal ions such as iron during anoxia/aglycemia. The increase in extracellular CSA in ischemic conditions may thus reflect enhanced formation of free radicals and could be used as an indirect measure of oxidative stress in that case. CSA however, like the parent molecule L-cysteine, is also a potent excitotoxin (see below).

Interactions of L-Cysteine with N-Methyl-D-Aspartate Receptors. Activation of the N-methyl-D-aspartate (NMDA) receptors evokes Ca^{2+} influx through the associated ionophore. While this Ca^{2+} signal is involved, e.g., in neuronal plasticity and memory processes (23), an excessive influx of Ca^{2+} is apparently the major cause of glutamate toxicity. Both prolonged overexcitation of NMDA receptors by excitatory amino acids and prolonged alleviation of Mg^{2+} or Zn^{2+} blocks of the receptor-coupled ionophores play crucial roles in neuronal death. A torrent of Ca^{2+} via the open channels and a secondary release of Ca^{2+} from the intracellular stores lead to metabolic cascades which are deleterious to cells (24,25). They cause further depolarization, passive Cl^- influx, cation and water entry, mitochondrial dysfunction and generation of free radicals via activation of lipases (26). Activation of lipases and proteinases (27) and the reduced

expression of the glutamate receptor GluRB subunit which controls the selectivity of non-NMDA ion channels are then the main cause for membrane damage, cytoskeletal disruption and osmotic lysis (28,29). In addition to this, cerebellar granule cells produce nitric oxide (NO) in response to stimulation of their NMDA receptors (30). The Ca^{2+} which enters into neurons by means of activated NMDA receptors stimulates the constitutive form of nitric oxide synthase (NOS), generating the second messenger NO, which exhibits both neuroprotective and neurodestructive properties (31,32). There are thus a number of reasons why factors and processes which modulate Ca^{2+} influx through the NMDA receptor are of interest.

L-Cysteine destroys neurons when administered orally at high doses to infant mice (9). Damage is typically restricted to circumventricular regions which lack the blood-brain barrier. It evolves rapidly and the end-stage neuronal necrosis is reached within 2–3 hours. Paradoxically, lower doses cause a more devastating neurotoxic syndrome, which develops more slowly, but damages a greater number of brain regions, including cerebral cortex, hippocampus, caudate nucleus and thalamus (9). The cerebral cortex and hippocampus are the most vulnerable areas and neurons seem to be initially selectively lesioned (33). L-Cysteine causes a similarly widespread pattern of damage in the fetal rodent brain when administered orally or subcutaneously to the pregnant dam in late gestation (9). In light or electron microscopy neurons undergoing L-cysteine-induced degeneration have an identical appearance to those undergoing degeneration after exposure to glutamate or excitotoxic analogs of glutamate (9). The deleterious effect of L-cysteine thus resembles that of glutamate and NMDA. L-Cysteine also enhances the glutamate- and NMDA-evoked neuronal influx of Ca^{2+} (Fig 1). The competitive NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) prevents both cysteine neurotoxicity (9) and the Ca^{2+} -influx-enhancing effect (Fig. 2). In our experiments, the non-competitive NMDA antagonist dizocilpine, as well as the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), were effective inhibitors of the NMDA plus cysteine-evoked influx of Ca^{2+} .

The NMDA receptors are thus the most obvious targets for L-cysteine both as a neuromodulator and as a neurotoxin. However, this is an enigmatic excitotoxin. The results cited above indicate that it can at low concentrations selectively activate glutamate receptors, in particular those of the NMDA class, but it is unclear how this effect is produced (9). L-Cysteine may be a very weak NMDA agonist in its own right (34), but this may

