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Effect of melatonin on methamphetamine- and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurotoxicity and methamphetamine-induced behavioral sensitization

Yossef Itzhak [a,*], Julio L. Martin [a], M. Dean Black [a], Syed F. Ali [b]

[a] *Department of Biochemistry and Molecular Biology (R-62a), University of Miami School of Medicine, PO Box 016129, Miami, FL 33101, USA*
[b] *Neurochemistry Laboratory, Division of Neurotoxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA*

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Abstract

Methamphetamine (METH)- and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity is thought to be associated with the formation of free radicals. Since evidence suggests that melatonin may act as a free radical scavenger and antioxidant, the present study was undertaken to investigate the effect of melatonin on METH- and MPTP-induced neurotoxicity. In addition, the effect of melatonin on METH-induced locomotor sensitization was investigated. The administration of METH (5 mg $kg^{-1} \times 3$) or MPTP (20 mg $kg^{-1} \times 3$) to Swiss Webster mice resulted in 45–57% depletion in the content of striatal dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, and 57–59% depletion in dopamine transporter binding sites. The administration of melatonin (10 mg kg^{-1}) before each of the three injections of the neurotoxic agents (on day 1), and thereafter for two additional days, afforded a full protection against METH-induced depletion of dopamine and its metabolites and dopamine transporter binding sites. In addition, melatonin significantly diminished METH-induced hyperthermia. However, the treatment with melatonin had no significant effect on MPTP-induced depletion of the dopaminergic markers tested. In the set of behavioral experiments, we found that the administration of 1 mg kg^{-1} METH to Swiss Webster mice for 5 days resulted in marked locomotor sensitization to a subsequent challenge injection of METH, as well as context-dependent sensitization (conditioning). The pretreatment with melatonin (10 mg kg^{-1}) prevented neither the sensitized response to METH nor the development of conditioned locomotion. Results of the present study indicate that melatonin has a differential effect on the dopaminergic neurotoxicity produced by METH and MPTP. Since it is postulated that METH-induced hyperthermia is related to its neurotoxic effect, while regulation of body temperature is unrelated to MPTP-induced neurotoxicity or METH-induced locomotor sensitization, the protective effect of melatonin observed in the present study may be due primarily to diminishing METH-induced hyperthermia.

Keywords: Methamphetamine; MPTP; Melatonin; Dopaminergic neurotoxicity; Free radicals; Locomotor activity; Sensitization

1. Introduction

Methamphetamine (METH) is a potent psychostimulant that causes a massive release of newly synthesized dopamine (DA) from presynaptic vesicles and an increase in extracellular DA concentration in the nigrostriatal system. High doses of METH result in long-lasting neurotoxicity associated with a marked decrease in tyrosine hydroxylase activity, DA and dopamine transporter (DAT) binding sites in the striatum (Kogan et al., 1976; Fuller and Hemrick-Luecke, 1980; Wagner et al., 1980; Wilson et al., 1996). The dopaminergic neurotoxicity produced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is thought to be initiated after the conversion of MPTP to 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase B. MPP^+ is taken up by the DAT and then accumulated

* Corresponding author. Tel.: +1 305 2434635; fax: +1 305 2433955; e-mail: YITZHAK@MEDNET.MED.MIAMI.EDU.

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in the mitochondria of dopaminergic neurons (Langston et al., 1983; Heikkila et al., 1984). Although the initial mechanisms of action of METH and MPTP are different, the damage caused to the nigrostriatal dopaminergic neurons by these agents is similar to that detected in Parkinson's disease (for review see Fornai et al. (1997)).

Recent studies now support the hypothesis that METH- and MPTP-induced dopaminergic neurotoxicity is associated with the formation of free radicals. The generation of oxygen-base free radicals (e.g., superoxide and peroxynitrite) in METH-induced neurotoxicity has been proposed by several investigators (Seiden and Vosmer, 1984; De Vito and Wagner, 1989; Cadet et al., 1994; Giovanni et al., 1995, Sheng et al., 1996; Itzhak and Ali, 1996; Cadet et al., 1997; Itzhak et al., 1998). MPP^+ neurotoxicity is thought to be primarily related to its accumulation in the mitochondria where it acts as a direct inhibitor of Complex I (Nicklas et al., 1985; Sonsalla and Nicklas, 1992; Desai et al., 1996) and Complex III and IV (Desai et al., 1996). The marked depletion of DA caused by MPTP is preceded by a decrease in adenosine triphosphate (ATP) (Chan et al., 1991). It is postulated that inhibition of mitochondrial function and the decrease in energy stores may result in an increase in the generation of free radicals (Hasegawa et al., 1990). MPP^+ was found to induce hydroxy radical formation (Chiueh et al., 1992) and peroxynitrite via nitric oxide synthase (NOS) (Schulz et al., 1995). The findings that transgenic mice over-expressing superoxide dismutase are resistant to MPTP- (Przedborski et al., 1992) and METH- (Cadet et al., 1994) induced neurotoxicity further support the role of free radicals in the neurotoxic effects of these agents.

Melatonin is a natural hormone produced by the pineal gland and thought to regulate the circadian rhythm. Recent studies suggest that melatonin acts also as a free radical scavenger and antioxidant (for review see Reiter et al. (1997)). It is believed that melatonin donates an electron and directly detoxifies free radicals such as the highly toxic hydroxy radical (Pierrefiche et al., 1993; Reiter, 1996; Gilad et al., 1997). In vivo and in vitro studies showed that melatonin protects cells, tissue and organs against oxidative damage caused by various free radical generating agents (Melchiorri et al., 1995; Escames et al., 1997). As an antioxidant, this hormone is effective in protecting nuclear DNA, membrane lipids and possibly cytosolic proteins from oxidative damage (Reiter et al., 1995).

