The Protective Role of L-Carnitine against Neurotoxicity Evoked by Drug of Abuse, Methamphetamine, Could Be Related to Mitochondrial Dysfunction

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ABSTRACT: There is growing evidence that suggests that brain injury after amphetamine and methamphetamine (METH) administration is due to an increase in free radical formation and mitochondrial damage, which leads to a failure of cellular energy metabolism followed by a secondary excitotoxicity. Neuronal degeneration caused by drugs of abuse is also associated with decreased ATP synthesis. Defective mitochondrial oxidative phosphorylation and metabolic compromise also play an important role in atherogenesis, in the pathogenesis of Alzheimer's disease, Parkinson's disease, diabetes, and aging. The energy deficits in the central nervous system can lead to the generation of reactive oxygen and nitrogen species as indicated by increased activity of the free radical scavenging enzymes like catalase and superoxide dismutase. The METH-induced dopaminergic neurotoxicity may be mediated by the generation of peroxynitrite and can be protected by antioxidants selenium, melatonin, and selective nNOS inhibitor, 7-nitroindazole. L-Carnitine (LC) is well known to carry long-chain fatty acyl groups into mitochondria for β-oxidation. It also plays a protective role in 3-nitropropioinc acid (3-NPA)-induced neurotoxicity as demonstrated *in vitro* and *in vivo*. LC has also been utilized in detoxification efforts in fatty acid–related metabolic disorders.

In this study we have tested the hypothesis that enhancement of mitochondrial energy metabolism by LC could prevent the generation of peroxynitrite and free radicals produced by METH. Adult male C57BL/6N mice were divided into four groups. Group I served as control. Groups III and IV received LC (100 mg/kg, orally) for one week. Groups II and IV received 4 × 10 mg/kg METH i.p. at 2-h intervals after one week of LC administration. LC treatment continued for one more week to groups III and IV. One week after METH administration, mice were sacrificed by decapitation, and striatum was dissected to measure the formation of 3-nitrotyrosine (3-NT) by HPLC/Coularry system. METH treatment produced significant formation of 3-NT, a marker of peroxynitrite generation, in mice striatum.

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Ann. N.Y. Acad. Sci. 965: 225–232 (2002).

The pre- and post-treatment of mice with LC significantly attenuated the production of 3-NT in the striatum resulting from METH treatment. The protective effects by the compound LC in this study could be related to the prevention of the possible metabolic compromise by METH and the resulting energy deficits that lead to the generation of reactive oxygen and nitrogen species. These data further confirm our hypothesis that METH-induced neurotoxicity is mediated by the production of peroxynitrite, and LC may reduce the peroxynitrite levels and protect against the underlying mechanism of METH toxicity, which are models for several neurodegenerative disorders like Parkinson's disease.

KEYWORDS: L-carnitine; methamphetamine; neurotoxicity

INTRODUCTION

There is a growing body of evidence suggesting that brain injury observed after amphetamine and methamphetamine (METH) administration is due to an increase in free radical formation and mitochondrial damage, which leads to a failure of cellular energy metabolism followed by a secondary excitotoxicity.[10,11,17,20] Neuronal degeneration is also associated with decreased ATP synthesis. The energy deficits in the central nervous system can lead to the generation of reactive oxygen and nitrogen species as indicated by increased activity of the free radical scavenging enzymes like catalase and superoxide dismutase.[1,4]

Recently, we have reported that METH-induced dopaminergic neurotoxicity may be mediated by the generation of peroxynitrite and can be protected by antioxidants selenium, melatonin, and selective nNOS inhibitor, 7-nitroindazole.[2,10,11,13,14] L-Carnitine (LC) is a well-known molecule that carries long-chain fatty acyl groups into mitochondria for β-oxidation (FIG. 1). It also plays a protective role in 3-nitropropionic acid (3-NPA)-induced neurotoxicity as demonstrated *in vitro*[18] and *in vivo*.[3,5] In addition, LC has been utilized in detoxification efforts in