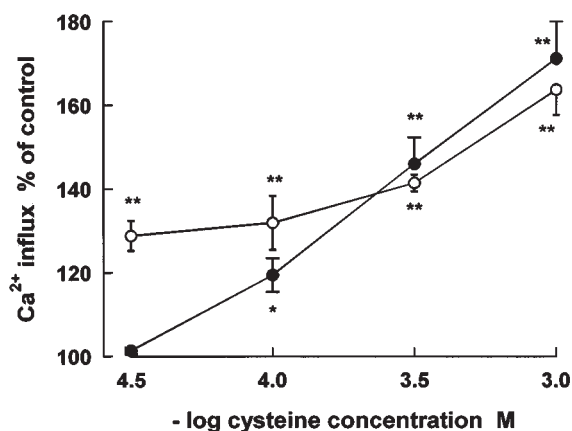


Fig. 1. Activation of NMDA- and glutamate-evoked calcium influx into cultured rat cerebellar granule cells by L-cysteine. Experiments with 1 mM glutamate (●) and 0.1 mM NMDA (○) in the presence of 0.81 mM $^{45}\text{CaCl}_2$ and varying concentrations of L-cysteine. Significantly different from the control: * $P < 0.05$; ** $P < 0.01$. Hermann et al. (53).

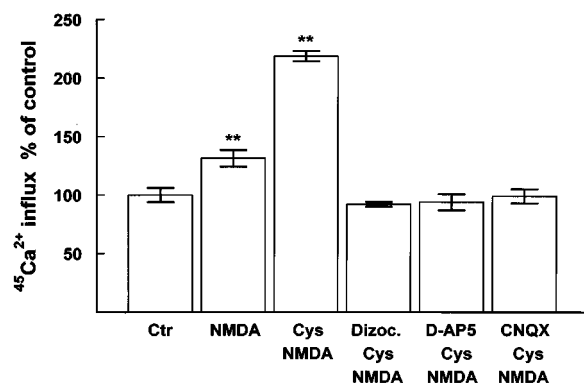


Fig. 2. Effects of NMDA and non-NMDA antagonists on the NMDA- and NMDA-plus cysteine-activated influx of $^{45}\text{Ca}^{2+}$ into rat cerebellar granule neurons. Concentrations: NMDA and cysteine 1 mM, dizocilpine, D-AP5 and CNQX 0.1 mM. Mean values with SEM of four independent experiments. Significantly different from the control: ** $P < 0.01$.

not explain its NMDA-like effects at low doses. In contrast to other excitotoxins, the L-cysteine molecule carries one acidic group instead of two and consequently does not obey the general structure-activity requirements of excitotoxins. However, the molecule has many other propensities, which may underlie its interaction with the NMDA receptors, as outlined in Fig. 3.

Free Radical Generation. L-Cysteine may generate oxygen free radical species by Haber-Weiss-type reactions when interacting with transition metal ions (35). The chemical reactivity of the sulfhydryl moiety may bring forth toxicity through auto-oxidation by generating

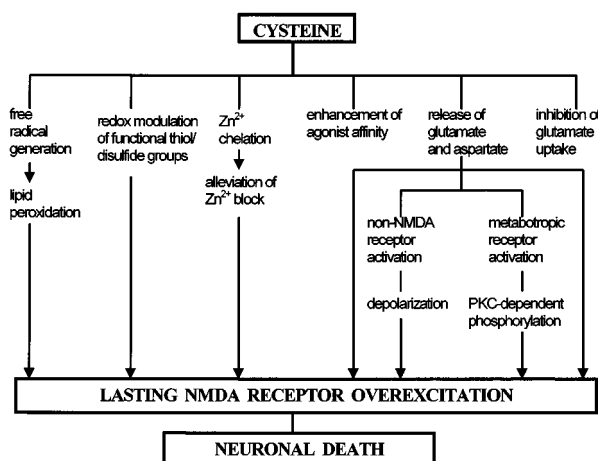


Fig. 3. Possible mechanisms of L-cysteine neurotoxicity mediated by overactivation of NMDA receptors.

