

Possible Mechanism for the Neuroprotective Effects of L-Carnitine on Methamphetamine-Evoked Neurotoxicity

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ABSTRACT: Some of the damage to the CNS that is observed following amphetamine and methamphetamine (METH) administration is known to be linked to increased formation of free radicals. This increase could be, in part, related to mitochondrial dysfunction and/or cause damage to the mitochondria, thereby leading to a failure of cellular energy metabolism and an increase in secondary excitotoxicity. The actual neuronal damage that occurs with METH-induced toxicity seems to affect dopaminergic cells in particular. METH-induced toxicity is related to an increase in the generation of both reactive oxygen (hydroxyl, superoxide, peroxide) and nitrogen (nitric oxide) species. Peroxynitrite (ONOO⁻), which is a reaction product of either superoxide or nitric oxide, is the most damaging radical. It can be reduced by antioxidants such as selenium, melatonin, and the selective nNOS inhibitor, 7-nitroindazole. METH-induced toxicity has been previously shown to increase production of the peroxynitrite stress marker, 3-nitrotyrosine (3-NT), *in vitro*, in cultured PC12 cells, and also *in vivo*, in the striatum of adult male mice. Pre- and post-treatment of mice with L-carnitine (LC) significantly attenuated the production of 3-NT in the striatum after METH exposure. LC is a mitochondriotropic compound in that it carries long-chain fatty acyl groups into mitochondria for β -oxidation. It was shown also to play a protective role against various mitochondrial toxins, such as 3-nitropropionic acid. The protective effects of LC against METH-induced toxicity could be related to its prevention of possible metabolic compromise produced by METH and the resulting energy deficits. In particular, LC may be maintaining the mitochondrial permeability transition (MPT) and modulating the activation of the mitochondrial permeability transition pores (mPTP), especially the cyclosporin-dependent mPTP. The possible neuroprotective mechanism of LC against METH-toxicity and the role of the mitochondrial respiratory chain and the generation of free radicals and their subsequent action on the MPT and mPTP are also being examined using an *in vitro* model of NGF-differentiated pheochromocytoma cells (PC12). In preliminary experiments, the pretreatment of PC12 cells with LC (5 mM), added 10 min before METH (500 μ M), indicated that LC enhances METH-induced DA depletion. The role of LC in attenuating METH-evoked toxicity is still under investigation and promises to reveal information regarding the underlying mechanisms and role of mitochondria in the triggering of cell death.

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ABBREVIATIONS:

| | |
|------|--|
| ALC | acetyl-L-carnitine |
| ANT | adenine nucleotide translocase |
| CoA | coenzyme-A |
| DA | dopamine |
| FA | fatty acid |
| LC | L-carnitine |
| METH | methamphetamine |
| mPTP | mitochondrial permeability transition pore |
| VDAC | voltage-dependent anion channel |

INTRODUCTION

Some of the damage to the CNS that is observed after amphetamine and methamphetamine (METH) administration is known to be linked to increased formation of free radicals. The METH-induced toxicity affects dopaminergic cells in particular. It is the release of the dopamine (DA) and formation of its metabolites that is thought to cause the augmentation of reactive oxygen species (ROS). In a recent *in vitro model*, METH toxicity in a clonal DA producing cell line (CATH.a) was shown to involve DA because DA release and DA synthesis inhibitors both attenuated METH toxicity.¹

This increase in ROS is thought to be related to mitochondrial dysfunction and/or cause the actual damage to the mitochondria (see FIGURE 1). Thus, it is the increased DA release which is thought to be the causal factor for the ensuing mitochondrial

