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Melatonin Attenuates Methamphetamine-Induced Toxic Effects on Dopamine and Serotonin Terminals in Mouse Brain

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ABSTRACT Methamphetamine (METH) is a drug of abuse that causes deleterious effects to brain monoaminergic systems. These toxic effects are thought to be due to oxidative stress. The pineal hormone, melatonin, has been shown to have neuroprotective effects against toxic quinones and oxidative stress produced by catecholamines. The present study was thus undertaken to assess possible protective effects of melatonin against METH-induced neurotoxic effects on the striatum and the nucleus accumbens by using autoradiographic techniques. Four dosages (5, 20, 40, 80 mg/kg) of melatonin were administered to mice intraperitoneally 30 minutes prior to the injections of METH (4 × 5 mg/kg) given at 2-hour intervals. The lowest doses of melatonin (5 mg/kg) had no significant effects against METH-induced toxicity. However, the higher doses (40 or 80 mg/kg) of melatonin significantly attenuated METH-induced toxic effects on both dopamine and serotonin systems. These data provide further evidence for a possible role of oxidative stress in METH-induced toxicity. **Synapse 30:150–155, 1998.**

INTRODUCTION

Methamphetamine (METH) is known to cause long-lasting neurotoxic effects on the dopamine (DA) and serotonin (5-HT) systems of rodents. Those are evidenced by long-lasting changes in several neuronal markers, including striatal DA level, tyrosine hydroxylase activity, DA transporters (Hotchkiss and Gibb, 1980; Steranka and Sanders-Bush, 1980; Wagner et al., 1980), as well as striatal 5-HT level, 5-HT synthesis, 5-HT transporters (Bahkit et al., 1981; Kovachich et al., 1989; Ricaurte et al., 1980; Seiden et al., 1988). These METH-induced neurotoxic effects are thought to be due to increased production of oxygen-based free radicals (Cadet and Brannock, 1998; Cubells et al., 1994; De Vito and Wagner, 1989). These ideas were supported by the demonstration that METH-induced striatal DA and 5-HT depletion is attenuated in transgenic mice that express the human Cu-Zn-superoxide dismutase (SOD) enzyme (Cadet et al., 1994; Hirata et al., 1995, 1996), thus identifying superoxide radicals as major culprits in the neurotoxicity of the drug.

Recent evidence has indicated that the pineal hormone, melatonin, has antioxidative properties by scavenging free radicals (Barlow-Walden et al., 1995; Hardeland and Rodriguez, 1995; Pieri et al., 1994) or via inhibition of nitric oxide synthesis (Pozo et al., 1994). We, thus, postulated that if melatonin is an antioxidant action, then METH-induced neurotoxicity might be attenuated by administration of melatonin. In the present study, we have performed receptor autoradiographic studies using [^{125}I]RTI-121- and [^{125}I]RTI-55-labeled DA and 5-HT transporters to evaluate the effects of several doses of melatonin against METH-induced neurotoxicity in mice.

MATERIALS AND METHODS

Male CD-1 mice aged 8 weeks were used in these experiments. All animal use procedures were according to the NIH guide for the Care and Use of Laboratory Animals and were approved by the local Animal Care Committee of NIDA.

Animal experiments

On the day of experiments, mice received 4 injections of 5 mg/kg of METH or saline (total amount per animal