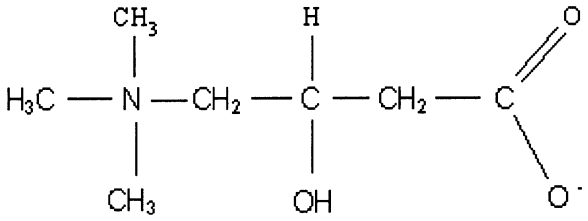

FIGURE 1. Structure of L-carnitine.

fatty acid–related metabolic disorders.[10] It has been shown to prevent mitochondrial damage induced in the rat choroid plexus by medium-chain fatty acid.[15]

In this study we have tested the hypothesis that enhancement of mitochondrial energy metabolism by LC could prevent the generation of peroxynitrite and free radicals produced by METH.

METHOD

Adult male C57BL/6N mice were divided into four groups:

- Group I served as control.
- Groups III and IV received LC (100 mg/kg, orally) for one week.
- Groups II and IV received 4 × 10 mg/kg METH i.p. at 2-h intervals after one week of LC administration.

LC treatment continued for one more week to groups III and IV.

One week after the METH administration, mice were sacrificed by decapitation, and striatum was dissected to measure the formation of 3-nitrotyrosine (3-NT) by HPLC/Coularry system.

RESULTS

The toxicity of METH has been shown previously to increase the production of 3-nitrotyrosine (3-NT) *in vitro,* for example, in cultured PC12 cells (FIG. 2) and also *in vivo,* as shown in the striatum of adult male mice (FIG. 3).

In the present experiment, METH treatment also produced significant formation of 3-NT, a marker of peroxynitrite generation, in the mice striatum (FIG. 4). The pre- and post-treatment of mice with LC significantly attenuated the production of 3-NT in the striatum resulting from METH treatment.

DISCUSSION

The *in vivo* toxicity of METH as manifested by the increase in the production of 3-NT in the striatum of mice was reduced by the pretreatment of the animals with LC. The METH-induced dopaminergic neurotoxicity is thought to be mediated by the generation of peroxynitrite radicals and can be protected by antioxidants selenium, melatonin, and selective nNOS inhibitor, 7-nitroindazole.[11,12] The protective effects by the compound LC in this study could be related to the prevention of the possible metabolic compromise by METH and the resulting energy deficits that lead to the generation of reactive oxygen and nitrogen species.

LC is required for the transport of activated acyls, namely, acyl-CoAs, across the inner mitochondrial membrane (FIG. 5). The LC requirement is related to the fact that fatty acids activated in the form of acyl-CoA outside the mitochondria cannot be imported into the mitochondrial matrix where the β-oxidation enzymes are located. In addition, by interacting with coenzyme A (CoA), LC exerts a role in any CoA-