The present study was undertaken to investigate whether melatonin is protective against METH and MPTP-induced neurotoxicity. First, we found that melatonin protected against METH- but not MPTP-induced dopaminergic neurotoxicity. Thus, we investigated whether melatonin prevents the development of locomotor sensitization to METH. The results indicate that melatonin protected only against METH-induced dopaminergic neurotoxicity, but it did not prevent METH-induced locomotor sensitization.

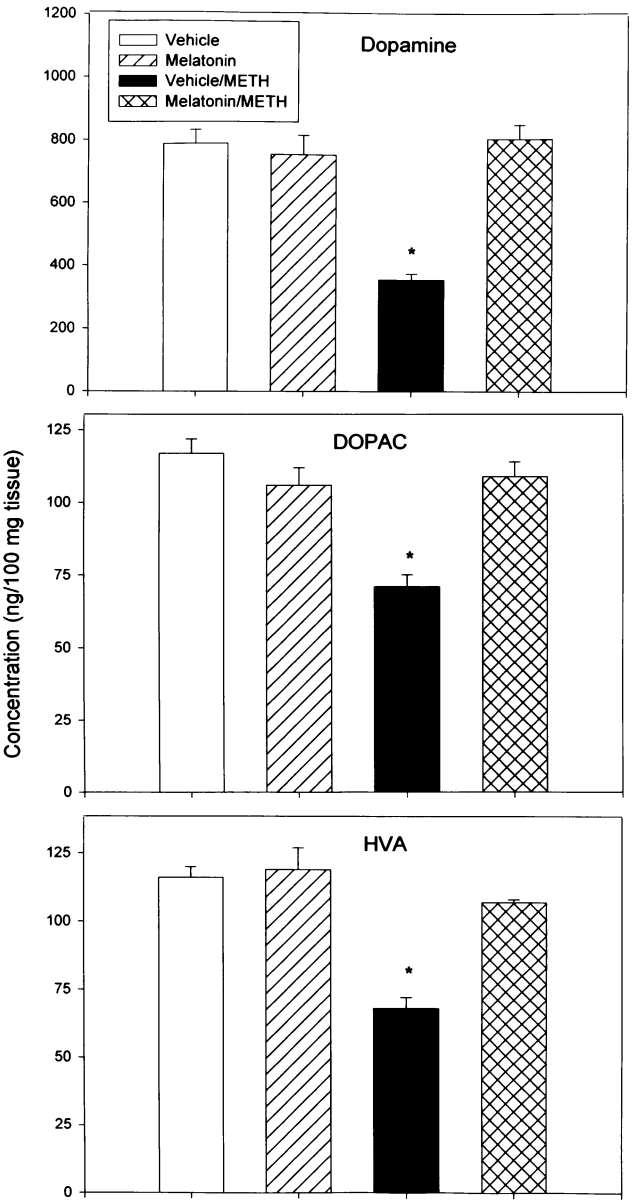

Fig. 1. Tissue content of dopamine, DOPAC and HVA in mouse striatum. Swiss Webster mice were administered either vehicle (3% ethanol × 3), melatonin (10 mg kg^{-1} × 3), vehicle/METH (5 mg kg^{-1} × 3) or melatonin/METH (× 3). The treatment with vehicle or melatonin was continued for two additional days (see Section 2) and animals were sacrificed on the third day. The content of striatal dopamine, DOPAC and HVA was then determined. Results represent the mean ± S.E.M. of 10–12 determinations for each drug or vehicle treatment. $*P < 0.05$ compared vehicle treatment.

Table 1
Binding parameters of [^{3}H]mazindol in mouse striatum

Treatment	B_{max} (fmol/mg protein)	K_d (nM)
Vehicle	1492 ± 108 (100%)	3.77 ± 0.32
Melatonin	1562 ± 78 (104%)	4.32 ± 0.23
Vehicle/METH	642 ± 51 (43%)[a]	4.36 ± 0.21
Melatonin/METH	1485 ± 78 (99%)	3.85 ± 0.27
Vehicle/MPTP	612 ± 22 (41%)[a]	4.15 ± 0.30
Melatonin/MPTP	685 ± 61 (46%)[a]	4.21 ± 0.28

Swiss Webster mice were administered either vehicle (3% ethanol × 3), melatonin (10 m kg^{-1} × 3), vehicle/METH (5 mg kg^{-1} × 3), melatonin/METH, vehicle/MPTP (20 mg kg^{-1} × 3) or melatonin/MPTP. The striatum was removed 72 h after the last injection was delivered and prepared for saturation binding experiments. Binding data were analyzed by the LIGAND program and represent the mean ± S.E.M. of 5–8 separate experiments. Numbers in parentheses represent the percent change from vehicle control value. B_{max} denotes maximal number of binding sites, and K_d denotes affinity constant.
[a] $P<0.05$ Neuman–Keuls test for comparison with vehicle control value.

2. Materials and methods

2.1. Drugs and chemicals

METH-HCl, Desipramine-HCl, benztropine mesylate, and melatonin were purchased from Sigma. MPTP-HCl was purchased from Research Biochemical International (Natick, MA). [^{3}H]Mazindol (24.0 Ci mmole^{-1}) was purchased from New England Nuclear (Wilmington, DE). METH and MPTP solutions were prepared in saline (0.9% NaCl), while melatonin was dissolved in 3% ethanol solution.