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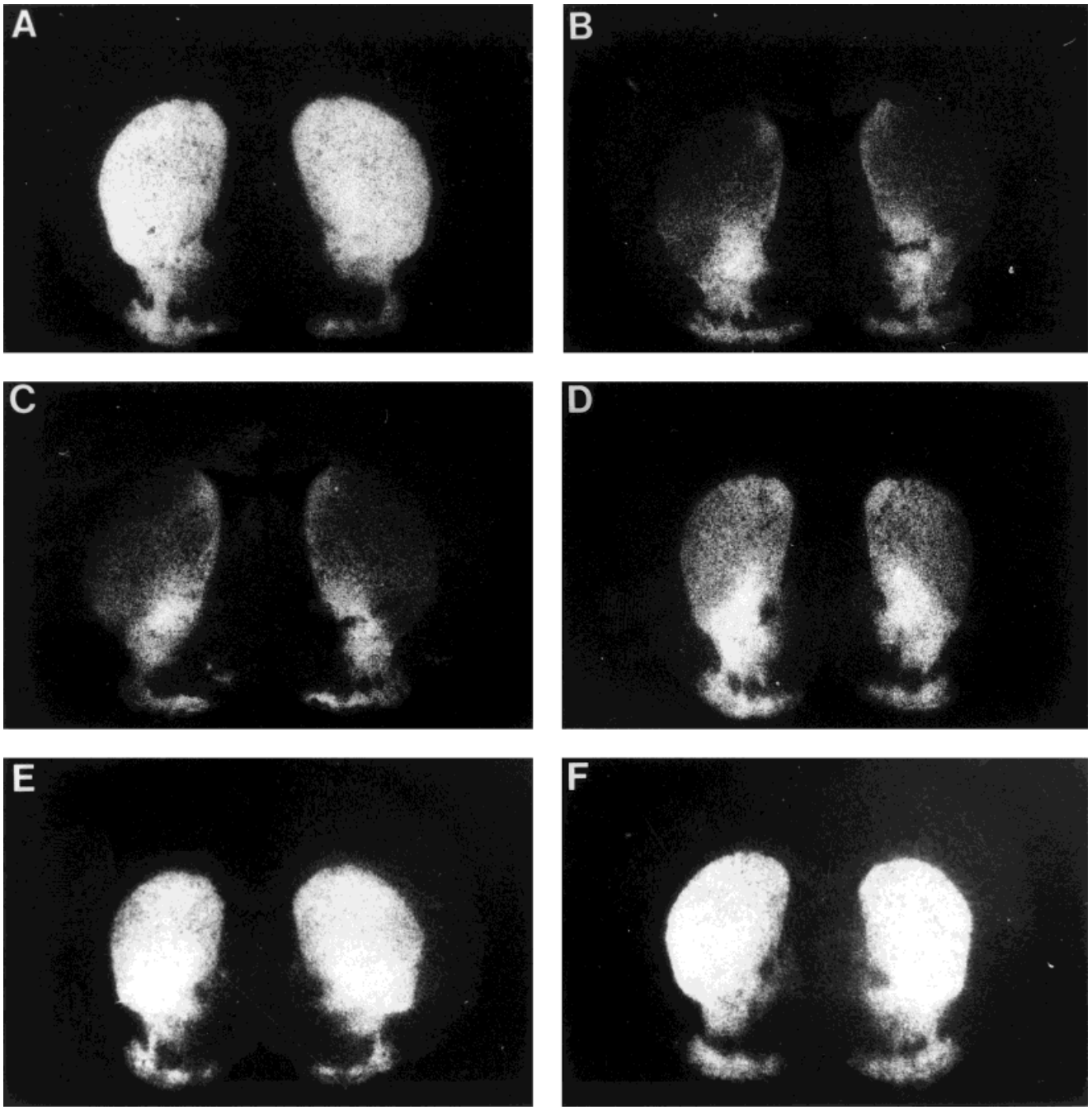

Fig. 1. Autoradiographic distribution of [^{125}I]RTI-121-labeled DA transporters in the striatum and the nucleus accumbens of METH (5 mg/kg × 4)-injected mice. **A:** Control. **B:** Treated with saline. **C,D,E,F:** Treated with 5, 20, 40, 80 mg/kg of melatonin, respectively, 30 minutes prior to METH injection. The lighter the image the greater the binding density. Note the obvious decreases in DA transporters caused by METH in saline-treated mice (B). These decreases were attenuated in high dose melatonin-treated mice (D,E,F). The quantitative data are given in Fig. 2.

was 20 mg/kg of METH) via the intraperitoneal route. Several dosages of melatonin (5, 20, 40, 80 mg/kg, Sigma, St. Louis, MO) or saline were also injected intraperitoneally 30 minutes prior to the administration of each dose of METH. METH and melatonin were given at 2-hour intervals for a total of 4 injections according to previously published protocols (Hirata et al., 1996). Two weeks later, the animals were sacrificed and their brains were rapidly removed, frozen in isopentane on dry ice, and stored frozen at −70°C. Sections (20 µm thick) were cut at −20°C and thaw-mounted on 3-aminopropylethoxysilane coated glass slides. The slides were kept at −70°C until used in the autoradiographic studies using [^{125}I]RTI-121 (SA:2200

Ci/mmol) and [^{125}I]RTI-55 (SA:2200 Ci/mmol) as described below.