hydrogen peroxide (36). The oxygen free radicals generated may cause lipid peroxidation and thence overactivation of NMDA receptors (see Fig. 3). On the other hand, cysteine may capture NO and form S-nitrosocysteine (cys-NO) (32). Cys-NO is neurotoxic in the absence of superoxide dismutase (SOD), because it generates NO^\bullet , which reacts with endogenous oxygen radicals, produces peroxynitrite (OONO^-) and consequently causes neuronal injury (37). Exposure of cultured hippocampal neurons to Cys-NO elevates intracellular Ca^{2+} and the recovery from the brief depolarization-induced increase in intracellular Ca^{2+} is delayed, indicating a perturbation of the cellular mechanisms maintaining Ca^{2+} homeostasis (38). The primary action of NO released from cys-NO is mimicked by oligomycin, an inhibitor of mitochondrial ATP synthetase. The effect is not additive to that of cys-NO. Brorson and colleagues (38,39) assume that NO released from cys-NO suffices to inhibit ATP synthesis by means of binding to Fe atoms in heme and Fe-S cluster-containing enzymes, or else that the generated peroxynitrite inactivates complexes I–III of the mitochondrial electron transport chain. In this manner the long-lasting release of NO and production of its by-product peroxynitrite can inhibit mitochondrial respiration and stop mitochondrial proton pumping, leading to rundown of the inner membrane potential which allows ATP synthesis and mitochondrial Ca^{2+} uptake via the Ca^{2+} uniporter (40,41).

The neurotoxicity of S-nitrosohomocysteine is paradoxically forestalled in rat cerebrocortical cultures by L-cysteine (42), which apparently acts as a NO sink via transnitrosylation. No release of free NO then occurs. SOD offers no further protection over excess L-cysteine at

millimolar concentrations. However, 1 mM of L-cysteine alone is precisely the killing threshold via NMDA receptors (42). In experiments in which L-cysteine itself is neurotoxic, it does not afford protection from S-nitrosohomocysteine toxicity. Neither does SOD block the neuronal death induced by the combination of S-nitrosohomocysteine and L-cysteine. In contrast, cysteine toxicity is abrogated by NMDA receptor antagonists such as dizocilpine and memantine (40).

Mild and short-lasting exposure to cys-NO leads to apoptosis, which is prevented by pretreatment with SOD and catalase (41). Moreover, in the presence of SOD, application of cys-NO attenuates the NMDA-evoked influx of Ca^{2+} , a prerequisite for NMDA receptor-mediated neurotoxicity (42,43). Under normal *in vivo* circumstances, SOD is present in the blood serum and extracellular fluid. The extracellular isoform of SOD is clearly different from its intracellular counterpart, but their functions are essentially the same, i.e., to inactivate reactive oxygen species (ROS) (44). In the presence of SOD, cys-NO ameliorates NMDA receptor-mediated neurotoxicity, because any NO^{\bullet} produced from homolytic cleavage of cys-NO is prevented by SOD from entering the neurotoxic pathway with $\text{O}_2^{\bullet-}$. Cys-NO can also react heterolytically and transfer NO^+ to thiol(s) in the redox modulatory site(s) of NMDA receptors, resulting in a nitrosothiol derivative. This process downregulates NMDA receptor activity, possibly through facilitation of disulfide formation (Fig. 4). If we think that NMDA receptor activation is followed by Ca^{2+} influx and consequent generation of NO via NOS activation, the small amounts of L-cysteine are neuroprotective by means of generation of cys-NO and a negative feedback mechanism. As we shall see, the greater amounts of L-cysteine, which are not buffered locally by NO, will be neurotoxic