2.2. Animals and schedule of drug administration for the determination of dopaminergic neurotoxicity

Male Swiss Webster mice (10–12 weeks of age 30–32 g; Charles River, Wilmington, MA) were maintained on a 12-h light–dark lighting schedule at room temperature of 21°C, and housed in groups of five with free access to food and water. The principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. Animals were divided into six groups (10–20 animals per group), and on the first day the following injections were given intraperitoneally three times (every 3 h): (a) vehicle (3% ethanol solution); (b) melatonin (10 mg kg^{-1}); (c) vehicle 30 min before METH (5 mg kg^{-1}); (d) melatonin (10 mg kg^{-1}) 30 min before METH (5 mg kg^{-1}); (e) vehicle 30 min before MPTP (20 mg kg^{-1}); and (f) melatonin (10 mg kg^{-1}) 30 min before MPTP (20 mg kg^{-1}). All injections were given in a volume of 0.1 ml/10 g of body weight. We have previously shown that these schedules of METH (Itzhak and Ali, 1996) and MPTP (Itzhak et al., 1997) resulted in moderate dopaminergic neurotoxicity (e.g. about 60% depletion of striatal DA and DAT binding sites). Since it has been recently reported that 10 mg kg^{-1} melatonin prevented MPTP-induced neurotoxicity (Acuna-Castroviejo et al., 1997), this dose was routinely used in the present study. On the next day, 16 h after the last METH or MPTP injections were given, all melatonin pretreated animals (groups b, d and f) received two injections of melatonin (10 mg kg^{-1}, each) in 10 h intervals. The vehicle pretreated animals (groups a, c and e) received two injections of 3% ethanol, also separated by 10 h interval. On the third day, the animals received the same treatment as on the second day. After 72 h, following the last METH or MPTP injection, animals were sacrificed, the brain was rapidly removed, and the striatum was dissected and stored at −80°C. Routinely, striatal tissue from one hemisphere was used for monoamine assays, and the tissue from the other hemisphere was used for [^{3}H]mazindol binding assays. Since for each binding experiment striatal tissue from three to four mice was pooled together, additional mice were treated as described above only for the purpose of DAT binding experiments.

2.3. Determination of neurotransmitter concentrations

Concentrations of DA and its metabolites DOPAC and HVA were quantitated by a modified method of high performance liquid chromatography (HPLC) combined with electrochemical detection as described by Ali et al. (1994). Each striatum was weighed in a measured volume (20% w/v) of 0.2 N perchloric acid containing 100 ng ml^{-1} of the internal standard 3,4-dihydroxybenzylamine. The tissue was then disrupted by ultrasonication, centrifuged at 4°C (15000 × g; 7 min), and 150 μl of the supernatant was removed and filtered through a 0.2 mm Nylon-66 microfilter (MF-1 centrifugal filter, Bioanalytical System, W. Lafayette, IN). Aliquots of 25 μl representing 2.5 mg of brain tissue were injected directly onto the HPLC/EC system for separation of the analytes. The concentration of DA, DOPAC and HVA were calculated using standard curves which were generated by determining in triplicate the ratio between three different known amounts of the amine or its metabolites and a constant amount of internal standard.

2.4. Binding of [^{3}H]mazindol to the dopamine transporter

Striatal tissue from one hemisphere of three to four mice was pooled together, homogenized in 20 volumes of Tris–HCl buffer (50 mM; pH 7.7) containing 300 mM NaCl and 5 mM KCl, and centrifuged (40000 × g; 15 min; 4°C). The pellet was resuspended in six volumes of sucrose (0.32 M) and aliquots were stored at −

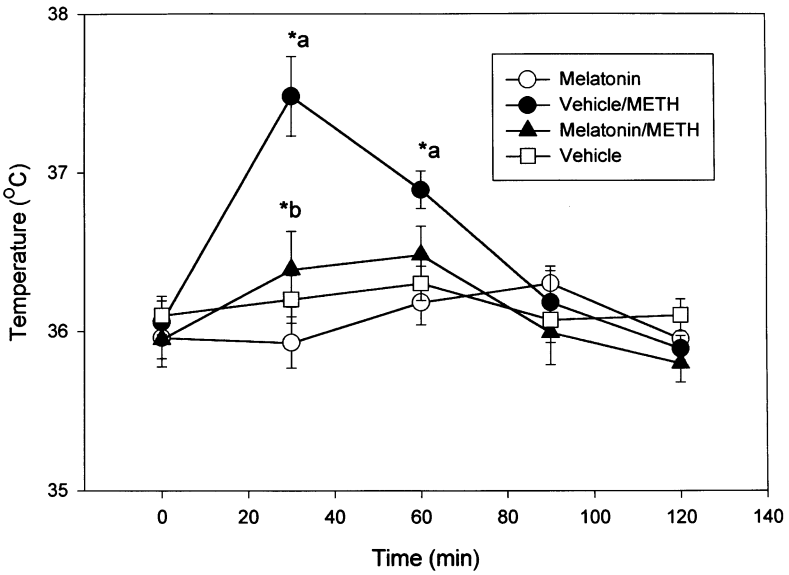

Fig. 2. Effect of METH and melatonin on animals' body temperature. The body temperature of Swiss Webster mice was measured 0–10 min before the administration of the following: melatonin (10 mg kg^{-1} dissolved in 3% ethanol), vehicle (3% ethanol) 30 min before METH (5 mg kg^{-1}) or melatonin 30 min before METH. Then temperature measurements were taken every 30 min for a total period of 120 min. Results represent the mean ± S.E.M. ($n = 10$ for each group). A significant increase in body temperature was observed at the 30 and 60 min time points in the vehicle/METH group (*a, $P < 0.05$ compared to temperature before METH administration). The pretreatment with melatonin significantly reduced METH-induced hyperthermia at the 30 min time point (*b, $P < 0.05$ compared to the 30 min time point of vehicle/METH group).