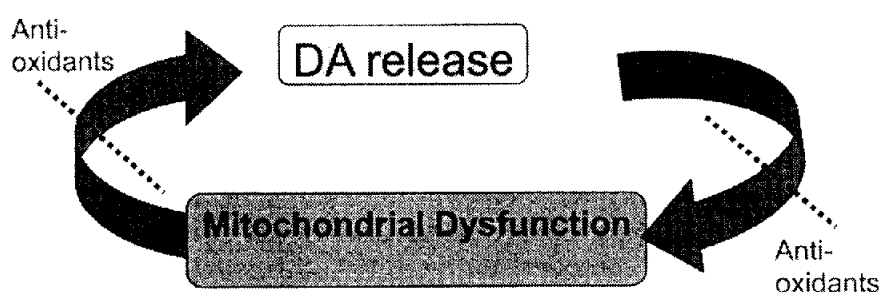


FIGURE 1. Methamphetamine toxicity causes dopamine release and mitochondrial dysfunction: which first? Since METH acts mainly at the dopaminergic nerve terminals the mechanism of its toxicity is thought to be via increased release of dopamine (DA). This extracellular DA would cause the formation of reactive free oxygen (OH , O_2^- , and H_2O_2) and nitrogen (NO) radicals, which damage the mitochondria. Both superoxide and nitric oxide can generate the most damaging radical—peroxynitrite (ONOO^-). However, METH may also cause mitochondrial dysfunction directly, which would lead to neuronal depolarization and, therefore, indirectly cause the release of DA. Both pathways would be attenuated by antioxidants.

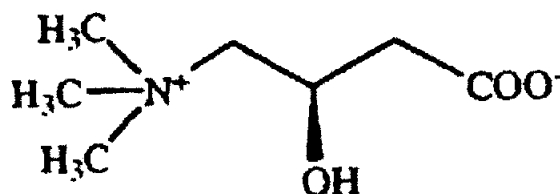


FIGURE 2. Structure of L-carnitine, a naturally occurring quaternary ammonium compound (trimethylamino β -hydroxybutyrate). It usually exists in solution as an amphiphilic zwitterion.

dysfunction and actual organelle damage, thereby leading to a failure of cellular energy metabolism and an increase in secondary excitotoxicity.^{2,3} The final result is a failure of cellular energy metabolism and an increase in secondary excitotoxicity. The other possibility is that METH may firstly cause mitochondrial dysfunction first that later leads to excessive DA release. In both cases the mitochondriotropic agents, such as L-carnitine (see FIGURE 2), may be able to exert their neuroprotective action against METH toxicity by action at the level of either DA release or mitochondrial function. This possibility is discussed in more detail in this paper.

METH-TOXICITY CAUSES DOPAMINE RELEASE AND MITOCHONDRIAL DYSFUNCTION; WHICH FIRST?

Investigators have suggested that the actual neuronal damage that occurs with METH-induced toxicity is linked with dopaminergic cells in particular.⁴ It has been suggested that this increase in ROS is related to the DA release and formation of its metabolites. It is also known that METH-induced toxicity is related to an increase in the generation of both reactive oxygen (hydroxyl, superoxide, peroxide) and nitrogen (nitric oxide) species (see FIGURE 3). It is the peroxynitrite (ONOO^-) radical, a reaction product of either superoxide or nitric oxide, that is the most damaging radical. These ROS species can be reduced by antioxidants, such as selenium, melatonin, and the selective nNOS inhibitor, 7-nitroindazole.^{3,6,7}

MITOCHONDRIAL DYSFUNCTION AND CELL DAMAGE

It is the peroxidation of lipids, proteins, and DNA that eventually leads to the mitochondrial and cellular damage. The outcome of the cellular damage could be that the cell is able to repair itself or it may go into a sort of survival mode (a less functional state or hibernation state). However, depending on the level of insult and the metabolic capacity of the cell, it may be overwhelmed and cellular death may occur.⁸

The consequences of mitochondrial dysfunction are very similar. They may force the cell to go into a less functional state or trigger apoptotic or finally necrotic cell death, depending on the severity of the insult.⁵

