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Dextromethorphan attenuates trimethyltin-induced neurotoxicity via σ_1 receptor activation in rats

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Abstract

We showed that dextromethorphan (DM) provides neuroprotective/anticonvulsant effects and that DM and its major metabolite, dextrorphan, have a high-affinity for σ_1 receptors, but a low affinity for σ_2 receptors. In addition, we found that DM has a higher affinity than DX for σ_1 sites, whereas DX has a higher affinity than DM for PCP sites. We extend our earlier findings by showing that DM attenuated trimethyltin (TMT)-induced neurotoxicity (convulsions, hippocampal degeneration and spatial memory impairment) in rats. This attenuation was reversed by the σ_1 receptor antagonist BD 1047, but not by the σ_2 receptor antagonist ifenprodil. DM attenuates TMT-induced reduction in the σ_1 receptor-like immunoreactivity of the rat hippocampus, this attenuation was blocked by the treatment with BD 1047, but not by ifenprodil. These results suggest that DM prevents TMT-induced neurotoxicity, at least in part, via σ_1 receptor stimulation.

Keywords: Dextromethorphan; Anticonvulsant; Trimethyltin; σ_1 Receptor

1. Introduction

Intoxication with trimethyltin (TMT) leads to profound behavioral and cognitive deficits in both humans (Fortemps et al., 1978) and experimental animals (Dyer et al., 1982). In rats, TMT induces degeneration of pyramidal neurons in the hippocampus and cortical areas connected to the hippocampus (Brown et al., 1979; Chang and Dyer, 1983; Shin et al., 2005a). TMT intoxication impairs performance in water maze and radial arm maze tests (Alessandri et al., 1994; Earley et al., 1992; Hagan et al., 1988; Ishida et al., 1997).

The σ_1 receptors appear to play an important neuromodulatory role in cholinergic neurotransmission (Maurice et al., 1999, 2001). TMT-induced learning impairment is attributable in part to σ receptor dysfunction (Maurice et al., 1999). Interestingly, systemic administration of the selective σ_1 ligand JO 1784 attenuates TMT intoxication (O'Connell et al., 1996). In the brain, σ sites are found primarily in the hippocampus and in regions associated with motor function, such as the red nucleus and substantia nigra (McLean and Weber, 1988).

Dextromethorphan (DM; 3-methoxy-17-methylmorphinan) is a non-narcotic morphinan derivative that has been used widely as an antitussive for almost 40 years. DM has also attracted attention because of its neuroprotective properties (Choi, 1987; Kim et al., 1996, 2001a,b, 2003a,b; Shin et al., 2004, 2005b; Tortella et al., 1988, 1989, 1994; Zhang et al., 2004). We have demonstrated that the anticonvulsant and neuroprotective effects of DM may be mediated in part by σ_1 receptor activation (Kamei et al., 1996; Kim et al., 2003a; Maurice et al., 1999; Shin et al., 2005b).

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In the present study, to extend our previous understanding of the pharmacological effects mediated by DM, we assessed the role of σ receptors in the pharmacological actions of DM on neurotoxicity (convulsive behaviors, neuronal degeneration, and learning impairment) induced by TMT. We observed that DM-induced activation of σ_1 receptors is important for preventing neurotoxicity induced by TMT in rats.

2. Material and methods

2.1. Animals and drug treatment

All animals were treated in strict accordance with the National Institutes of Health (NIH) Guide for the Humane Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1985; www.dels.nas.edu/ila). The Fischer 344 rat is known to be highly sensitive to TMT-induced neurotoxicity (MacPhail et al., 2003), and the TMT toxicity is enhanced in older rodents (Scallet et al., 2000). Consequently, we used 12-month-old Fischer 344 rats (Bio Genomics, Inc., Charles River Technology, Gapyung-Gun, Gyeonggi-Do, Korea) in this study. The rats were maintained on a 12 h light:12 h dark cycle and were fed *ad libitum*. They were allowed to adapt to these conditions for 2 weeks before TMT administration (8 mg/kg, i.p.). DM (12.5 or 25.0 mg/kg, s.c.) was administered twice at an interval of 6 h; at 30 min after the second administration, TMT was administered. The rats received DM once per day for 26 days following TMT administration and were sacrificed at 30 min after the final dose of DM. Control rats received an equal volume of saline.

For antagonism experiments, σ_1 receptor antagonist BD 1047 dihydrobromide (1 or 2 mg/kg, i.p.; Tocris Cookson Ltd., Bristol, UK) or σ_2 receptors antagonist ifenprodil (5 or 10 mg/kg, i.p.; Tocris Cookson) was administered 15 min before every dose of DM. The doses of the drugs used are comparable with those used by Shin et al. (2005b).

2.2. Radioligand binding

To assay (+) [^{3}H]SKF-10047 and [^{3}H]DTG binding, frozen brains (without the cerebellum) from male Fischer 344 rats were thawed for 1 h in 25 volumes of ice-cold 5 mM Tris–HCl/10 mM K^+-EDTA (pH 8.0 at 25 °C) and then homogenized for 15 s. Homogenates were centrifuged (45,000 × *g*, 15 min, 4 °C). The supernatant was discarded, and the pellets were resuspended in fresh ice-cold buffer and recentrifuged once. The final pellets were resuspended in ice-cold 5 mM Tris–HCl (pH 8.0 at 25 °C) (Munson and Rodbard, 1980).