80°C. For binding assays, the tissue was resuspended in 15 volumes of the buffer to yield protein concentration of 0.3–0.4 mg ml^{-1}. Saturation binding experiments were carried out in a final volume of 0.5 ml containing various concentrations of [^{3}H]mazindol (1.0–20.0 nM) and desipramine (300 nM; to occlude binding to the norepinephrine transporter). Nonspecific binding was determined in the presence of 20 μM benztropine. The reaction mixture was incubated for 60 min at 4°C, and stopped by a rapid vacuum filtration (Brandel M-12) through Whatman GF/B filters presoaked for 15 min in buffer containing polyethylenimine (0.05%). The filters were washed twice with 4 ml ice-cold buffer, and radioactivity was determined by scintillation counting. Protein concentration was determined by the method of Lowry et al. (1951). The binding parameters of [^{3}H]mazindol, e.g., the maximal number of binding sites (B_{max}) and the dissociation constant (K_d) were determined by the LIGAND program, version 2.3.10.

2.5. Measurement of body temperature

Each animal's body temperature was measured before the first injection of either melatonin (10 mg kg^{-1}), METH (5 mg kg^{-1}) or melatonin 30 min prior to METH, and every 30 min thereafter, for a total period of 120 min. The temperature measurement was taken by Thermoscan Instant Thermometer (Thermoscan, San Diego, CA) which was positioned in the animal's ear for only 1 s. The variation between the readings from each ear usually did not exceed 0.2°C, and the average temperature determined from both ears was considered as $n = 1$.

2.6. Measurement of METH-induced locomotor activity

Male Swiss Webster mice (30–32 g) were divided into four groups each containing ten animals. Each animal was placed in the test cage; this was a standard transparent rectangular rodent cage (42 × 24 × 20 cm high) and allowed a 30 min habituation period to the new environment before the test drug or the vehicle was delivered. Based on our previous studies, the 30-min habituation period is sufficient to allow animals' locomotor activity to stabilize at a relatively low pace compared to the initial period in the test cage (Itzhak, 1997). The investigation of the development of sensitization to the psychomotor stimulating effect of METH, as well as the test for conditioned locomotion, were adapted from our previous study (Itzhak, 1997). On day 1, animals were administered either: (a) vehicle (3% ethanol); (b) melatonin (10 mg kg^{-1}); (c) vehicle 30 min before METH (1 mg kg^{-1}); or (d) melatonin (10 mg kg^{-1}) 30 min before METH (1 mg kg^{-1}). Animals' locomotor activity was then recorded for a 60 min period. On days 2, 3, and 4, animals received the drug or vehicle treatments in their home cage, and on day 5 in the test cage. Thus, drug or vehicle administrations were conducted once a day for 5 consecutive days. To determine if context-dependent locomotor sensitization developed, on day 8, after a 3-day of drug free period, animals were placed in the test cage and after 30

min they received a saline injection. Locomotor activity was recorded for a 30 min period. Animals were then returned to their home cage. To investigate if melatonin prevented the induction of METH-induced locomotor sensitization, on day 12—after a 7-day drug free period—all animals were challenged with a single METH injection (1.0 mg kg^{-1}) following the 30 min habituation period. Subsequently, locomotor activity was recorded for a 60 min period.

Animals' activity was monitored by activity meter, Opto-Varimex Mini (Columbus Instruments, Columbus, OH), which consists of an array of 15 infrared emitter–detector pairs, spaced at 2.65 cm intervals, measuring activity along a single axis of motion. Each emitter and detector were mounted alongside the length of the cage (42 cm long). Both total counts and ambulatory counts were recorded and transferred by a computer interface to an IBM computer. Ambulatory counts correspond to horizontal activity, while the difference between the total and ambulatory counts corresponds to vertical activity. Based on our previous observations (Itzhak, 1997) and the current results, it appears that the fraction of non-ambulatory counts (25–30% of the total counts) increases or decreases in parallel to corresponding changes in ambulation counts. Because of this relationship, only ambulatory counts are reported. Counts were registered every 10 min for a period of 30 or 60 min.

2.7. Statistical analysis

The neurochemical results and the behavioral outcome of the multiple drug treatments were analyzed by one way ANOVA followed by post hoc Neuman-Keuls test. The effect of melatonin on METH-induced hyperthermia at various time intervals after drug administration was analyzed by a two-way ANOVA (drug treatment × time) with time as a repeated measure. Bonferroni multiple comparisons adjustment was performed to determine differences between various time points. A P value less than 0.05 was considered as a statistically significant difference.

3. Results

3.1. Effect of melatonin on METH-induced depletion of striatal dopamine and its metabolites and dopamine transporter binding sites

The administration of METH (5 mg $kg^{-1} \times 3$) to Swiss Webster mice resulted in a significant decrease in the content of striatal DA, DOPAC and HVA (44, 59 and 56% of control values, respectively, $P < 0.05$; Fig. 1). The treatment with melatonin, before and after the administration of METH, completely prevented METH-induced depletion of striatal DA, DOPAC and HVA (Fig. 1). In addition, while the administration of vehicle/METH (5 mg $kg^{-1} \times 3$) caused a 57% reduction in the number of DAT binding sites labeled with [^{3}H]mazindol, the pretreatment with melatonin completely precluded the depletion of DAT binding sites (Table 1). The administration of melatonin alone had no significant effect on the content of striatal DA,