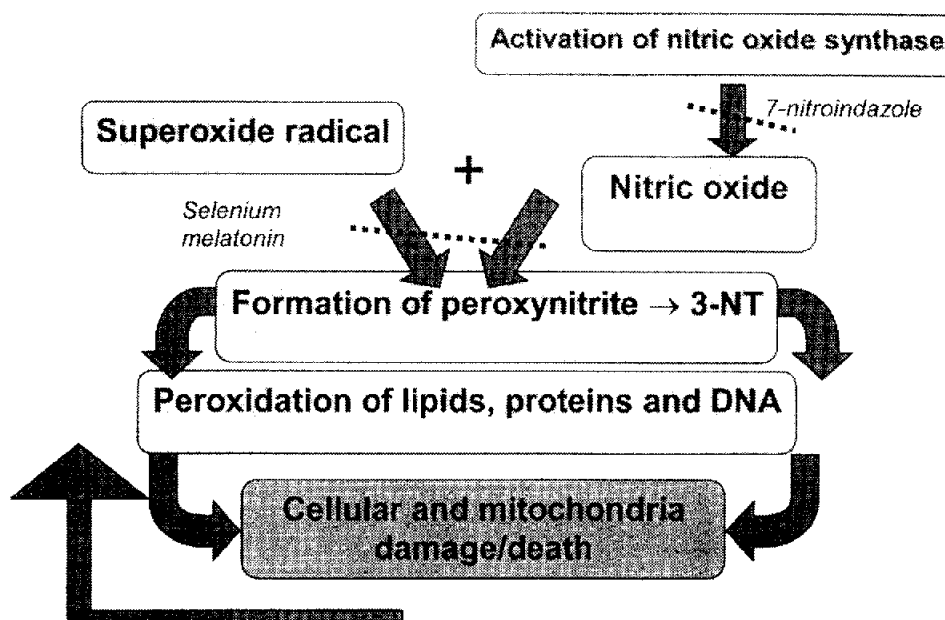


FIGURE 3. Factors leading to mitochondrial dysfunction. METH-induced toxicity is related to a cascade of events that generate both reactive oxygen (hydroxyl, superoxide, peroxide) and nitrogen (nitric oxide) species. It is the formation of peroxynitrite (ONOO^-), a reaction product of either superoxide or nitric oxide, that is the most damaging radical. The peroxynitrite radical can be detected by looking for the 3-nitrotyrosine (3-NT) marker. The generation of the various radicals can be reduced by antioxidants, such as selenium, melatonin, and the selective nNOS inhibitor, 7-nitroindazole. (Adapted from Ref. 5.)

CAN FACTORS ANTAGONIZING MITOCHONDRIAL DYSFUNCTION PREVENT OR REDUCE METH-TOXICITY?

METH-induced toxicity has been previously shown to increase production of the peroxynitrite stress marker, 3-nitrotyrosine (3-NT), *in vitro*, in cultured PC12 cells, and also *in vivo*, in the striatum of adult male mice.³ Pre- and post-treatment of mice with L-carnitine (LC) significantly attenuated the production of 3-NT in the striatum after METH exposure.⁹ METH-evoked toxicity was also attenuated by substrates of energy metabolism, for example, decylubiquinone or nicotinamide.^{10,11}

POSSIBLE MECHANISM BY WHICH L-CARNITINE REDUCES METH-TOXICITY

LC is a mitochondriotropic compound in that it carries long-chain fatty acyl groups into mitochondria for β -oxidation (see FIGURE 4). It was shown also to play a protective role against various mitochondrial toxins, such as 3-nitropropionic acid.^{12,13} The protective effects of LC against METH-induced toxicity could be related to its prevention of possible metabolic compromise produced by METH and the resulting energy deficits.

The primary physiological functions of LC are:

- it serves as the “carrier” to transport long-chain fatty acids (FA) across the inner mitochondrial membrane and into mitochondria where they can be used as an energy substrate;
- it increases free coenzyme-A (CoA) that is essential for lipid and carbohydrate utilization by combining with acetyl and transporting it out of the mitochondria; and
- it transports potentially toxic acyl compounds out of the mitochondria and cell, enabling their subsequent excretion in urine.

LC is also important in maintaining cell membrane stability through their involvement in acetylation of membrane phospholipids and amphiphilic actions.^{9,13} Thus, part of the protection provided by the L-carnitines could be linked to the amphiphilic nature of this molecule and its interaction with the surface charges on the cell membrane.^{14,15} The carnitines have at least two binding sites, one recognizing the trimethylamino group and a second, the carboxylic group, that can interact directly with the charges on the membrane phospholipids, glycolipids, and proteins.¹⁵ The amphiphilic properties of LC, acetyl-L-carnitine (ALC), and propionyl-L-carnitine enable interaction with these binding sites on the membranes, thereby affecting the membrane negative surface potential and other membrane characteristics affecting expression of receptors in the membrane or the interaction of other compounds with the cell membrane. Alternatively, others have proposed that ALC may affect the membrane directly and in some way make it more resistant to damage.¹⁴⁻¹⁶ Part of this process

