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Disparity in the temporal appearance of methamphetamine-induced apoptosis and depletion of dopamine terminal markers in the striatum of mice

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Abstract

Methamphetamine (METH) causes damage in the striatum at pre- and post-synaptic sites. Exposure to METH induces long-term depletions of dopamine (DA) terminal markers such as tyrosine hydroxylase (TH) and DA transporters (DAT). METH also induces neuronal apoptosis in some striatal neurons. The purpose of this study is to demonstrate which occurs first, apoptosis of some striatal neurons or DA terminal toxicity in mice. This is important because the death of striatal neurons leaves the terminals in a state of deafferentation. A bolus injection (i.p.) of METH (30 mg/kg) induces apoptosis (TUNEL staining) in approximately 25% of neurons in the striatum at 24 h after METH. However, in contrast to apoptosis, depletion of TH (Western blotting) begins to appear at 24 h after METH in dorsal striatum while the ventral striatum is unaffected. The peak of TH depletion (approximately 80% decrease relative to control) occurs at 48 h after METH. Autoradiographic analysis of DAT sites showed that depletion begins to appear 24 h after METH and peaks at 2 days (approximately 60% depletion relative to control). Histological analysis of the induction of glial fibrillary acidic protein (GFAP) by METH in striatal astrocytes revealed an increase at 48 h after METH that peaked at 3 days. These data demonstrate that striatal apoptosis precedes the depletion (toxicity) of DA terminal markers in the striatum of mice, suggesting that the ensuing state of deafferentation of the DA terminals may contribute to their degeneration.

Keywords

Neurotoxicity; Apoptosis; Methamphetamine; Striatum; Tyrosine hydroxylase; Dopamine transporter

1. Introduction

Methamphetamine (METH) is an addictive drug that is becoming popular in the USA [19] posing a serious health threat because METH induces neural damage in the central nervous system. The work of Escalante and Ellinwood [9] showed that chronic exposure to amphetamine induced chromatolysis in neurons of the cat brain. Soon after this finding, another group reported that exposure to a chronic high dose of METH produced depletion of DA (up to 80%) in the caudate nucleus of rhesus monkeys for up to 6 months post-treatment [30]. That

same study reported loss of some noradrenalin as well. These seminal studies indicated that METH was neurotoxic and thereby led to the systematic analysis of the impact of METH on monoaminergic systems of the brain. Conclusive evidence demonstrating METH-induced neuronal damage came from the application of the silver stain. METH was shown by this method to induce DA terminal degeneration in the forebrain [26,27]. METH-induced depletion of monoamines is long-lasting given that chronic exposure to METH in non-human primates led to reductions of DA and 5-HT in the caudate 4 years after withdrawal from METH [40]. METH has also been shown to induce tolerance in experimental animals [14,18,28,33].

Dose-dependent decreases in binding of [^{11}C]WIN-35,428 to caudate DAT sites at various doses of METH was studied by PET scanning in baboons [34]. The loss of binding to DAT observed in vivo was confirmed by autoradiography postmortem. The same study also demonstrated decreased levels of DA, DOPAC, and serotonin in the caudate [34]. Studies with humans also revealed METH-induced deficits in monoaminergic systems of the forebrain. For example, a postmortem study of METH users (unknown amount of consumption of the drug) reported significant reduction in the levels of DAT, TH, and DA levels in the caudate and putamen [39]. These reductions may be due to toxicity (fewer DA terminals) rather than neuroadaptation (diminished number of DAT sites against an unchanged number of DA terminals) since a PET scan involving METH abusers showed reduced levels of DAT in the caudate after 3 years of abstinence from METH [21]. Moreover, detoxified METH abusers showed decreased levels of DAT binding and decreased glucose utilization in the caudate and putamen [35,36]. METH abusers showed only partial recovery of striatal glucose metabolism after protracted abstinence lasting up to 17 months [37]. The deficits in DAT found in the striatum of METH abusers have been associated with decrements in motor speed and verbal learning [36].

The above studies provide unequivocal evidence demonstrating toxicity to METH by the pre-synaptic DA terminals of the striatum in both experimental animals and humans. In recent years, it was discovered that METH induces apoptotic cell death in the striatal neurons that are post-synaptic to the DA terminals [7,8,24,42,45]. Thus, in the light that METH induces both pre- and post-synaptic neural damage, a question that arises is whether METH-induced apoptotic cell death and DA terminal toxicity appear simultaneously or whether these events peak at different times after METH. This is an important question because neuronal apoptosis in the striatum may cause instability of the DA terminals contributing to the degeneration of the terminals. In this study, we demonstrate that METH-induced apoptosis (TUNEL) peaks at 24 h while depletion of the DA terminal markers (TH and DAT) peak at 2 days after METH. That is, METH-induced apoptosis precedes both DA terminal toxicity and the induction of GFAP in striatal astrocytes.