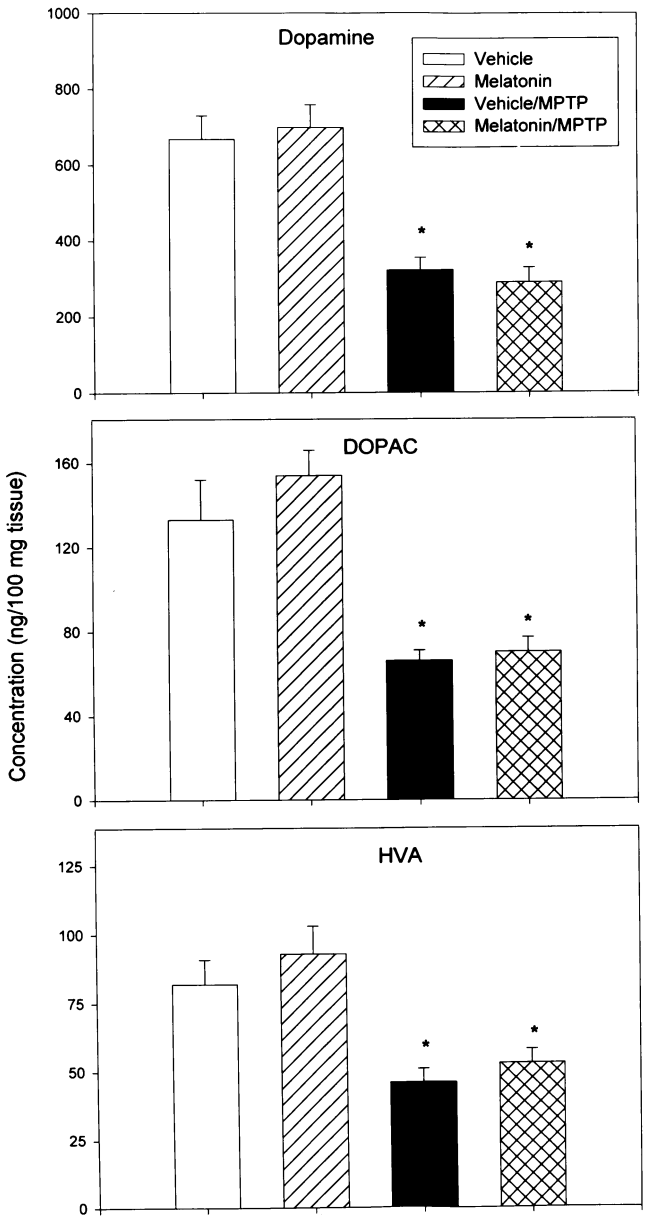

Fig. 3. Tissue content of dopamine, DOPAC and HVA in mouse striatum. Swiss Webster mice were administered either vehicle (3% ethanol × 3), melatonin (10 mg $kg^{-1} \times 3$), vehicle/MPTP (20 mg $kg^{-1} \times 3$) or melatonin/MPTP (× 3). The treatment with vehicle or melatonin was continued for two additional days (see Section 2) and animals were sacrificed on the third day. The content of striatal dopamine, DOPAC and HVA was then determined. Results represent the mean ± S.E.M. of 10–12 determinations for each drug or vehicle treatment. *$P < 0.05$ compared vehicle treatment.

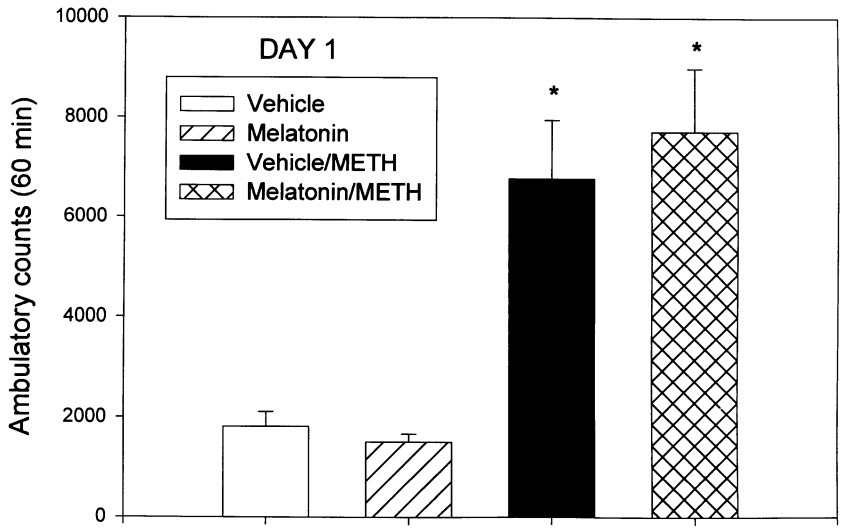

Fig. 4. Animals' response to acute administration of vehicle (3% ethanol), melatonin (10 mg kg^{-1} dissolved in 3% ethanol), vehicle/METH (1 mg kg^{-1}) or melatonin (10 mg kg^{-1})/METH (1 mg kg^{-1}). On day 1, the intensity of METH-induced locomotor activities recorded for the vehicle and melatonin pretreated mice were very similar, and significantly higher than vehicle control mice (*$P < 0.05$). The pretreatment with melatonin had no significant effect on METH-induced locomotor activity. Also, melatonin alone had no significant effect on animals' locomotor activity compared to vehicle control treated animals. Results represent the mean ± S.E.M. ($n = 10$ for each group).

DOPAC and HVA (Fig. 1) and DAT binding sites (Table 1) compared to the values of control vehicle treatment. It also appears that the vehicle used, 3% ethanol, had no significant effect on the dopaminergic markers measured since they did not differ significantly from results we obtain after the administration of saline alone to Swiss Webster mice (Itzhak et al., 1997). These findings suggest that melatonin protected against METH-induced dopaminergic neurotoxicity.