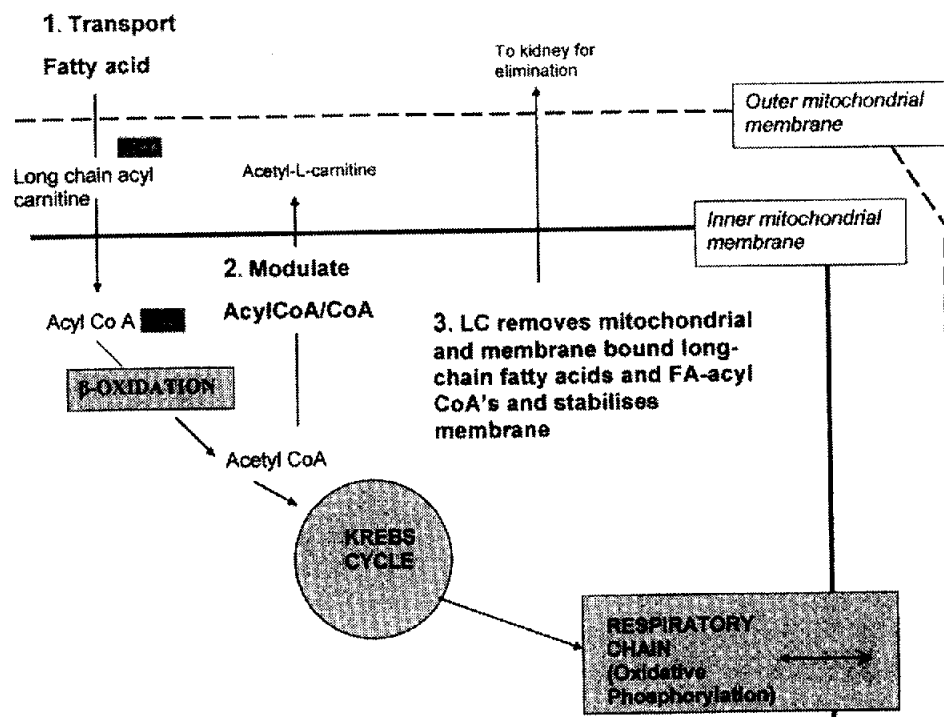


FIGURE 4. The major functions of L-carnitine at the metabolic level in the cell and mitochondria are to transport fatty acids into mitochondria, modulate acyl-CoA levels, and remove long-chain fatty acids and acyl-CoAs from the mitochondria.

* may also involve changes in membrane fluidity, thereby affecting the membrane phase transition behavior.¹⁵ ALC has also been reported to increase fluidity in rat brain microsomes and liposomes.¹⁷

LC prevents the detergent-like effect of long-chain fatty acids by clearing them from the mitochondria and preventing their interaction with the phospholipids as mentioned above. Rats treated with octanoic acid displayed extensive ultrastructural disruptions in the epithelial cells of the choroid plexus.¹⁸ Octanoic acid disrupts the mitochondria structure and inhibits the organic anion transport system in the choroid plexus. The activity of the mitochondrial enzyme cytochrome oxidase was greatly reduced following octanoic acid (1,000mg/kg), but this was prevented by the pre-treatment with an equimolar dose of LC given i.p. 30min prior to the octanoic acid injection. This study suggested that LC supplementation could restore mitochondrial function of the choroid plexus subjected to toxic organic anions in metabolic disorders, and may, therefore, be useful in the prevention of metabolic encephalopathy.

The carnitine system also plays a role in the mitochondrial elongation-desaturation of the omega 3 polyunsaturated fatty acids to DHA.¹⁹ Thus ALC may increase the synthesis of phospholipids required for membrane formation and integrity. It plays a role in the membrane repair by reacylation of phospholipids.²⁰

CELL FUNCTION AND ALTERATION OF MITOCHONDRIAL PERMEABILITY TRANSITION PORES

A number of cellular functions are affected by the changes in the mitochondrial membrane permeability; indeed this process could be a major trigger in the METH-evoked cell toxicity process (see FIGURE 5). Indeed, excess DA metabolites may trigger apoptosis by this process. A recent study showed that the permeability changes evoked by dopamine were linked to its toxicity.²¹ They found that DA reduced the mitochondrial membrane potential. Cyclosporin A, a blocker of mPTP, prevented this decrease but did not rescue the cells from dopamine toxicity.