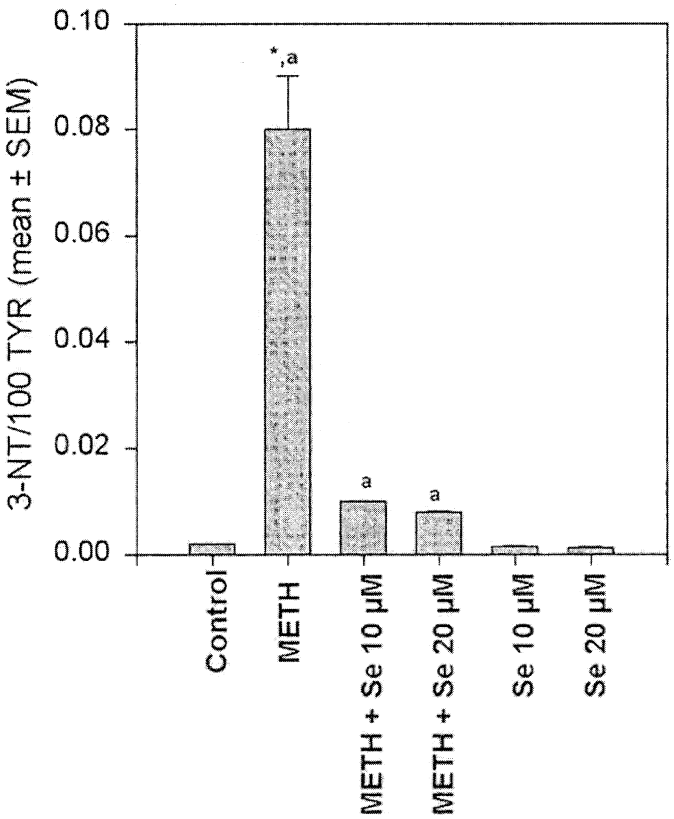

FIGURE 2. Methamphetamine-evoked (200 µM) production of 3-nitrotyrosine (3-NT) in PC 12 cells after 24 h in culture. (Adapted from Imam and Ali.[10])

dependent process. An increase in CoASH availability or a decrease in acyl CoA levels expands the roles of LC to substrate choice, removal of inhibitory metabolites, and modulation of key enzymatic steps.

Defective mitochondrial oxidative phosphorylation and metabolic compromise play an important role in atherogenesis, in the pathogenesis of Alzheimer's disease, Parkinson's disease, diabetes, and aging.[7] Various compounds, known to be detrimental, act on the respiratory chain. Thus, cholic acid in experimental atherogenic diets inhibits Complex IV; cocaine inhibits Complex I; the poliovirus inhibits Complex II; ceramide inhibits Complex III; azide, cyanide, chloroform, and methamphetamine inhibit Complex IV.[6,21] The METH-evoked toxicity has been shown to be attenuated by substrates of energy metabolism such as with decylubiquinone or

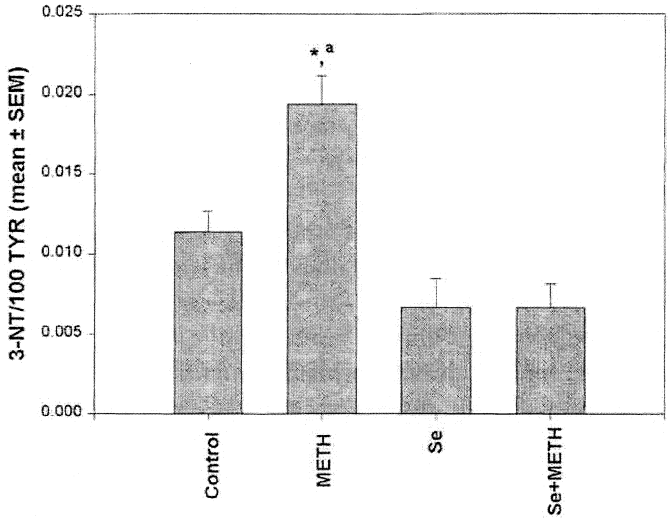

FIGURE 3. Methamphetamine-evoked (4 × 100 mg/kg i.p.) production of 3-nitrotyrosine in the striatum of adult male mice. (Adapted from Imam and Ali.[10])

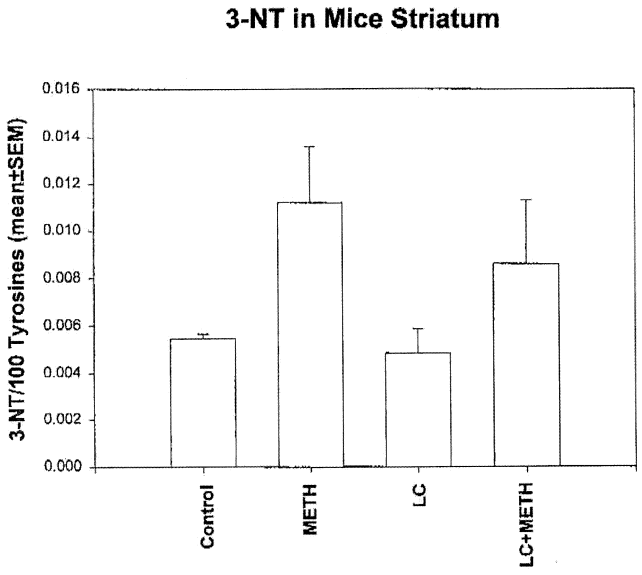

FIGURE 4. Protective effect of L-carnitine on the methamphetamine-evoked toxicity in mice striatum.