2. Materials and methods

2.1. Animals, drugs, and drug administration

Single intraperitoneal (i.p.) injections of METH (30 mg/kg) (Sigma, St. Louis, MO) were given to male ICR mice 10–11 weeks old (Taconic, Germantown, NY). All animals were housed individually with food and water available ad libitum on a 12-h light/dark cycle. Animals were habituated for 2 weeks before any drug treatment. After drug treatment, animals were sacrificed by decapitation at 16 h, 24 h, 2 days, or 3 days. The brains were dissected out and immediately frozen on dry ice. Brain tissue was stored at -80°C until used. All procedures of animal use were according to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Hunter College of the City University of New York.

2.2. TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) histochemistry

The method is adapted from [7] with minor modifications. In brief, fresh frozen 20- μ m coronal sections were collected between bregma 0.38 mm $\pm$ 0.1 mm and fixed in 4% paraformaldehyde for 30 min. After a wash with phosphate-buffered saline, pH 7.6 (PBS), the sections were immersed in 0.4% Triton X-100 in PBS for 5–10 min at 70 °C. Sections were washed and TUNEL reactions (Roche Applied Science, Indianapolis, IN) were applied directly onto the sections and incubated for 1 h in a humidified chamber. After TUNEL staining, sections were counterstained with DAPI. Stained sections were washed in PBS and a coverslip was applied on the tissue with Vectashield (Vector Laboratories, Burlingame, CA). Images were taken with a Nikon Eclipse E400 epifluorescent scope attached to a Hamamatsu digital camera C4742-95 using FITC filters.

2.3. Autoradiographic analysis of DAT

Fresh frozen 20- μ m coronal sections were dried in a dessicator and then incubated in 0.073 nM [125 I]RTI-121 (2200Ci/mmol, New England Nuclear, Boston, MA) buffered solution (137 mM NaCl, 2.7 mM KCl, 10.14 mM Na₂HPO₄, 1.76 mM KH₂PO₄, 10 mM NaI) for 1 h at room temperature. Nonspecific binding was determined using 10 μ M GBR-12909 (Sigma, St. Louis, MO). After incubation, sections were washed twice with chilled buffer for 20 min and then quickly rinsed with chilled distilled water. Slides were allowed to air-dry overnight and exposed on Hyperfilm MP (Amersham Pharmacia, Piscataway, NJ) together with a [125 I] micro-scale. Binding of [125 I]RTI-121 was quantified by densitometry with use of a computer-based NIH image analysis system.

2.4. GFAP immunohistochemistry

Fresh frozen 20- μ m coronal sections from bregma 0.38 mm $\pm$ 0.1 mm were air-dried and fixed in absolute ethanol at –20 °C for 10 min. Sections were allowed to air-dry again and washed in PBS. Nonspecific sites were blocked with M.O.M.[®] Mouse Ig Blocking Reagent (Vector Laboratories, Burlingame, CA) in 0.3% Triton X-100/PBS for 1 h. After being washed with PBS, sections were incubated with M.O.M.[®] diluent for 15 min and then with Cy3-conjugated mouse anti-GFAP (1:25, Sigma, St. Louis, MO) for 3 h. After washing, slides were mounted with Vectashield (Vector Laboratories, Burlingame, CA). All incubations were performed at room temperature with gentle rocking. Images were taken with a Nikon Eclipse TE200 inverted-epifluorescent scope attached to a CE 3.2.0 digital camera using rhodamine filters.