Thus, a possible mechanism for the neuroprotective action of LC could be at this mitochondrial level. The protective effect of LC against METH-neurotoxicity may be via effects on mitochondrial oxidative phosphorylation and reduced formation of free radical species. In particular, LC may be maintaining the mitochondrial permeability transition (MPT) and modulating the activation of the mitochondrial permeability transition pores (mPTP), especially the cyclosporin-dependent mPTP.²²

Physiologically hormones such as thyroid hormone regulate mPTP (see FIGURE 6) and therefore cellular metabolism; furthermore these alterations in the mPTP affect cytochrome C, procaspases, ROS, and apoptosis inducing factor (AIF) release and, therefore, apoptosis. A number of other factors, such as mitochondrial depolarization, oxidative damage, and other cell factors, for example, long-chain FAs, can cause mitochondrial membrane permeability transition (mPT). The actual permeability was thought to depend on pores that are contact sites between the inner and outer membranes. They were thought to be non-specific pores, permeant to any molecule of less than 1.5kDa. However, recent research suggests that these pores are in fact junctional complexes of the adenine nucleotide translocase (ANT; inner membrane) and the voltage-dependent anion channel (VDAC; outer membrane) (see FIGURE 7).

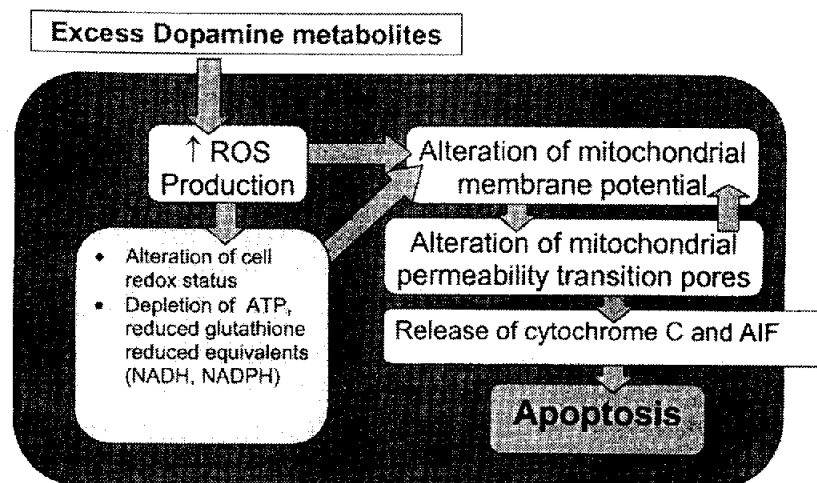


FIGURE 5. Dopamine and mitochondrial dysfunction. The possible sequence of events that underlie the toxicity due to excessive DA release in the CNS. The DA metabolites are thought to cause an increase in the reactive oxygen load, which leads to alterations in the mitochondrial membrane and opening of the pores. This allows the escape of cytochrome C, procaspases, ROS, and the apoptosis inducing factor (AIF) into the cell cytoplasm, triggering apoptosis. (Adapted from Ref. 8.)