3.2. Effect of melatonin on METH-induced hyperthermia

Since several studies have documented a relationship between METH-induced dopaminergic neurotoxicity and METH-induced hyperthermia (Bowyer et al., 1992; Ali et al., 1994; Miller and O'Callagahan, 1994; Kuperman et al., 1997), the effect of melatonin on METH-induced hyperthermia was investigated. Results in Fig. 2 depict that 30 and 60 min after the administration of METH (5 mg kg^{-1}) there was a significant increase in animals' body temperature (1.67 ± 0.03 and 0.91 ± 0.03°C, respectively; $P < 0.05$ compared to pre-drug administration). Neither the vehicle (3% ethanol) nor melatonin (10 mg kg^{-1} dissolved in 3% ethanol) had a significant effect on animals' body temperature as determined during the 2 h period (Fig. 2). However, the pretreatment with melatonin markedly diminished METH-induced hyperthermia. There was a significant decrease of 1.31 ± 0.04°C in animals' body temperature at the 30 min time point following METH administration in the melatonin pretreated animals compared to the vehicle pretreated animals ($P < 0.05$; Fig. 2). Similar findings were observed after the third administration of melatonin and METH (data not shown). These findings suggest a relationship between the protective effect of melatonin and its ability to diminish METH-induced hyperthermia.

3.3. Effect of melatonin on MPTP-induced depletion of striatal dopamine and its metabolites and dopamine transporter binding sites

The administration of MPTP (20 mg $kg^{-1} \times 3$) to Swiss Webster mice resulted in a significant decrease in the content of striatal DA, DOPAC and HVA (41, 48 and 57% of control values, respectively, $P < 0.05$; Fig. 3). The treatment with melatonin (10 mg kg^{-1}) before and after MPTP administration did not provide any protection against the depletion of DA and its metabolites (Fig. 3). Similarly, melatonin had no effect on MPTP-induced decrease in the number of striatal [3H]mazindol binding sites (Table 1). MPTP caused a 59 and 54% decrease in DAT binding sites in vehicle and melatonin treated animals, respectively (Table 1). In an additional experiment animals ($n = 7$) were treated with 20 mg kg^{-1} melatonin prior and after MPTP (20 mg $kg^{-1} \times 3$) administration (according to the 10 mg kg^{-1} melatonin schedule; see Section 2). Results from the binding of [^{3}H]mazindol to DAT indicated that the 20 mg kg^{-1} melatonin schedule also did not afford protection against MPTP-induced depletion of DAT binding sites (data not shown).

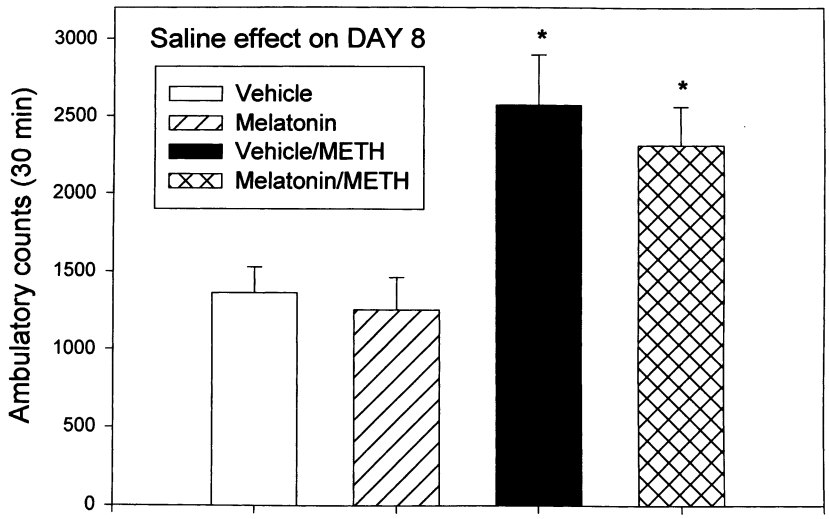

Fig. 5. Test for conditioned-locomotion. Swiss Webster mice were treated with either vehicle (3% ethanol), melatonin (10 mg kg^{-1} dissolved in 3% ethanol), vehicle/METH (1 mg kg^{-1}) or melatonin (10 mg kg^{-1}) /METH (1 mg kg^{-1}) for 5 days. On day 1 and 5 drug or vehicle administration were paired with the test cage. After a 3-day drug free period, on day 8, animals received a saline injection in the test cage and their locomotor activity was measured. The intensity of locomotor activities recorded for the vehicle/METH and melatonin/METH groups were very similar and significantly higher than vehicle control group ($*P < 0.05$). Results represent the mean ± S.E.M. ($n = 10$ for each group).

3.4. Effect of melatonin on METH-induced locomotor sensitization

Since we found that melatonin protected against METH-induced dopaminergic neurotoxicity we sought to investigate whether melatonin attenuates first, METH-induced locomotor activity, and second, METH-induced locomotor sensitization. Fig. 4 depicts the initial acute effects of vehicle (3% ethanol), melatonin (prepared in 3% ethanol), vehicle and METH and melatonin and METH. Results indicate first, that 1 mg kg^{-1} METH produced marked locomotor activity compared to vehicle treated animals. Second, melatonin (10 mg kg^{-1}) pretreatment did not affect METH-induced locomotor activity. Third, melatonin alone (10 mg kg^{-1}) did not cause any change in locomotor activity compared to the vehicle treated animals. These findings indicate that 10 mg kg^{-1} melatonin affected neither spontaneous nor METH-induced locomotor activity.

The animals were paired with the drug (e.g., METH or melatonin/METH) and the test cage twice, on day 1 and day 5 that was the last day of drug administration. We have previously shown that this paradigm of psychostimulant administration results in context-dependent locomotor activity or conditioning (Itzhak, 1997). Thus, on day 8, after a 3-day drug free period, a saline injection was paired with the test cage and animals' activity was recorded. Results presented in Fig. 5 indicate the following. First, animals pretreated with vehicle/METH showed a significant greater activity compared to animals treated with vehicle alone or melatonin alone ($P < 0.05$). Second, animals pretreated with melatonin/METH showed a similar intensity of locomotor activity as the vehicle/METH group. Third, the locomotor activities of the animals pretreated with vehicle or melatonin for 5 days did not differ from each other. These findings indicate that melatonin did not prevent the development of METH-induced context-dependent locomotor activity.