2.5. TH Western blot

Striatal tissue was dissected out after decapitation and separated into DM, DL, VM, and VL compartments (dorsal–medial, dorsal–lateral, ventral–medial, and ventral–lateral, respectively). Tissues were then homogenized with lysis buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 320 mM sucrose, 5 mM HEPES, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 1 mM DTT, 1% Inhibitor Cocktail [1.04 mM AEBSF, 0.8 μ M aprotinin, 0.02 mM leupeptin, 0.04 mM bestatin, 0.015 mM pepstatin A, 0.014 mM E-64 (Sigma, St. Louis, MO)]). Homogenates were centrifuged at 800 $\times$ g for 5 min at 4 °C. Supernatants were further centrifuged at 3000 $\times$ g, and supernatants were then used for Western blot analysis. After protein concentration was determined by the Bradford assay, samples were denatured in Laemmli sample buffer containing 20% β -mercaptoethanol for 10 min at 85 °C. Samples were then subjected to 10% SDS-PAGE gel, and proteins were transferred onto PVDF membranes. Membranes were blocked with 5% non-fat dry milk and probed with mouse anti-TH (1:1000, Chemicon, Temecula, CA) overnight at 4 °C with gentle rocking. Membranes were washed with TBS and incubated with HRP-conjugated goat anti-mouse (1:1000, Santa Cruz Biotech, Santa Cruz, CA) for 1 h at room temperature. After a wash with TBS, proteins were detected with the use

of the SuperSignal[®] West Pico Chemiluminescent Substrate (Pierce, Rockford, IL) and apposed onto Hyperfilm[™] ECL film (Amersham Bio-sciences Corporation, Piscataway, NJ). For internal standards, membranes were stripped and reprobed with mouse anti- β -actin (1:10,000, Sigma, St. Louis, MO). Densitometry was performed using a computerized image analysis system with NIH software. The relative density of each band was normalized against that of β -actin.

2.6. Cell counts and quantification

All coronal sections were taken from bregma 0.38 ± 0.1 mm. Cells were counted from 20- μ m-thick coronal sections in an area of 0.26 mm² for each region of the striatum (DM, DL, VM, VL). Average neuronal cell counts done previously were then used to quantify percentage of TUNEL-positive neurons. TUNEL cell counts were averaged from five 20- μ m serial sections per animal. The number of GFAP-positive cells was quantified from three 20- μ m serial sections per animal.

2.7. Statistical analysis

Analysis was performed from mean $\pm$ SEM. Differences between groups were analyzed by ANOVA followed by post hoc comparison using Fisher's protected least significance test. Significance was set at $P \leq 0.05$.

3. Results

3.1. METH-induced striatal apoptosis

We assessed METH-induced apoptosis in coronal sections of striatal tissue utilizing an in situ method that labels the nuclei of apoptotic cells. Apoptotic cells display green fluorescence because they have accumulated numerous nicks on their DNA that become tagged by FITC-labeled dUTPs using terminal deoxynucleotidyl transferase (referred to as TUNEL). Fig. 1A shows representative photomicrographs of TUNEL-stained cells from the striatum of mice that had been exposed to increasing doses of METH and were euthanized 24 h later (b–e). Apoptotic cell death becomes detectable at a dose of 20 mg/kg of METH and reaches significant levels at 30 mg/kg (Fig. 1B). At 40 mg/kg of METH, neither the amount of TUNEL staining nor the mortality rate changes appreciably from 30 mg/kg of METH. The dorsal–medial aspect of the striatum consistently displays lower levels of METH-induced apoptosis relative to other striatal quadrants (Fig. 1B). METH-induced striatal apoptosis displays inter-animal variability ranging from 0 to 85% in the dorsal lateral striatum at 30 mg/kg of METH as shown in the scatter graphs (Fig. 1C). A few animals do not display apoptosis in the striatum. In the light of the above, we chose the 30 mg/kg dose of METH for subsequent experiments in this study.

In order to determine the time at which apoptosis peaks in the striatum, we assessed multiple time points after the injection of METH. Apoptotic cells become detectable by TUNEL at 16 h after METH. The peak of apoptosis occurs at 24 h after METH in all aspects of the striatum (Fig. 2A). METH-induced apoptosis is barely detectable at 2 days post-METH. However, some TUNEL staining is apparent at 3 days post-METH (Fig. 2A). This is due to one animal that displayed an abnormally high level of apoptosis on day 3 (Fig. 2B). This animal may represent a delayed onset of apoptosis or a second phase of apoptosis. The majority of the animals displayed only residual levels of apoptosis on day 3 (Fig. 2B), possibly due to secondary effects from the initial apoptosis.