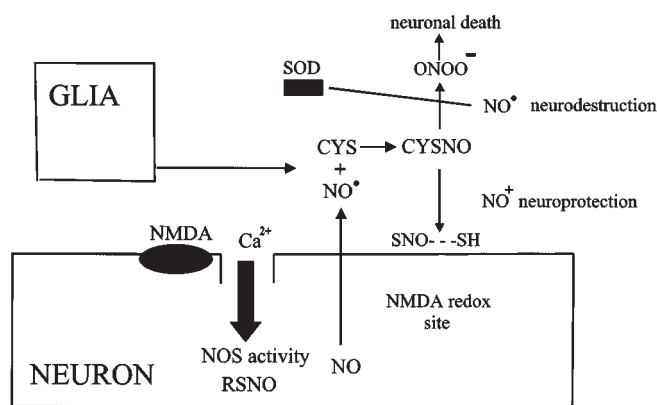


Fig. 4. Mechanism of neuroprotection and neurodestruction by S-nitrosocysteine. Modified from Lipton et al. (43) and Lipton and Stamler (32).

via chelation of Zn^{2+} or via a direct interaction with disulfide group(s) in the NMDA-receptor redox site(s). A definite balance between the neuronal release of glutamate and NO and the neuronal or glial release of L-cysteine as a cotransmitter or neuromodulator may thus settle the normal activation of NMDA receptors.

Accumulation of Glutamate in the Extracellular Space. The neurotoxicity of L-cysteine may also be mediated by means of elevated extracellular concentrations of glutamate. The rationale in this suggestion is that L-cysteine has been shown to inhibit glutamate reuptake in neurons (45), and production of free radicals may stimulate the release (46) or inhibit the uptake (47) of glutamate. Moreover, L-cysteine inhibits glutamate decarboxylase in the mouse brain *in vivo*, which allows an extracellular accumulation of glutamate (48). In our *in vitro* experiments L-cysteine evoked the release of preloaded labeled glutamate from hippocampal slices (Fig. 5A) and enhanced the release evoked by K^+ depolarization (Fig. 5B). However, the *in vivo* data of Puka-Sundvall and associates (33) are somewhat contradictory. They measured by means of microdialysis the extracellular levels of glutamate and other amino acids in the cerebral cortex after injection of a toxic dose of L-cysteine and found only minor changes in the glutamate levels. These authors therefore held it unlikely that L-cysteine could elicit neurotoxicity by increasing extracellular glutamate.

Direct Interaction with the Redox Site(s) in the NMDA Receptor. L-Cysteine may enhance the actions of excitatory amino acids at NMDA receptors by reducing the functional thiol/disulfide groups in the receptor-ionophores (32,49,50). Puka-Sundvall and coworkers (33)

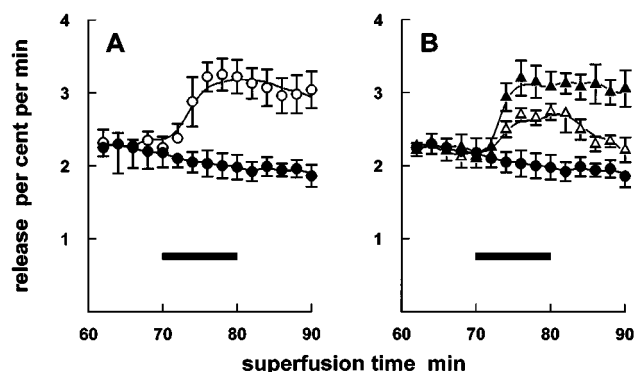


Fig. 5. Effects of L-cysteine on the (A) basal and (B) K^+ -stimulated release of $[^3\text{H}]$ glutamate from rat hippocampal slices. The bars indicate the presence of 1 mM cysteine and/or 50 mM K^+ from 70 to 80 min of superfusion. Control experiments without L-cysteine and K^+ stimulation (\bullet) and the effects of 1 mM L-cysteine (\circ), K^+ stimulation (Δ) and K^+ stimulation plus L-cysteine (\blacktriangle). Mean values with SEM of four independent experiments.

suggest that there obtains synergism between L-cysteine and glutamate, the main mechanism being potentiation of glutamate toxicity by cysteine-evoked reduction of the glutamate-activated NMDA receptor-ionophore complexes, as depicted in Fig. 3. However, relatively large concentrations of L-cysteine are apparently needed to cause any reduction in the NMDA receptors (49).

