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Short communication

High-dose dextromethorphan produces myelinoid bodies in the hippocampus of rats

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ABSTRACT

Dextromethorphan (DM) administered at supra-antitussive doses produce psychotoxic and neurotoxic effects in humans. We administered DM (80 mg/kg) to rats intraperitoneally to determine the ultrastructural change induced by DM, because intraperitoneal route is sensitive for the behavioral responses. Treatment with DM resulted in mitochondrial dysfunction and formation of myelinoid bodies in the hippocampus. MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate] attenuated DM-induced cytosolic oxidative burdens. However, neither MK-801 nor naloxone affected DM-induced mitochondrial dysfunction and formation of myelinoid bodies, indicating that the neurotoxic mechanism needs to be further elucidated. Therefore, the spectrum of toxicological effects associated with DM need to be reassessed.

Dextromethorphan (DM, 3-methoxy-17-methylmorphinan) is a dextrorotatory optical isomer of levomethorphan, a typical morphine-like opioid. With increasing DM doses, the user experiences dysmetria and an inability to respond to pain and other external stimuli (1). Five teenagers who ingested large doses of DM that they obtained over the internet for recreational purposes died as a result of its direct toxic effects (2). Fatal poisonings due to large amounts of DM and zipeprol have been reported in Korea (3). Within an antitussive dose range, DM is an effective cough suppressant with negligible side effects; however, at very high doses, DM produces a very complex pharmacological profile (2, 4–6).

An earlier report showed that a single exposure to a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, produced vacuolar degeneration in the posterior cingulate cortex and the retrosplenial cortex in the rat brain (7). In contrast, another NMDA receptor antagonist, DM, did not produce neuropathologic changes when administered orally (8). Therefore, we investigated whether DM induces neuropathological changes in the brain when taken via the i.p. route. Because DM binding sites are mainly located in the CA1 area of the hippocampus (9), herein, we focused on the CA1 region. Unexpectedly, we observed that DM produces myelinoid bodies with a concentrically laminated membrane in the CA1 region of the rat hippocampus. Since Miao et al. (10) suggested that formation of myelinoid bodies might be related to mitochondrial degeneration, we investigated whether DM produces mitochondrial dysfunction in the present study.

Rats received 80 mg/kg, i.p. of DM, and then were sacrificed 1 day later (Fig. 1A). Rats were anesthetized with sodium

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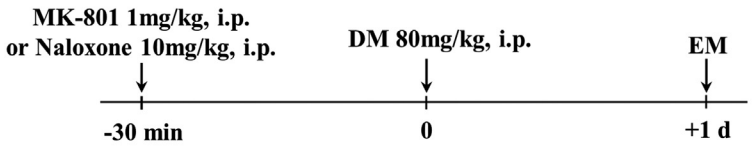
A



Treatment	Myelinoid body formation/ animal number
Naloxone	0/4
MK-801	0/4
DM	3/4
Naloxone + DM	3/4
MK-801 + DM	3/4

B

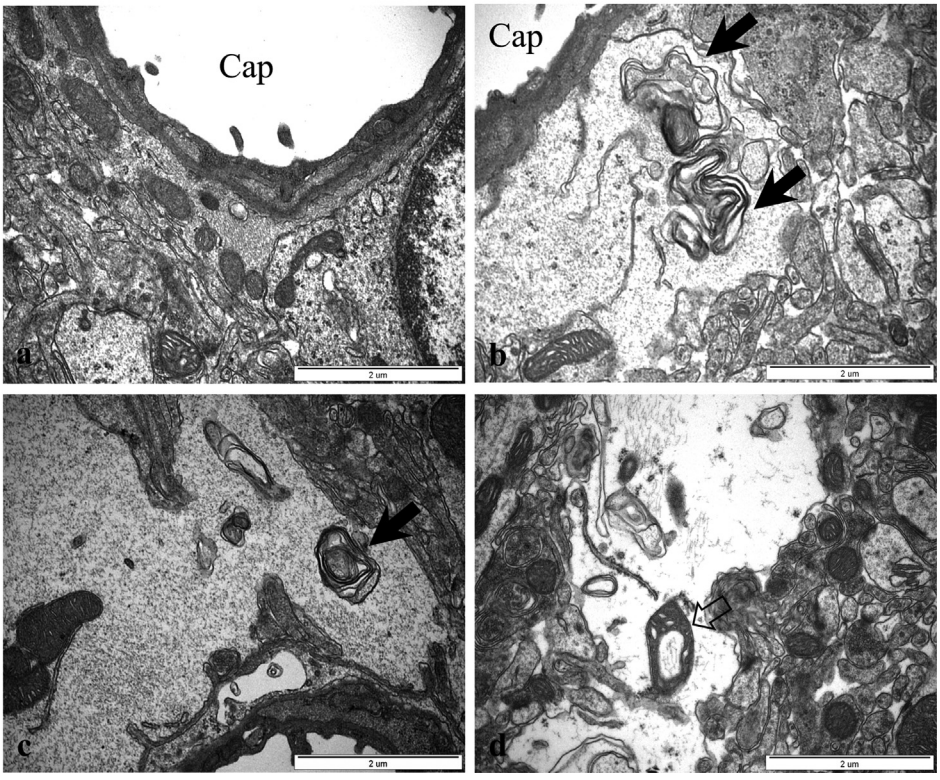


Fig. 1. Experimental design for evaluating DM-induced ultrastructural changes; effects of naloxone or MK-801 on DM (80 mg/kg, i.p.)-induced formation of myelinoid bodies (A), and representative photomicrographs of ultrastructural changes in the CA1 area of the hippocampus from rats in the presence of DM (B); Saline-treated animal showed a well-preserved ultrastructural architecture (a). DM (80 mg/kg, i.p.)-induced ultrastructural changes in the neuropil around capillaries (b), and mitochondria (d; open arrow). Myelinoid bodies (b and c; closed arrows) were seen in the processes close to vessels in the hippocampus one day after DM administration. Cap = capillary.

pentobarbital and perfused transcardially with PBS followed by 2.5% glutaraldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The hippocampal tissues were cut into small pieces and fixed in the same solution for 4 h at 4 °C and then they were washed overnight in PB and post-fixed in 1% osmium tetroxide in PB for 1 h at 4 °C. The tissues were dehydrated in a graded series of acetone dilutions and finally embedded in Epon 812 epoxidic resin. Ultra-thin sections (40 nm) were stained with uranyl acetate and lead citrate and observed with a Philips EM400 transmission electron microscope operating at 100 kV.

We isolated cytosolic and mitochondrial fraction, and examined lipid peroxidation reactive oxygen species as we previously described (11). Mitochondrial transmembrane potential was measured with the dye 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolycarbocyanine iodide (JC-1; Molecular Probes), which exists as a green fluorescent monomer at low membrane potential, but reversibly forms red fluorescent "J-aggregates" at polarized mitochondrial potentials. Briefly, aliquots of 250 μg of isolated mitochondrial protein were suspended in respiration buffer 250 mM sucrose, 20 mM HEPES, 2 mM $MgCl_2$, 2.5 mM