Autoradiographic assays

Binding assays for DA transporters were performed according to a published protocol (Boja et al., 1995; Hirata et al., 1996). Briefly, slide-mounted sections were incubated for 60 minutes at room temperature (RT) with radioiodinated 3β-[4 (trimethylstannyl) phenyl]-tropane-2β-carboxylic acid isopropyl ester ([^{125}I]RTI-121, 100,000 cpm/ml) using a binding buffer consisting of 137.0 mM NaCl, 2.7 mM KCl, 10.41 mM Na_2HPO_4, 1.76 mM Na_2HPO_4, and 10 mM NaI. Specific binding was determined in the presence of 10 µM GBR-12909 and represented greater than 90% of total binding. At the end of the incubation period, the slides were washed twice in fresh buffer for 20 minutes at RT, dipped in ice-cold distilled water, and dried under a stream of cool air.

Binding assays for 5-HT transporters were performed according to a published protocol (Hirata et al., 1995). In brief, slide-mounted sections were incubated for 90 minutes at 4°C with [^{125}I]RTI-55 (120,000 cpm/ml) in a binding buffer (BB) consisting of 55.2 mM sodium phosphate buffer, pH 7.4. The radioligand was made in a protease inhibitor cocktail containing BB, 1 mg/ml BSA, chymostatin (25 µg/ml), leupeptin (25 µg/ml), EDTA (100 µM), and EGTA (100 µM). LR1111 (10 µM) was used to block binding of [^{125}I]RTI-55 to DA transporters. Specific binding was determined in the presence of 10 µM paroxetine and represented greater than 90% of total binding. At the end of the incubation period, the slides were washed twice in fresh buffer for 5 minutes at RT, dipped in ice-cold distilled water, and dried under a stream of cool air.

The slides were then apposed to radiosensitive films (Hyperfilm, Amersham, Buckinghampshire, UK) with plastic standards ([^{125}I] microscales, Amersham) for 3 days (DA transporter) or 7 days (5-HT transporter) at 4°C. The films were then developed according to routine procedures. [^{125}I]RTI-121 and [^{125}I]RTI-55 bindings were quantified on both sides of the mice brains using a Macintosh computer-based image analysis system (Image, NIH) using standard curves generated from the [^{125}I] microscales. Non-specific binding was at the level of the film background.

Statistical analyses

Data are presented as means ± SEM. Statistical analyses were done using analysis of variance (ANOVA) followed by posthoc Fisher's PLSD test.

RESULTS

Figures 1 and 2 show the effects of melatonin against the neurotoxicity of METH (5 mg/kg) on [^{125}I]RTI-121-labeled DA transporters in the striatum and the nucleus accumbens. In saline-injected mice, METH caused 68 and 31% decreases of DA transporter in the striatum and the nucleus accumbens compared to the control mice (Fig. 2). Low-dose of melatonin (5 mg/kg) had no significant effects against the toxic effects of METH on DA terminals. In contrast, 40 and 80 mg/kg of melatonin significantly attenuated the deleterious effects of the drug. For example, mice which received melatonin (40 or 80 mg/kg) prior to the administration of METH only showed 19 and 16% or 11 and 5% decreases of DA transporters in the striatum and the nucleus accumbens, respectively (Fig. 2).