Chelation of Zn Ions. L-Cysteine may chelate the NMDA receptor-ionophore blocker Zn^{2+} , thus alleviating the voltage-independent Zn^{2+} block of the receptor. This is followed by a torrent of Ca^{2+} into the neurons (9,16,51–53). In our cerebellar granule cell cultures (53), the marked potentiation of NMDA-evoked Ca^{2+} influx (Fig. 1) and the glutamate-evoked elevation of intracellular free Ca^{2+} by L-cysteine (Fig. 6) are markedly attenuated by both the presence of Zn^{2+} and pretreatment with Zn^{2+} . In the experiments of Eimerl and Schramm (51), L-cysteine effectively potentiated glutamate toxicity and $^{45}\text{Ca}^{2+}$ influx. Almost maximal potentiation was already achieved at the 20- μM concentration. At such a low concentration it seems reasonable to ascribe the potentiating effect to the well-known ability of L-cysteine to bind metal ions and not to its reducing activity. For instance, the concentration of dithiothreitol must be in the order of 1 mM to cause comparable effects, and that compound is nevertheless markedly more potent than L-cysteine as a reducing agent (49).

Generation of Toxic Oxidized L-Cysteine Derivatives. In addition to the above possible mechanisms, L-cysteine is metabolized to toxic oxidized products, e.g., S-sulfocysteine (54), CSA and cysteate (13,19,22,55). Some of these also act at both ionotropic and metabotropic glutamate receptors (19,56,57) and may thus mediate the neurotoxic effects of L-cysteine (13). Other excitotoxic metabolites such as L-cysteine-S-sulfate and

L-cysteine sulfinic acid may mediate a minor part of this neurotoxicity (9). L-Cysteine sulfinic acid is a broad spectrum agonist at all excitatory amino acid receptors (58). The structural similarity to kainate is striking (Fig 7). Sulfocysteine is an excitotoxic glutamate analog, application of which to primary cultures of mouse neocortical and cerebellar neurons results in an increase in *c-fos* mRNA induction, which is accompanied by a release of cytosolic lactate dehydrogenase, used as an indicator of excitotoxicity (59).

L-Cysteine and L-cysteate have been reported to be harmless to hippocampal slices with a plentiful supply of oxygen and glucose but toxic to energy-deprived slices (20). In the latter, cysteine is twice as toxic as cysteate, probably due to a more broad-spectrum mechanism of action. The neurotoxicity of both compounds is antagonized by the competitive NMDA antagonist D-AP5, while the glycine antagonist 7-chlorokynureate blocks only cysteine toxicity. Neither of them is toxic in the absence of Ca^{2+} , while a high concentration of Mg^{2+} blocks only the neurotoxic effect of cysteate. This lack of inhibition by Mg^{2+} has been presumed to result from possible interactions of L-cysteine with the agonist-specific site which opens the NMDA-governed ion channel and with the Mg^{2+} -binding site which prevents the blockade of the NMDA receptor by divalent cations, or from the ability of L-cysteine to chelate Mg^{2+} (20). All direct evidence of both mechanisms is lacking, however. The ability of L-cysteine to chelate Zn^{2+} may be a more plausible explanation which also covers the stronger toxicity of L-cysteine when compared to that of L-cysteate (51). Cysteine and cysteate may also interact with different subtypes of NMDA receptors.

