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INDUCTION BY 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE OF LIPID PEROXIDATION IN VIVO IN VITAMIN E DEFICIENT MICE

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1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is neurotoxic to dopaminergic neurons in the midbrain and their projections into the striatum [1]. It has been speculated that the mechanism of dopaminergic neurotoxicity involves at least two potential components: mitochondrial damage and oxidative stress. There is no question that MPTP is a mitochondrial toxin in vitro [2]. It is also widely accepted that MPTP induces oxidative stress in the lung in vivo [3,4]. What is in question is which of these mechanisms might predominate in the neurotoxicity of MPTP.

MPTP has been shown not to induce lipid peroxidation in vivo in the striatum [5]. Since lipid peroxidation may be a component of the toxicity of some oxidative stress-inducing agents, the lack of lipid peroxidation with MPTP might argue against oxidative stress as part of the neurotoxic mechanism. This study was performed to reexamine the induction of lipid peroxidation by MPTP, as measured by conjugated diene formation, in various brain regions as well as the striatum. In addition, vitamin E deficient mice were used since they are known to be more susceptible to oxidative stress than normal mice [6].

MATERIALS AND METHODS

Male C57 BL/6 mice (25 g, 13 weeks old) served as control mice and were given regular laboratory rodent chow. Vitamin E deficient C57 BL/6 mice were raised from 3-week-old male weanlings and fed a vitamin E deficient diet for 16 weeks. All mice were housed in plastic cages in groups of 3 or 4 under an artificial light-dark cycle (light from 8:00 a.m. to 8:00 p.m.) and at a constant temperature (23°) and relative humidity (55%). Mice were allowed free access to food and water. Mice were randomly divided into two groups, for MPTP treatment or saline treatment. The MPTP group received four intraperitoneal injections of the hydrochloride salt of MPTP dissolved in 0.9% saline (15 mg/kg) at 2-hr intervals.

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This dose of MPTP was chosen since higher doses were lethal to vitamin E deficient mice. The saline group received 0.04 mL of saline with the same schedule. Animals were decapitated after cervical dislocation at 1, 24 or 48 hr after the last injection. Brains were removed and dissected into striatum, midbrain, thalamus, cortex and cerebellum (20-70 mg/sample). Vitamin E levels in the various brain regions were measured by HPLC with fluorescence detection [7].

Samples were prepared for conjugated diene analysis of extracts of tissue homogenates as described by Recknagel et al. [8]. Analysis was performed by second derivative absorption spectrophotometry as described by Corongiu and Milia [9]. Quantitation of the peak between 233 and 242 nm was done with the extinction coefficient 2.5×10^4 [10]. This is a useful approach since Corongiu and Milia [9] observed that the height of the second derivative peak is directly proportional to the concentration of peroxidized lipid.

RESULTS AND DISCUSSION

Vitamin E levels were measured in various brain regions of control and vitamin E deficient mice. Control levels were as follows (N = 3): striatum, 6.1 ± 1.0 μg/g tissue; midbrain, 5.2 ± 0.5; cortex, 4.3 ± 0.3; and cerebellum, 4.4 ± 1.1. Vitamin E deficient mice were 35-43% depleted of vitamin E: striatum, 3.5 ± 0.2 μg/g tissue; midbrain, 3.3 ± 0.3; cortex, 2.8 ± 0.5; and cerebellum, 2.6 ± 0.2. Vitamin E levels in the thalamus were not measured, but were probably as depleted as the rest of the brain.

Preliminary experiments demonstrated that lipid peroxidation was detectable in the midbrains of vitamin E deficient mice and was maximal at 24 hr after the last dose of MPTP. The cortex and cerebellum did not produce more lipid peroxides than controls after MPTP. The spectra produced were very similar to published spectra [5,9]. The results in Table 1 show that lipid peroxidation was produced by MPTP in the midbrains of vitamin E deficient mice only. Vitamin E sufficient mice demonstrated no increase in lipid peroxidation above control levels in any brain region. However, the dose of MPTP used in these experiments was neurotoxic to all mice, since our previous work has demonstrated that dopamine and its metabolites were depleted by this dose of MPTP in the brains of all mice, both vitamin E deficient and sufficient [11]. In fact, vitamin E deficiency significantly enhances the ability of MPTP to alter DOPAC and dopamine in the midbrain, but not the striatum. Therefore, vitamin E deficiency may make mice more sensitive to the dopaminergic neurotoxicity of MPTP in the substantia nigra but not the striatum.

Table 1. Conjugated diene levels in control and vitamin E deficient mice and the effects of MPTP

Group	Diene level (x 10^7 mol/g)		
	Striatum	Thalamus	Midbrain
control	0.5 ± 0.1	1.3 ± 0.2	1.3 ± 0.3
MPTP	0.5 ± 0.1	1.6 ± 0.3	1.1 ± 0.3
Vit E-	0.4 ± 0.2	1.4 ± 0.5	1.0 ± 0.5
Vit E- MPTP	0.7 ± 0.4	1.7 ± 0.4	3.5 ± 0.8*

Groups are: control; MPTP-treated normal mice; Vit E-, vitamin E deficient; and Vit E- MPTP, vitamin E deficient mice treated with MPTP. Animals were treated as described in Materials and Methods and killed 24 hr after the last dose of MPTP. Values are means ± SEM, N = 5.

*Significantly different from Vit E- ($P < 0.05$) by Student's paired t-test. Data from vitamin E sufficient mice were not compared to vitamin E deficient mouse data.

The results presented here argue in favor of oxidative stress induction by MPTP in the mouse midbrain. MPTP produced lipid peroxidation only in vitamin E deficient mouse midbrain. Vitamin E deficient mice are known to be much more susceptible to oxidative stress-inducing agents than normal mice [6]. It is commonly found that oxidative stress-inducing agents do not induce lipid peroxidation *in vivo* in normal animals [6]. Usually, the animals must be vitamin E deficient or selenium deficient before lipid peroxidation is measurable as the result of oxidative stress [6]. Therefore, the findings reported here with MPTP correspond with findings with other oxidative stress-inducing agents. Lipid peroxidation was not produced in the striatum where the dopaminergic neurons of the midbrain project. This may be because the dopaminergic terminals represent only a small fraction of the tissue present in the striatum. It is also possible that differences occur between mouse brain regions in terms of the ability to form the toxic metabolite of MPTP, such as has been observed with monkey brain regions. Perhaps the midbrain is exposed to higher levels of the toxic metabolite than the striatum. However, the mouse striatum and midbrain demonstrate no difference in the ability to metabolize MPTP to form protein bound metabolic products [12]. On the other hand, it could be possible that MPTP has somewhat different mechanisms of toxicity in the two brain regions. The differences between the striatum and the substantia nigra should be examined to find possible biochemical differences that may support different mechanisms in the two brain regions.

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