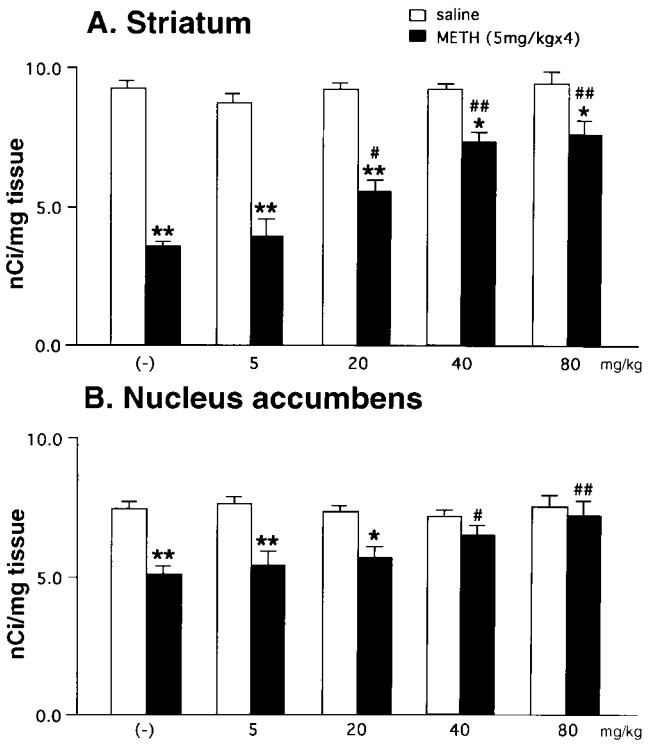

Fig. 2. Effects of melatonin on DA transporters in the striatum (**A**) and the nucleus accumbens (**B**) of METH (5 mg/kg × 4)-injected mice. The mice were treated with saline or several dosages of melatonin 30 minutes prior to the administration of METH. Autoradiographic studies were carried out as described in Materials and Methods. The values represent means ± SEM (nCi.mg tissue) of 5–6 animals per group. $^{*}P < 0.01$ in comparison to control mice, injected same dosage of melatonin. $^{**}P < 0.001$ in comparison to control mice, which injected same dosage of melatonin. $^{\#}P < 0.01$ in comparison to the METH-injected mice. $^{\#\#}P < 0.001$ in comparison to the METH-injected mice. (−) refers to animals treated with vehicle.

Figures 3 and 4 show the effects of melatonin against the neurotoxic effects of METH (5 mg/kg) on [^{125}I]RTI-55-labeled 5-HT transporters in the striatum and the nucleus accumbens. In saline-injected mice, METH caused 30 and 17% decreases of 5-HT transporter in the striatum and the nucleus accumbens, compared to the control mice (Fig. 4). The lowest dose of melatonin (5 mg/kg) had no significant effects against METH-induced neurotoxicity on 5-HT terminals. However, 40 and 80 mg/kg of melatonin caused significant attenua-