CSA may also act via inhibition of glutamate uptake (60) or via selective effects on phospholipase D-coupled glutamate receptors (61), provided that sufficiently high local concentrations are reached (22). Although the neurotoxic actions of oxidized cysteine derivatives are well

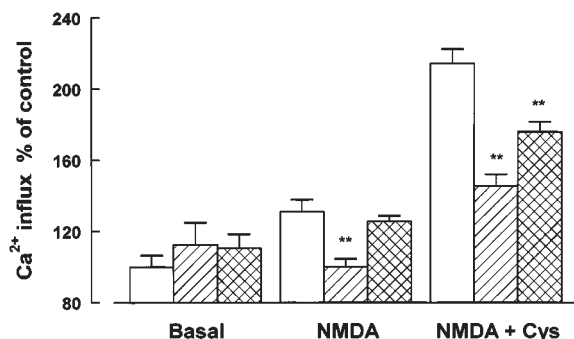


Fig. 6. Effect of 1 mM Zn^{2+} application (hatched bars) and pretreatment with 1 mM Zn^{2+} (cross-hatched bars) on the NMDA- and L-cysteine-evoked influx of $^{45}\text{Ca}^{2+}$ into cultured rat cerebellar granule cells. Control experiments (open bars). Significant effects of Zn^{2+} treatments: ** $P < 0.01$, Hermann et al. (53).

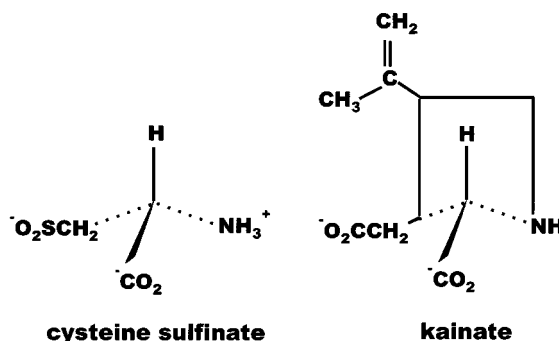


Fig. 7. The steric similarities of cysteine sulfinic acid and kainate. Modified from Max (65).

documented (13,55,62,63), results on their mediator role in cysteine-evoked neurotoxicity are still not consistent (19,20).

Generation of Cysteine α -Carbamate. CO_2 and L-cysteine may form an excitotoxic carbamate, though this is a hypothesis backed by only circumstantial evidence (9,64). Olney and his group (9) report that the NMDA-like neurotoxicity of L-cysteine is potentiated by physiological concentrations of hydrogen carbonate. The second pK_a of L-cysteine (associated with the amine function) is 8.37. Consequently, a proportion of L-cysteine will be deprotonated at physiological pH and be able to form an α -carbamate by reacting with the physiological hydrogen carbonate buffer system. The pK_a values of amino acids lacking the sulfhydryl group exclude the possibility of forming carbamates (65). In the case of L-cysteine, however, Nunn and associates (64) have confirmed by ^1H and ^{13}C NMR spectroscopy that a new molecular species is formed upon addition of hydrogen carbonate to solutions containing L-cysteine. They suggest that this indicates the formation of an α -carbamate. There is indeed a striking structural resemblance between the α -carbamate of L-cysteine and NMDA (Fig. 8). Activation of NMDA receptors by cysteine α -carbamate may thus be an important but so far largely overlooked mechanism for excitotoxicity. Such a mechanism of neurotoxicity has nevertheless been surmised to form the basis for chronic neurological degeneration in man (64).

Owing to an early reasoning of Olney and associates (13), it is often assumed that L-cysteine neurotoxicity is a consequence of its oxidation to cysteine sulfinic acid by way of cysteine dioxygenase (see above, Fig. 7). However, since the excitotoxicity of L-cysteine is increased in the presence of hydrogen carbonate (9), the alternative that the toxicity is due to carbamate formation must be seriously taken into account. These two mechanisms may not be mutually exclusive and certain data suggest that both of them may be operative (65). As discussed above, high levels of L-cysteine cause neuronal damage within 2–3 h and lower levels produce even more damage over 4–6 h, but in more numerous regions of the brain (9).