Next we investigated whether melatonin pretreatment attenuated the induction of sensitization to METH. After a 1 week drug free period, animals were challenged with METH (1 mg kg^{-1}) and their locomotor activity was recorded. Results in Fig. 6 indicate that the response to the challenge METH injections of animals pretreated for 5 days with vehicle/METH was about twice as high as the response of control vehicle treated animals. In addition, the locomotor activity produced by a challenge METH injection to the melatonin/METH pretreated group did not differ significantly from the vehicle/METH group. These findings indicate that melatonin did not prevent the induction of locomotor sensitization to METH. Also, the responses of the vehicle and melatonin pretreated groups to the challenge METH injection were very similar (Fig. 6).

4. Discussion

The present study indicates that melatonin has a differential effect on METH- and MPTP-induced neurotoxicity. Melatonin protected Swiss Webster mice from METH-induced depletion of striatal DA, DOPAC, HVA, and DAT binding sites. However, the hormone had no effect on MPTP-induced neurotoxicity, despite the fact that the degree of depletion in the dopaminergic markers produced by the two neurotoxic

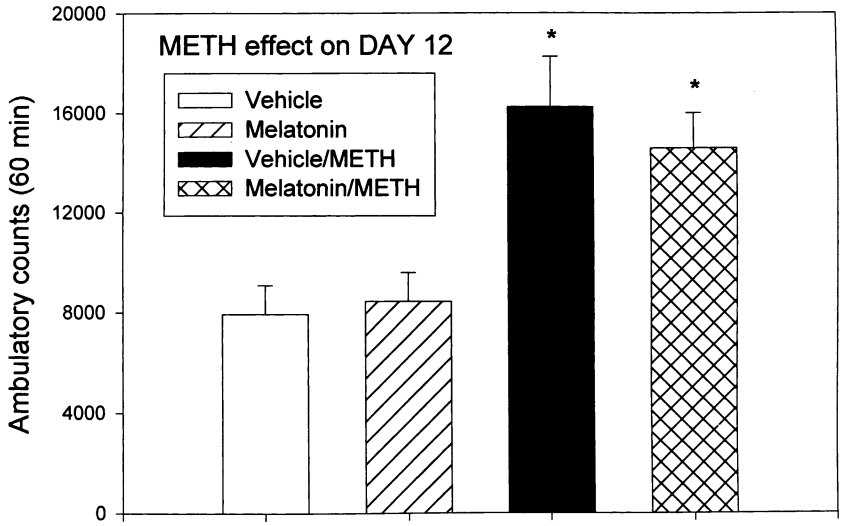

Fig. 6. Test for METH-induced sensitization. Swiss Webster mice were treated with either vehicle (3% ethanol), melatonin (10 mg kg^{-1} dissolved in 3% ethanol), vehicle/METH (1 mg kg^{-1}) or melatonin (10 mg kg^{-1})/METH (1 mg kg^{-1}) for 5 days. After a 1 week drug free period, on day 12, animals were challenged with 1 mg kg^{-1} METH. The intensity of locomotor activities recorded for the vehicle/METH and melatonin/METH groups were very similar and significantly higher than vehicle control group ($*P < 0.05$). The responses of the vehicle and melatonin pretreated groups to 1 mg kg^{-1} METH were not significantly different from each other. Results represent the mean ± S.E.M. ($n = 10$ for each group).

agents was very similar (Figs. 1 and 3Table 1). In a recent study, it has been reported that melatonin (10 mg kg^{-1}) protected C57BL/6 mice against MPTP-induced increase in lipid peroxidation and MPTP-induced decrease in striatal tyrosine hydroxylase immunoreactive nerve terminals (Acuna-Castroviejo et al., 1997). Although our MPTP results seem to be in contrast with the above report, there are at least three points to be considered. First, the markers used to determine the dopaminergic neurotoxicity in our study (e.g., content of striatal DA and its metabolites and DAT binding sites) are different from those used in the above study. Second, while we used Swiss Webster mice, Acuna-Castroviejo and co-workers used C57BL/6 mice. Third, in our study animals were treated three times with 20 mg kg^{-1} MPTP, while in the Acuna-Castroviejo et al. (1997) study only a single injection of 20 mg kg^{-1} MPTP was delivered. It is likely that a single MPTP injection produces less severe neurotoxicity than the three MPTP injections (used in our study), and melatonin may protect against a milder neurotoxic effect. However, the schedule of MPTP used in our study is quite common among other investigators, and in fact it did not produce severe neurotoxicity in Swiss Webster mice which are known to be less susceptible to MPTP than C57BL/6 mice. As indicated in Fig. 3, our regimen of MPTP resulted in a moderate, but not a severe, depletion of DA and its metabolites. Similarly, the depletion in DAT binding sites was about 60% (Table 1). Moreover, it appears that the degree of MPTP-induced neurotoxicity was very similar to that produced by METH administration. Yet, melatonin protected against METH- but not MPTP-induced depletion of dopaminergic markers.

During the preparation of this manuscript Gibb et al. (1997) reported that melatonin did not protect against METH-induced serotoninergic and dopaminergic neurotoxicity in rats. Although the discrepancy between our results and the report by Gibb et al. is not clear, again, there are several differences in the methodology and design of the two studies. First, Gibb et al. had used rats, and in fact METH alone did not produce any dopaminergic neurotoxicity but only serotoninergic neurotoxicity. Second, Gibb et al. administered melatonin in a 30% ethanol solution while we dissolved melatonin in 3% ethanol. Clearly, the 30% ethanol solution by itself may be toxic to the animals. Third, while we treated the mice with melatonin before and after METH administration (e.g., on the first, second and third day following METH administration) Gibb et al. had a completely different schedule of drug administration (for both METH and melatonin). Fourth, while we clearly demonstrated that the vehicle alone (3% ethanol) had no effect on body temperature (Fig. 2), there was no data in Gibb's study to demonstrate the effect of either melatonin or the 30% ethanol solution on animals' body temperature. Therefore, it seems difficult to compare between our study and Gibb's study.