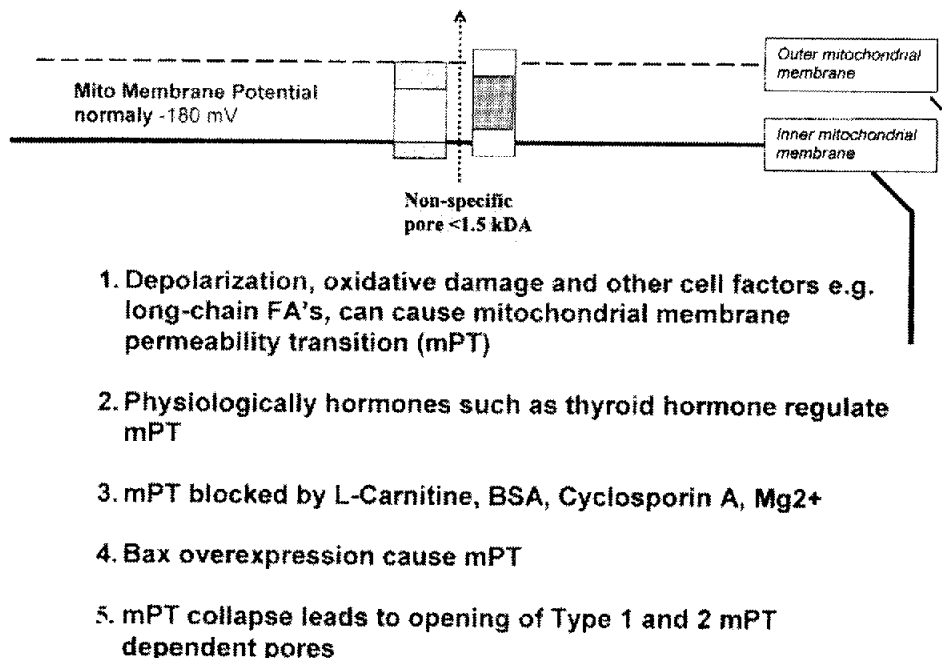
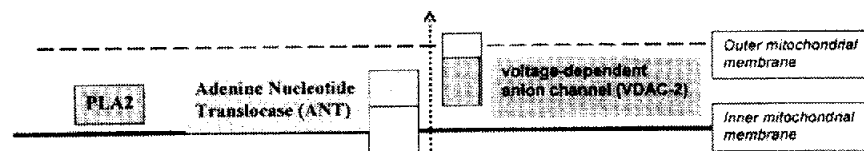


FIGURE 6. Various factors affect the mitochondrial membrane permeability transition and the subsequent opening of the mPTP. The level of membrane depolarization and state of membrane fluidity are the major determining factors. This process is modulated physiologically by hormones, such as thyroid hormone, to effect changes in mitochondrial metabolism, but can also be affected by a number of other cellular factors and pharmacological agents. Bovine serum albumin (BSA), L-carnitine, cyclosporin A, and magnesium (Mg^{2+}) are able to block the mitochondrial membrane transition.



1. Pores are junctional complexes of the ANT and VDAC
2. Act as multifunctional recruitment centres, binding proteins involved in energy and lipid metabolism + other less well defined proteins.
3. mPTP is affected by accumulation of pre-proteins e.g. mtHSP70, HSP60, MnSOD, RM12 as well as \uparrow Bax and \downarrow chaperone proteins
4. Opening of mPTP, the life-or-death switch of cells, \uparrow permeability
5. Result \downarrow in adenine nucleotides and energy production
6. Escape of apoptogenic factors - cytochrome c, apoptosis inducing factors (AIFs), procaspases and ROS. This triggers **APOTOSIS**
7. \uparrow glyco-peroxidation and lipoperoxidation products
8. Loss of Ca^{2+} buffering and activation of mitochondrial phospholipase A(2) \uparrow hydrolysis of phospholipids
9. Swelling of mitochondria and rupture of outer mito membrane. This causes cell death by **NECROSIS**

FIGURE 7. The sequence of events following the opening of the mPTP can lead to cell death by apoptosis or necrosis. The junctional complex that forms the pore (mPTP) can be modulated by a number of proteins involved in energy and lipid metabolism and the increased opening of these pores could lead to escape of proapoptotic factors triggering cell death by apoptosis. Further loss in permeability control leads to accumulation of intra-mitochondrial calcium (Ca^{2+}) and activation of phospholipase, which eventually causes a swelling and rupture of mitochondrial membrane and cell death by necrosis.