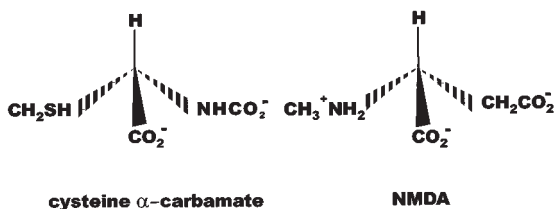


Fig. 8. Stereochemical similarities between the α -carbamate of cysteine and NMDA, a consequence of their opposite chiralities. Modified from Nunn et al. (64).

These two consequences may represent different toxicity mechanisms, because the NMDA antagonist D-AP5 blocks the neurotoxicity of low doses of cysteine, while that evoked by high doses is blocked by CNQX (Fig. 2). The toxicity of high doses of L-cysteine may thus involve 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors. Max (65) speculates that the low-dose mechanism may involve carbamate formation or chelation of Zn^{2+} (see above), while the high-dose mechanism involves diffusion of L-cysteine to additional sites of toxicity, followed by enzymatic oxidation to cysteine sulfinic acid. To his theory we would add the possibility that at high doses L-cysteine could elevate the extracellular concentration of glutamate (see above), which in turn can locally activate non-NMDA classes of glutamate receptors sensitive to CNQX (see Fig. 3).

Interaction of L-cysteine with catecholamines

Catecholamines such as dopamine, norepinephrine and epinephrine play essential roles in the control of emotion and movement, but can generate neurotoxins under certain conditions. In particular, dopamine induces cytotoxicity by means of the products of auto-oxidation, reactive free radicals and quinone. Reactive free radicals such as superoxide and hydroxy radical can damage lipids, proteins or DNA and cause cell death. The free radical-mediated toxicity of dopamine is witnessed by the sensitivity of neurons to antioxidants such as catalase, SOD, ascorbate and N-acetyl-L-cysteine (66,67). However, the possibility has also been raised that the cytotoxic effects of catecholamines may be mediated by covalent binding of quinones to cysteine residues in proteins (68).

Here, however, there remains an unsettled conflict. Cysteinylcatechols are toxic to P19 neuroglial cultures and to pyramidal neurons in organotypic cultures of the hippocampus (69), but dopamine neurotoxicity is attenuated by the thiol antioxidant N-acetyl-L-cysteine (68). While the SH group in N-acetylcysteine can detoxify reactive oxygen species, N-acetylcysteine and other thiol-containing compounds can attenuate the cytotoxicity of *o*-quinone, the oxidation product of dopamine, via covalent binding (70). Otherwise, the conjugation of *o*-quinone to the cysteine residues in proteins triggers neuronal apoptosis (68). In this respect, N-acetylcysteine is neuroprotective. In contrast to N-acetylcysteine, L-cysteine itself is neurodestructive in catecholaminergic systems, forming cysteinylcatechols by nucleophilic addition of cysteine to oxidized catechols (Fig. 9). In particular, 5-S-cysteinyl-3,4-dihydroxyphenylacetate

(cysdopac) has been shown to be particularly cytotoxic in differentiated P 19 neuroglial cultures (69). Cysdopac is also neurotoxic to hippocampal pyramidal neurons. Selective NMDA receptor antagonists ablate this effect. Electrophysiological experiments have failed to demonstrate NMDA receptor agonist activity for cysdopac, nor does cysdopac inhibit glutamate uptake. Instead, cysdopac has been proved to be an indirect excitotoxin, because it is a potent inhibitor of mitochondrial complex I activity. Mitochondrial dysfunction with impaired energy metabolism can then increase sensitivity to endogenous NMDA receptor agonists, thereby leading to indirect excitotoxic neurodegeneration by Ca^{2+} -dependent mechanisms (69). Catechylcatechol formation thus yields novel neurotoxins which could contribute to excitotoxic neurodegeneration. Such a mechanism, if operative in the pathogenesis or progression of Parkinson's disease, may explain the therapeutic efficacy of NMDA receptor antagonists in some Parkinsonian patients (71).