3.2. METH-induced DA terminal toxicity

The striatal DA terminal markers TH and DAT were assessed at various times after a single administration of METH (30 mg/kg). TH, the rate-limiting enzyme of catecholamine

biosynthesis, was assessed by Western blotting of total protein from four striatal quadrants (Fig. 3A). After stripping the blots and reprobing for β -actin, the bands corresponding to TH protein were quantitated by image analysis and normalized against β -actin. TH protein levels decrease below control at 16 h (24%) and 1 day (31%) after METH in the dorsal striatum (both medial and lateral aspects). In contrast, TH protein levels are not appreciably decreased at these time points in the ventral striatum (Fig. 3B). At days 2 and 3 post-METH, TH protein levels are decreased approximately 80% below control in all aspects of the striatum (Fig. 3B). Note that when the peak of TH depletion is reached at day 2, the peak of apoptosis preceded it by approximately 24 h (compare Figs. 2A and 3B).

Striatal DAT sites were assessed by autoradiography and the levels of binding of [125 I]RTI-121 were determined by image analysis (see Fig. 4A). Similar to TH, DAT sites were decreased at 1 day post-METH but the peak of depletion was reached 2 days after METH (Fig. 4B).

In addition to the DA terminal markers, we also assessed the induction of GFAP in striatal astrocytes at various times after METH by immunohistofluorescence (Fig. 5A). We counted the number of reactive astrocytes in an area of 0.26 mm² in each striatal quadrant. In sections from control mice, the levels of GFAP are at the threshold of detection by immunohistofluorescence with the commercial antibody that we used (Fig. 5A,a, c, e, and g). Thus, we arbitrarily set the control as zero number of astrocytes. The induction of GFAP in astrocytes is significantly above control at day 2 post-METH. The peak of GFAP induction occurs at 3 days after METH (Fig. 5B). This contrasts sharply with the peak of apoptosis (Fig. 2A).

4. Discussion

The present study measured the temporal emergence of METH-induced deficits of TH and DAT (DA terminal markers) and striatal apoptosis (post-synaptic to the DA terminals) in mice using an acute high dose regimen of METH. We also measured the induction of the astrocytic marker GFAP in the striatum. The results demonstrate that striatal apoptosis appears first, soon followed by deficits in TH and DAT while GFAP induction reaches peak levels at day three post-METH at which time the DA terminal markers have reached their nadir. We used a single high dose of METH at 30 mg/kg instead of a binge delivered 4 $\times$ at 2-h intervals [31] because we have recently observed that the former is approximately 4 $\times$ more effective inducing striatal apoptosis than 10 mg/kg 4 $\times$ at 2-h intervals [46]. However, both are as effective inducing deficits of DA terminal markers in the striatum of mice [42,43,44]. Both dose schedules of METH induce GFAP in striatal astrocytes [12]. However, a study suggests that this astrocytic response may be due to degenerating corticostriatal fibers since GFAP induction was observed in the absence of depletion of striatal TH [24]. Our results suggest that degenerating striatal neurons may also contribute to the induction of the astrocytic response since TUNEL staining precedes the peak of GFAP induction in striatal astrocytes. We utilize a higher dose of METH that produces a higher amount of injury to striatal neurons.

The neural damage induced by METH is likely to result from the impact of more than one mechanism acting at the pre-synaptic and/or post-synaptic sites of the striatum. For example, the DAT of rats exposed to a single high dose of METH (15 mg/kg) becomes inactivated by an oxidative mechanism because DA uptake in striatal synaptosomes is decreased by 48% at 6 h and fully recovers by 24 h after METH [10,11]. In the present study involving mice, we did not observe a decrease in binding of [125 I]RTI-121 by autoradiography to striatal DAT sites at 16 h after METH. However, significant decreases were observed at 24 h and the nadir of binding occurred at 48 h after METH. It is conceivable that the decrease of DAT activity (DA uptake) seen during the first few hours after METH represents damage by reactive oxygen species that inactivate the DAT without affecting its capacity to bind [125 I]RTI-121.

Subsequently, around 24 h after METH, incidentally during the peak of apoptosis, other changes take place that diminish the levels of DAT protein in the DA terminals. We have observed loss of DAT protein by Western blot analysis of total striatal protein using 10 mg/kg 4× at 2-h intervals of METH [43]. In the striatum of mice, decreases of binding of [¹²⁵I]RTI-121 to DAT sites correlate with loss of protein from the terminals [43].