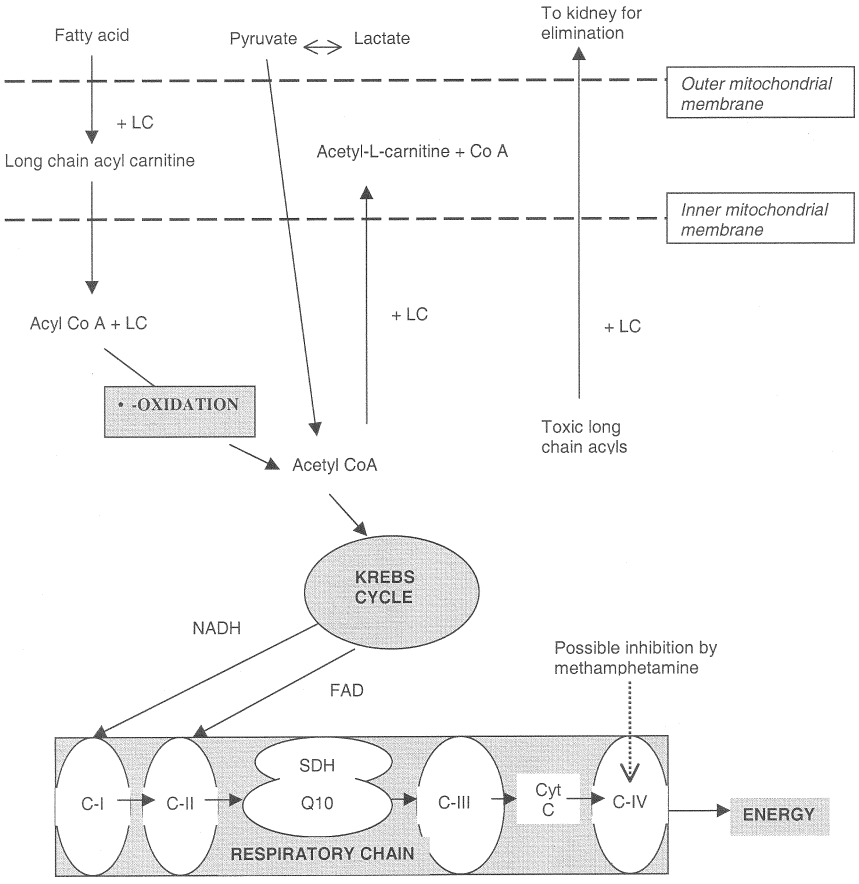

FIGURE 5. Schematic representation of the role of L-carnitine in mitochondrial metabolism. Cell toxicity and damage can be attenuated by energy substrates such as L-carnitine (LC). LC enhances fatty-acid metabolism and allows the transport of acetyl groups and coenzyme A (CoA) out of the mitochondria because the inner membrane is impermeable. LC would also enhance pyruvate metabolism by maintaining appropriate levels of acetyl CoA. In addition, LC removes toxic long-chain acyl groups from the mitochondria. The Krebs cycle feeds reducing energy in the form of nicotineamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) into the respiratory chain. Coenzyme Q10 (Q10) is located in the electron transport system of the mitochondria, linking complexes I (C-I) and II (C-II) with complex III (C-III) of the respiratory chain. Cytochrome c (Cyt C) links complex III to complex IV (C-IV). Methamphetamine is thought to inhibit C-IV activity.

nicotinamide.[9,17] A similar mechanism probably underlies the protective efficacy of the carnitines.[18,19] The protective effect of LC against METH-neurotoxicity may be through effects on mitochondrial oxidative phosphorylation and reduced formation of free radical species.

In summary, these data further confirm our hypothesis that METH-induced neurotoxicity is mediated by the production of peroxynitrite, and LC may improve the mitochondrial oxidative phosphorylation and/or scavenge the peroxynitrite and protect against the underlying mechanisms of METH toxicity, which are models for several neurodegenerative disorders such as Parkinson's disease.

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