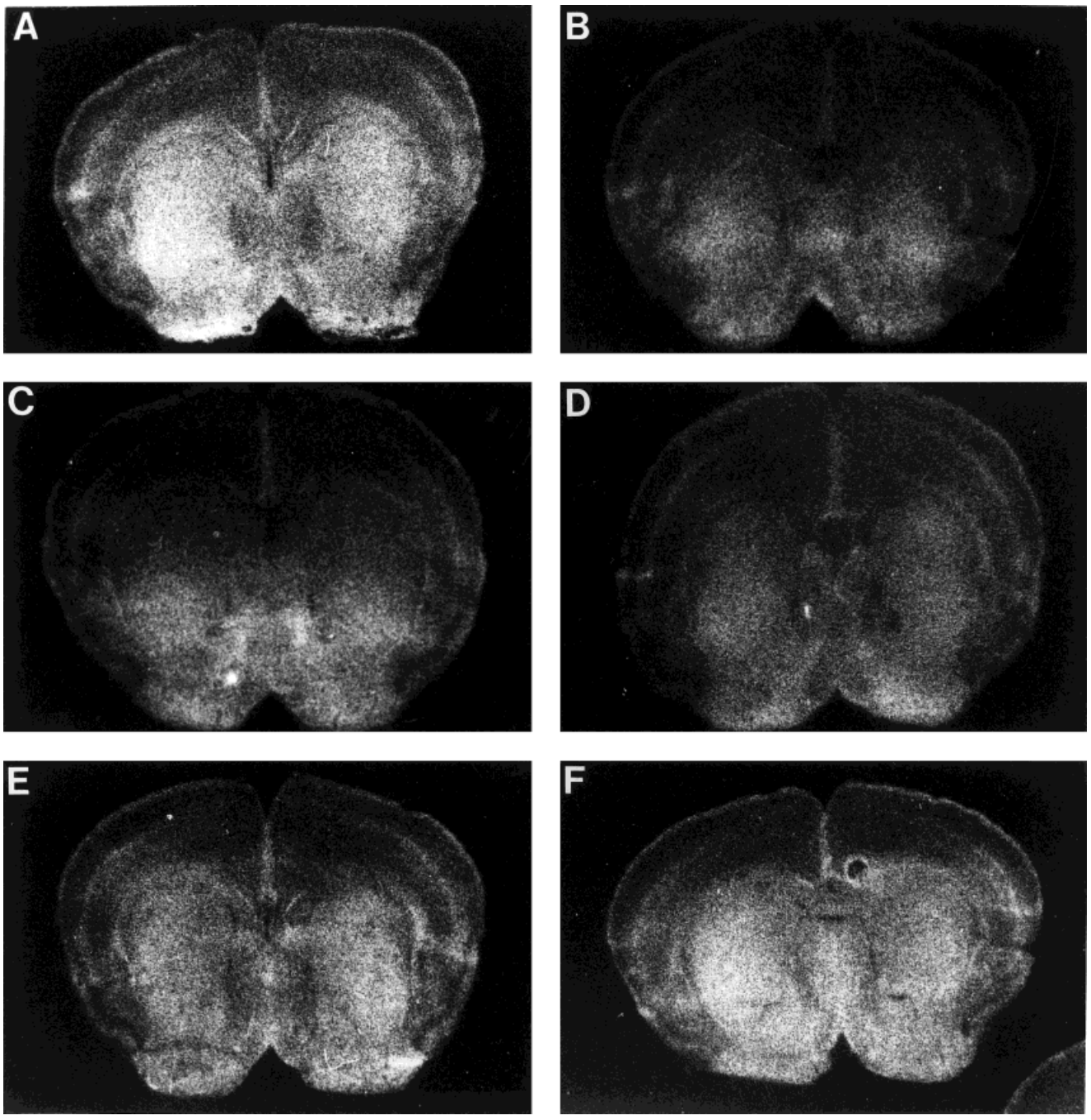

Fig. 3. Autoradiographic distribution of [^{125}I]RTI-55-labeled 5-HT transporters in the striatum and the nucleus accumbens of METH (5 mg/kg × 4)-injected mice. **A:** Control. **B:** Treated with saline. **C,D,E,F:** Treated with 5, 20, 40, 80 mg/kg of melatonin, respectively, 30 minutes prior to METH injection. The lighter the image the greater the binding density. Note the obvious decreases in 5-HT transporters caused by METH in saline-treated mice (B). These decreases were attenuated in high dose melatonin-treated mice (E,F). The quantitative data are given in Figure 4.

tion against the toxic effects of METH (Fig. 4). For example, there were only very small decreases (19 and 13%) in 5-HT transporters in the striatum and no significant decreases (6 and 5%) of 5-HT transporters in the nucleus accumbens of mice pre-injected with 40 or 80 mg/kg of METH, respectively (Fig. 4).

DISCUSSION

This is the first demonstration of neuroprotective effects of melatonin against METH-induced neurotoxicity in the DA and 5-HT systems of mice. These results will be discussed in view of the reported antioxidative effects of melatonin.