In a final volume of 0.5 mL, membrane suspensions were incubated with radioligand in the presence of 5 mM Tris–HCl (pH 8.0 at 25 °C) for 60 min. The σ_1-binding assays were performed in the presence of 300 nM unlabeled MK-801 to block binding of [^{3}H]-(+)-SKF-10047 (final concentration, 5 nM; New England Nuclear, Boston, MA; specific activity, 46.5 Ci/mmol) to PCP receptors. In all σ_2-binding assays, 3 μM unlabeled (+)-SKF 10047 was included to block binding of [^{3}H]-DTG (final concentration, 5 nM; New England Nuclear; specific activity, 40.8 Ci/mmol) to σ_1 receptors. The reactions were terminated by the addition of 5 mL ice-cold 5 mM Tris–HCl (pH 8.0), followed by rapid filtration through Whatman GF/B filter papers (presoaked in 0.5% polyethyleneimine in H_2O to reduce non-specific binding) using a Brandel cell harvester (Gaithersburg, MD). The filters were then rinsed twice with 5 mL aliquots of the same buffer. Absolute ethanol and Beckman Ready Value scintillation cocktail were added to the vials, which were counted the next day at an efficiency of about 36%. The K_i values were determined using LIGAND software (Calderon et al., 1994; Munson and Rodbard, 1980).

To assay [^{3}H]TCP binding, whole brains minus the cerebellum were rapidly removed and disrupted in 45 volumes of ice-cold 5 mM Tris–HCl buffer (pH 7.4) using a Brinkmann polytron homogenizer. The homogenates were centrifuged (20,000 × *g*, 29 min, 5 °C). The pellet was washed in fresh buffer, centrifuged twice more, resuspended in 45 volumes of assay buffer, and kept on ice until used (Calderon et al., 1994).

Binding to homogenates was determined in a 1 mL incubation volume consisting of 900 μL tissue (containing ~0.8 mg protein), 50 μL [^{3}H]TCP (44.8 Ci/mmol; New England Nuclear) for a final concentration of 4 mM, and 50 μL buffer, test compound, or 10 μM TCP (for determination of non-specific binding). After a 90 min incubation at 5 °C, the reaction was terminated by rapid filtration using a Brandel cell harvester. The filters were washed with 5 mL aliquots of ice-cold assay buffer, placed in counting vials with 4 mL of CytoScint ES scintillation cocktail (ICN Biomaterials, Inc., Irvine, CA), and allowed to stand overnight before counting. The inhibition constants (K_i) for the various compounds were calculated using GraphPAD software (ISI Software, Philadelphia, PA) (Calderon et al., 1994). The protein content was determined by the Coomassie protein assay (Pierce, Rockford, IL), using bovine serum albumin as the standard.

2.3. Convulsive behavior

Using a convulsion meter (CONVULS-1 Columbus Instruments, Columbus, OH), convulsive impulse counts were measured over a 20 min period at 1 h, 2 h, 4 h, 1 day, 2 days, 3 days, 4 days, 1 week, 2 weeks, and 3 weeks after TMT treatment (Shin et al., 2005a).

2.4. Spatial reference memory test: Morris water maze task

A hidden platform test was started at 21 days after TMT injection. The apparatus was a circular water tank, 140 cm in diameter and 60 cm in height. During testing, the tank was filled with water (23 ± 2 °C) that was clouded with powdered milk. A transparent platform was set inside the tank with its top submerged 2 cm below the water surface in the center of one of the four quadrants of the maze, at a constant position throughout the test. The tank was located in a large room with four extra-maze cues that were constant throughout the study (Jhoo et al., 2004; Zou et al., 2006). The movements of the animal in the tank were monitored with a video tracking system (EthoVision, Noldus, Netherlands). For each trial, the rat was put into the pool at one of the five positions, with the sequence of positions being selected randomly, and the time taken to reach the hidden platform was recorded. Each rat was allowed 120 s to find the platform; if the rat was unable to find it within 120 s, the trial was terminated, and a maximum score of 120 s was assigned. Trials were conducted for 5 consecutive days, four times a day. The results were analyzed statistically using a two-way ANOVA test for repeated measures, followed by Bonferroni's test.

2.5. Associative memory test: passive avoidance task

Passive avoidance was measured using a Gemini Avoidance System (San Diego Instruments, San Diego, CA), which consists of two-compartment shuttle chambers with a constant current shock generator. On an acquisition trial (21 days after TMT administration), each rat was placed into the start chamber, which remained darkened. After 20 s, the chamber light was illuminated and the door was opened for the rat to move into the dark chamber freely. Immediately after the rat entered the dark chamber, the door was closed and an inescapable scrambled electric shock (0.8 mA, 5 s, once) was delivered through the floor grid. The rat was then returned to its home cage. Twenty-four hours later (22 days after TMT administration), each rat was again placed in the start chamber (retention trial). The interval between the placement in the lighted chamber and the entry into the dark chamber was measured as the step-through latency in the retention trial (maximum 300 s) (Jhoo et al., 2004).

2.6. Fluorescent Nissl staining

At 26 days after TMT injection, the rats were perfused transcardially with 50 mL of 50 mM phosphate-buffered saline (PBS), followed by 500 mL of 4% paraformaldehyde in PBS, under pentobarbital anesthesia. The brains were removed, post-fixed for 24 h in the same fixative at 4 °C, and then immersed in 30% sucrose in PBS until they sank. The brains were cut into 35-μm transverse free-floating sections, using a horizontal sliding microtome. Fluorescent Nissl staining was performed using NeuroTrace 530/615 red fluorescent Nissl stain solution (N-21482, Invitrogen), which is considered to be a specific marker for neurons (Rao et al., 2003). Briefly, sections were incubated in PBS containing 0.1% Triton® X-100 (Sigma–Aldrich, St. Louis, MO) for 10 min and incubated in a 100-fold dilution of NeuroTrace solution for 20 min. The sections were then washed in PBS for 2 h and mounted with an anti-fade agent (Fluoromount-G, Southern Biotech, Birmingham, AL). Digital images of fluorescent Nissl-stained