The extracellular levels of GSH and cysteine *in vivo* have remained unaltered when the rat striatum and substantia nigra were perfused with the active metabolite 1-methyl-4-phenylpyridinium (MPP^+) of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (72). However, a massive but transient increase in GSH occurred when the perfusion was discontinued, followed by a slightly delayed and smaller elevation in extracellular cysteine which could be blocked by the γ -GT inhibitor acivicin. The release and γ -GT and dipeptidase-mediated hydrolysis of GSH to glutamate, glycine and cysteine may thus be important factors in the degeneration of dopamine neurons (72). The toxicity of glutamate and glycine mediated by means of overactivated NMDA receptors is well documented, but the role of cysteine is intriguing in this animal model of Parkinson's disease. Damaged dopamine neurons should take up cysteine if they attempt to replenish

their intraneuronal GSH. The oxygen radical-mediated oxidation of dopamine generates dopamine-*o*-quinone, which reacts avidly with cysteine to form 5-S-cysteinyl-dopamine. We may assume that cysdopac is formed in a similar manner. In addition to this, the generation of several other potentially toxic cysteinylcatechols has been suggested (72). 5-S-Cysteinyl-dopamine is further oxidized by oxygen radicals to the dihydrobenzothiazine precursor 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxylate (DHBT-1). The final products, benzothiazines (72), can be accumulated by brain mitochondria (73) and be further metabolized to electrophilic intermediates which evoke a time-dependent irreversible inhibition of NADH-CoQ1 reductase by covalent modification of the key sulfhydryl residues in mitochondrial complex I (74). These toxic benzothiazines may thus contribute, together with cysdopac, to the delayed adverse effects on complex I and thence to degeneration of dopaminergic neurons.

CONCLUDING REMARKS

Although L-cysteine is a poorly elucidated excitotoxin, it may nevertheless be of significance in neurodegenerative disorders. L-Cysteine concentrations increase in the brain during ischemia and the extracellular levels are also elevated. Certain neurodegenerative diseases are associated with increased plasma concentrations of L-cysteine. Here we review several mechanisms which may underlie cysteine neurotoxicity. The cerebral concentration and distribution of L-cysteine may be a critical determinant for the outcome of glutamate-mediated overexcitation. The neurotoxicity of L-cysteine involves both pre- and postsynaptic interactions with glutamatergic neurotransmission. The enhanced release and production of excitatory agents catalyzed by cysteine dioxygenase is paralleled by the enhanced affinity of NMDA receptors for these agonists. These agents then act at the NMDA-governed ionophores, converting them to an open state by means of alleviation of Zn^{2+} , Mg^{2+} and disulfide blocks. The possible accumulation of extracellular glutamate may also activate metabotropic glutamate receptors, resulting in protein kinase C-dependent phosphorylation and activation of the NMDA receptor ionophores. Activation of non-NMDA receptors may help to alleviate the Mg^{2+} block by depolarization. These alterations result in a torrent of Ca^{2+} into neurons which is lethal. In the presence of catecholamines, cysteinylcatechols may also inhibit the mitochondrial respiration, leading to an increased sensitivity to endogenous NMDA agonists.

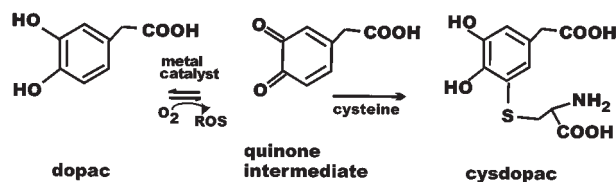


Fig. 9. Formation of 5-S-cysteinyl-3,4-dihydroxyphenylacetate (cysdopac). Repeated single electron oxidation of catechols to their corresponding quinones is catalyzed by metal ions such as Fe^{3+} , Cu^{2+} , and Mn^{2+} . The process is accompanied by the production of partially reduced oxygen species (ROS). The quinone readily accepts nucleophiles to form a catechol preferentially substituted at position 5. Modified from Montine et al. (69).

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