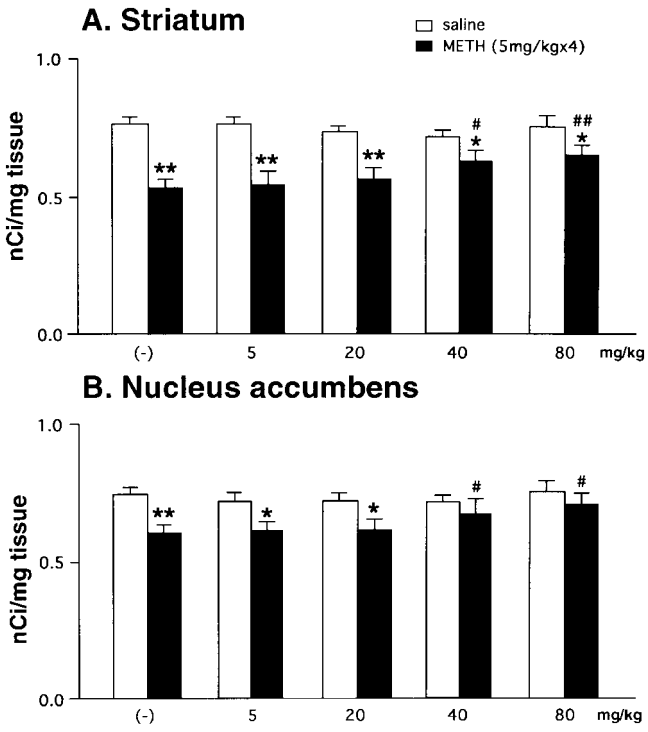

Fig. 4. Effects of melatonin on 5-HT transporters in the striatum (**A**) and the nucleus accumbens (**B**) of METH (5 mg/kg × 4)-injected mice. The mice were treated with saline or several dosages of melatonin 30 minutes prior to the administration of METH. Autoradiographic studies were carried out as described in Materials and Methods. The values represent means ± SEM (nCi.mg tissue) of 5–6 animals per group. $^{*}P < 0.01$ in comparison to control mice, which injected same dosage of melatonin. $^{**}P < 0.001$ in comparison to control mice, injected same dosage of melatonin. $^{\#}P < 0.01$ in comparison to the METH-injected mice. $^{\#\#}P < 0.001$ in comparison to the METH-injected mice. (−) refers to animals treated with vehicle.

METH is known to cause marked increases of the release of DA in the rodent striatum (Baldwin et al., 1993). The metabolic breakdown of DA leads to the generation of oxygen-based free radicals such as the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) with subsequent formation of hydroxyl radicals (Giovanni et al., 1995). Previous observations from this laboratory (Cadet et al., 1994; Hirata et al., 1995, 1996) and from those of other investigators (Cubells et al., 1994) have supported the ideas that oxygen-based radicals are intimately involved in METH-induced toxicity.

More recently, a role for nitric oxide in METH-induced DA damage has been suggested (Taraska and Finnegan, 1997). These ideas are based on a number of converging results. For example, Sonsalla et al. (1989) have shown that N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, attenuates METH-induced neurotoxicity. Other investigators have also shown that administration of METH can cause an increase glutamate release in the striatum (Nash and Yamamoto, 1992). Subsequent activation of NMDA receptor, by causing increases in intracellular calcium levels, could have stimulated nitric oxide synthase (NOS) activity in animals treated with METH. This idea is supported by observations that NOS inhibitors, such as 7-nitroindazole or nitroarginine, prevent METH-induced neurotoxicity in vivo (Di Monte et al., 1996; Itzhad and Ali, 1996) and METH-induced DA cell death in vitro (Sheng et al., 1996).

In order to further test the free radical idea, we have made use of the pineal hormone, melatonin, which has been shown to have antioxidative properties. For example, melatonin attenuates kainic acid-induced neuronal cell death in the hippocampus (Ut et al., 1996), and L-cysteine-induced seizures and lipid peroxidation in the brain of mice (Yamamoto and Tang, 1996). These protective effects of melatonin are probably related to: (1) endogenous free radical scavenging properties of the hormone (Hardeland and Rodriguez, 1995; Pieri et al., 1994); (2) stimulation of the activity of antioxidative enzymes such as glutathione peroxidase (Barlow-Walden et al., 1995); or (3) inhibition of NOS activity (Pozo et al., 1994). Thus, when taken together, these observations suggest that melatonin might provide protection against the toxic effects of METH by interfering with the effects of oxygen-based radicals and the synthesis of nitric oxide.

It is, nevertheless, possible that other mechanisms might also play a role in the protective effects of melatonin. These include competition of melatonin with METH for entry into the brain as well as melatonin-induced cellular and molecular neuroadaptive changes that might have rendered DA and 5-HT terminals more resistant to the toxic effects of METH. It is also possible that melatonin might have triggered endogenous trophic or repair mechanisms that participate in the regeneration of monoaminergic systems. Further studies will need to evaluate these issues.

In summary, the present study indicates that the pineal hormone, melatonin, provides protection against METH-induced neurotoxicity in the DA and 5-HT systems of mice. These findings may have implications for the elucidation of the cellular mechanisms involved in toxic effects of METH and, by extension, in neurodegenerative diseases such as Parkinson's disease, which involves pathology in the nigrostriatal DA pathway.

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