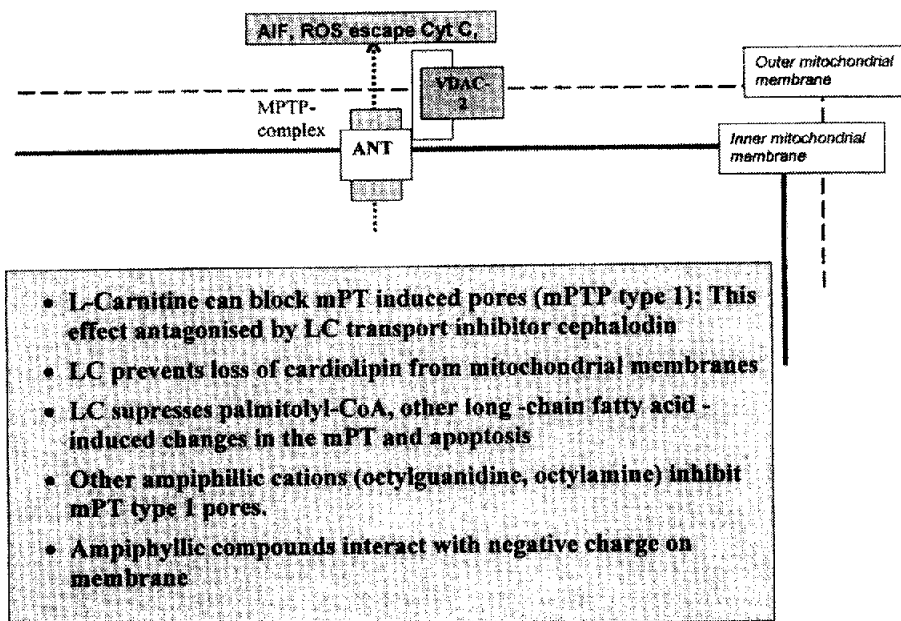


FIGURE 8. Possible mechanism by which L-carnitine (LC) may modulate and attenuate cell toxicity via effects on mPTP. LC can modulate mitochondrial membrane transition (mPT) by directly blocking mPTP as well as preventing mPT due to accumulation of long-chain fatty acids. This property is probably related to the amphiphilic nature of the LC molecule.

These act as multifunctional recruitment centers, binding a range of proteins involved in energy and lipid metabolism. Also, other less well defined proteins are recruited. Thus, mPTP is affected by accumulation of preproteins, for example, mtHSP70, HSP60, MnSOD, and RM12, as well as increase in Bax and decrease in chaperone proteins. The opening of mPTP is thought to be the life-or-death switch of cells. The sequence of events following the opening of the mPTP can lead to cell death by apoptosis or necrosis. In the final steps, the loss of Ca^{2+} buffering and activation of mitochondrial phospholipase A(2) would lead to the hydrolysis of phospholipids and the swelling of mitochondria and rapture of the outer mitochondrial membrane.

ROLE OF L-CARNITINE IN MPTP OPENING AND NEUROPROTECTION

LC may be maintaining the mitochondrial permeability transition (mPT) and modulating the activation of the mitochondrial permeability transition pores (mPTP), especially the cyclosporin-dependent mPTP (see FIGURE 8).²² L-Carnitine can block mPT induced pores (mPTP type 1) and this effect is antagonized by LC transport inhibitor cephalodin. Other ways in which it may modulate mPTP could be preventing loss of cardiolipin from mitochondrial membranes and LC also suppresses palmitoyl-CoA and other long-chain fatty acid-induced changes. Other amphiphilic cations (octylguanidine, octylamine) also inhibit mPT type 1 pores. Amphiphilic compounds like LC may also affect the mitochondrial membrane properties directly by interacting with negative charges on membrane.

CONCLUSION

Thus, the modulation of mitochondrial membrane permeability and pore (mPTP) opening by LC may be a possible mechanism by which it modulates cell toxicity caused by METH. This possible neuroprotective mechanism of LC against METH-toxicity and the role of the mitochondrial respiratory chain and the generation of free radicals and their subsequent action on the MPT and mPTP is being examined using an *in vitro* model of NGF-differentiated pheochromocytoma cells (PC12). In preliminary experiments, the pretreatment of PC12 cells with LC (5mM), added 10min before METH (500 μM), indicated that LC enhances METH-induced DA depletion. The role of the carnitines, especially LC in attenuating METH-evoked toxicity, is still under investigation and promises to reveal information regarding the underlying mechanisms and role of mitochondria in the triggering of cell death.

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