The increase of extracellular DA induced by METH can lead to the oxidative damage of proteins. DA can auto-oxidize to the *ortho*-quinone that can readily react with cysteine residues in proteins such as DA transporters [38]. In synaptosomal preparations the, auto-oxidation of DA leads to the inhibition of DA uptake by the DAT [3] and inhibition of glutamate uptake by the glutamate transporter [2], and in the presence of tyrosinase, DA induces the covalent modification and inactivation of TH [41]. These harmful effects of DA auto-oxidation may result from the covalent modification of cysteinyl residues of proteins. For example, intrastriatal injection of DA resulted in the accumulation of protein-bound cysteinyl DA 24 h post-injection [15]. These findings may account in part for the inhibition of vesicular DA uptake observed in vitro soon after exposure to METH [4] and may lead to the instability of the terminals resulting in the loss of DAT and TH observed 24 h after METH.

Exposure to METH produces deleterious effects on DA levels [30] and on the rate-limiting enzyme of catecholamine biosynthesis TH [16]. Rats given one single injection of METH (40 mg/kg) displayed decreased levels of TH protein at 6 h (29% decrease) with the lowest depletion reached at 72 h (76% decrease) after METH [6]. In the present study involving mice, we observed small decreases at 16 h and 24 h after METH in dorsal striatum only. Two days after METH, the nadir of decrease was reached for both dorsal and ventral aspects of the striatum. The more rapid decrease in levels of striatal TH protein in rats may be due to the higher dose of METH used (40 versus 30 mg/kg in our study) or to an inherent species difference.

Another factor impacting on the striatal neurons and their synaptic contacts is the degeneration of cortical–striatal neurons induced by D-amphetamine [29]. Recent evidence demonstrates the degeneration of cortical neurons by METH [8,25]. Thus, it is plausible to suspect that the striatal apoptosis induced by METH may be initiated by the excessive release of glutamate. Two pieces of evidence support this hypothesis, (1) METH induces the striatal overflow of glutamate as measured by in vivo microdialysis [23], and (2) antagonists of the NMDA receptor attenuate METH-induced DA terminal injury in the striatum [20,22,32].

Degeneration of the striatal DA terminals is a complex phenomenon that may be accounted for by multiple mechanisms operating in parallel or separately at different times after METH. One such mechanism may involve the generation of reactive oxygen species and the induction of lipid peroxidation and modification of proteins (protein carbonyls). Recent studies have detected soon after the systemic injection of METH, the induction of lipid peroxidation in the striatum, hippocampus, and prefrontal cortex [1,13]. METH induces lipid peroxidation in the striatum as early as 30 min after a single i.p. injection of 15 mg/kg [1] that persists for up to 24 h post-METH [13]. In the same study, it was found that protein carbonyls were elevated 4 h after METH and remained elevated for up to 24 h in the striatum and hippocampus, but not in the cortex [13]. One disturbing aspect of these studies is that there is a mismatch between the magnitude of METH-induced lipid peroxidation and terminal damage in these brain regions. The same mismatch exists for protein carbonyls. For example, METH does not induce the production of either lipid peroxidation or protein carbonyls in the prefrontal cortex, yet METH induces significant and long-lasting depletion of serotonin in this brain region [13]. Moreover, the hippocampus displays significantly higher levels of lipid peroxidation and protein carbonyls than the striatum, but the striatum contains several times more DA than the hippocampus [13]. These observations suggest the possibility that, although the generation of

reactive oxygen species is an important contributor to METH-induced toxicity of the striatal DA terminals, other factors will play a role. Our results suggest that weakening of the synapses between the DA terminals and the striatal neurons may be such a contributing factor, especially in the light of our observation that the bulk of apoptosis precedes DA terminal deficits.

We have consistently observed the peak of apoptosis at 24 h after METH. However, a recent study reported the peak of METH-induced apoptosis of some striatal neurons at 3 days after METH [17]. One plausible explanation for this discrepancy may be the age of the mice since the doses of METH are not very different. We inject our mice with METH when they are 11 weeks old compared to 9–11 weeks in the other study [17]. The same group reports the release from the mitochondria of AIF (apoptosis-inducing factor) at 30 min after METH and accumulation of proteolytic fragments of lamin A at 8 h after METH [17]. A study by a different group reported a decrease of cytochrome *c* oxidase staining in striatal tissue (rat) 2 h after METH, suggesting that METH-induced disruption of mitochondrial function occurs very soon after the administration of METH [5]. Our results are consistent with an early onset of apoptosis, an event that may lead to the weakening of the contacts between the dopaminergic terminals and the projection neurons of the striatum. We have conclusive histological evidence demonstrating that METH-induced apoptosis spares the cholinergic, somatostatin, and GABA-parvalbumin interneurons, but impacts the projection neurons (manuscript in preparation).