The basis for the differential effect of melatonin on METH- and MPTP-induced neurotoxicity we observed is not quite clear. As indicated in Section 1, several studies have suggested that the dopaminergic neurotoxicity produced by these agents involves the generation of free radicals. The finding that melatonin has the properties of a free radical scavenger and antioxidant supports the notion that this hormone may protect

against the effects of both METH and MPTP. An example for the hypothesis that similar mechanisms may underlie METH- and MPTP-induced neurotoxicity is the finding that blockade of the neuronal nitric oxide synthase (nNOS) by 7-nitroindazole (7-NI) protected against both MPTP- (Schulz et al., 1995) and METH- (Itzhak and Ali, 1996) induced neurotoxicity. Moreover, mutant mice deficient of the nNOS gene are protected against both MPTP- (Przedborski et al., 1996) and METH- (Itzhak et al., 1998) induced neurotoxicity. A putative relationship between NOS and melatonin stems from reports suggesting that melatonin prevents NO-induced damage in brain and also inhibits brain NOS activity (Pozo et al., 1994; Bettahi et al., 1996; Pozo, 1997). Taken together, these findings could support the theory that melatonin may prevent both METH- and MPTP-induced neurotoxicity.

One of the major differences, however, between METH- and MPTP-induced dopaminergic neurotoxicity is the finding that the former is frequently associated with the hyperthermia produced by METH. Several studies have documented that agents which diminish METH-induced hyperthermia also protect against METH-induced neurotoxicity (Bowyer et al., 1992; Ali et al., 1994; Miller and O'Callagahan, 1994; Kuperman et al., 1997). These studies also demonstrated that an increase in ambient temperature increased METH-induced neurotoxicity while a decease in the ambient temperature reduced METH neurotoxicity. No such relationship has been reported for MPTP-induced neurotoxicity. The results of the present study indicate that although melatonin alone did not produce any significant changes in animals' body temperature, the hormone significantly reduced the hyperthermia caused by METH (Fig. 2). Thus, if protection against METH-induced neurotoxicity is related to inhibition of METH-induced hyperthermia, then results of the present study may support this hypothesis. However, it appears that other agents may protect against METH-induced neurotoxicity without affecting METH-induced hyperthermia. For instance we (Itzhak and Ali, 1996) and others (Di Monte et al., 1996) have reported that the nNOS inhibitor 7-NI which protected against METH-induced dopaminergic neurotoxicity did not abolish METH-induced hyperthermia. In addition, the spin trapping agent, phenyl-*N*-*tert*-butyl nitrone (PBN) also provided protection against METH-induced neurotoxicity without affecting animals' body temperature (Cappon et al., 1996). Thus, although several studies, including the present study, support the notion that negating METH-induced hyperthermia may provide protection, other evidence does not always support this hypothesis.

Nevertheless, if temperature regulation may be one of the mechanisms which underlies METH-induced neurotoxicity, it is possible that the protective effect of melatonin observed in the present study is simply due to diminishing METH-induced hyperthermia. In this case, the hormone is not expected to protect against MPTP-induced neurotoxicity which is unrelated to temperature regulation. This assumption may also explain the finding that melatonin had no effect on METH-induced behavioral sensitization.

The development of behavioral sensitization to amphetamines is usually investigated by the administration of intermittent relatively low, non-neurotoxic, doses of amphetamines. Behavioral sensitization to amphetamines has been linked to amphetamine-induced psychosis in humans (Robinson and Becker, 1986; Segal and Kuczenski, 1997) and the development of drug craving (Robinson and Berridge, 1993). In the present study we sought to investigate the effect of melatonin on METH-induced locomotor sensitization because melatonin prevented METH-induced dopaminergic neurotoxicity. Using the same schedule of METH administration to Swiss Webster mice we have recently shown that inhibition of nNOS by 7-NI prevented not only the development of METH-induced neurotoxicity (Itzhak and Ali, 1996), but also METH-induced locomotor sensitization (Itzhak, 1997). Similarly, mice deficient in the nNOS gene were protected against both METH-induced neurotoxicity and locomotor sensitization (Itzhak et al., 1998). Therefore, initially we anticipated that melatonin may protect against the development of sensitization to METH. However, as indicated in Figs. 4 and 6, melatonin affected neither the acute response to METH (on day 1) nor the induction of behavioral sensitization to METH as determined on day 12. We have previously shown that this paradigm of METH administration also resulted in context-dependent locomotor activity (e.g. conditioned locomotion; Itzhak, 1997). Results presented in Fig. 5 indicate that METH pretreated animals indeed developed context-dependent locomotion which was resistant to the effect of melatonin. Taken together, the behavioral experiments indicate that melatonin had no effect on METH-induced locomotor activity and sensitization. The differential effect of melatonin on METH-induced neurotoxicity and behavioral sensitization may be due to the antioxidant and 'anti-hyperthermia' properties of the hormone. If the protective effect of melatonin was a result, for instance, of attenuating METH-induced dopamine release, then the behavioral outcome of METH administration is also expected to be diminished. However, if melatonin acted as a free radical scavenger and–or an inhibitor of METH-induced hyperthermia, then it is anticipated that the hormone may protect against neurotoxicity but not necessarily against the behavioral effects of METH.

In summary, the present study demonstrates that melatonin had a differential effect on METH- and MPTP-induced dopaminergic neurotoxicity. This finding and the observation that melatonin had no effect on

METH-induced locomotor activity suggest that the protection afforded against the neurotoxic effect of METH may be due primarily to the ability of the hormone to diminish METH-induced hyperthermia.

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