In conclusion, our data demonstrate that striatal deficits of TH, DAT, and induction of GFAP are preceded by striatal apoptosis. This observation suggests the possibility that depletion of DA terminal markers may be causally associated or enhanced by striatal apoptosis. Loss of striatal neurons must render a significant number of dopaminergic synapses devoid of contacts. Experiments in progress in our laboratory are attempting to elucidate this interesting possibility.

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Fig. 1.

Comparison of the dose of METH and the induction of apoptosis in the striatum. The mice received one bolus injection of METH (i.p.) and were sacrificed 24 h after the injection. Coronal sections of brain tissue at the level of the striatum were processed for TUNEL staining. (A) Scanning confocal micrographs of TUNEL-positive cells in the striatum of mice treated with increasing doses of METH (10–40 mg/kg, i.p.) (a–e). Scale bar = 50 μ m. (B) Bars show mean $\pm$ SEM percent of TUNEL-positive neurons for dorsal – medial (DM), ventral–medial (VM), dorsal – lateral (DL), and ventral–lateral (VL) regions of the striatum. * P < 0.005, ** P < 0.01, *** P < 0.05 compared to saline control. (C) Scattergraphs indicate percent of TUNEL-positive neurons for each animal within each treatment group. Each dot represents one animal. ● Saline,

● METH 10 mg/kg, ● METH 20 mg/kg, ● METH 30 mg/kg, ● METH 40 mg/kg. $n = 10-11$ animals per experimental group.

Fig. 2.

Time course of METH-induced apoptosis in the striatum. The animals received a single injection of METH at 30 mg/kg (i.p.) and were sacrificed at various times after the treatment. Coronal sections of brain tissue were processed for TUNEL as described under Materials and methods. (A) Percent of TUNEL-positive neurons (mean $\pm$ SEM) for dorsal-medial (DM), ventral-medial (VM), dorsal-lateral (DL), and ventral-lateral (VL) regions of the striatum at 16 h, 1 day, 2 days, and 3 days post-METH treatment. * $P < 0.0001$ compared to all saline treatment groups and regions. $^!P < 0.0001$, $^{!!}P < 0.0005$, $^{!!!}P < 0.001$, compared to corresponding region of METH at 1 day. No significance was found between striatal regions.

(B) Scattergraphs show percent of TUNEL-positive neurons for each animal within each treatment group. Each dot represents one animal.

Fig. 3.

Time course of METH-induced depletion of tyrosine hydroxylase (TH) in the striatum. The mice received a single injection of METH (30 mg/kg, i.p.) and were sacrificed at the times indicated. (A) Western blots of TH and β -actin from four different quadrants of the striatum [dorsal-medial (DM), ventral-medial (VM), dorsal-lateral (DL), and ventral-lateral (VL)] at 16 h, 1 day, 2 days, and 3 days post-METH treatment. (B) Quantification of optical densities (O.D.) (mean $\pm$ SEM) of protein bands corresponding to TH from 6 separate Western blots, a different animal per blot. At 16 h post-treatment, METH induced a loss of 24.2%, 12.7%, 30.9%, 12.9% in the DM, VM, DL, VL quadrants of the striatum, respectively. At 1 day post-treatment, METH induced a loss of 25.9%, 8.1%, 37.5%, 13.5% in the DM, VM, DL, VL

quadrants of the striatum, respectively. At 2 days post-treatment, METH induced a loss of 78.2%, 77.4%, 83.8%, 77.6% in the DM, VM, DL, VL quadrants of the striatum, respectively. At 3 days post-treatment, METH induced a loss of 87.8%, 81.8%, 84.8%, 86.2% in the DM, VM, DL, VL quadrants. * $P < 0.0001$, ** $P < 0.005$, *** $P < 0.01$ compared to corresponding regions of saline treatment groups. [†] $P < 0.0001$ compared to corresponding regions of METH at 16 h. [!] $P < 0.0001$ compared to corresponding regions of METH at 1 day. [#] $P < 0.05$ compared to the DM region within each experimental group. [@] $P < 0.05$ compared to the DL region within each experimental group. $n = 6$ animals per experimental group.

Fig. 4.

Appearance of deficits of dopamine transporters (DAT) in the striatum after METH. Animals received a single injection of METH (30 mg/kg, i.p.) and were sacrificed at the times indicated after METH. Coronal sections of brain tissue were processed for receptor autoradiographic analysis of DAT. (A) Autoradiographs of coronal sections through the striatum showing METH-induced depletion of DAT sites in the striatum at times 16 h, 1 day, 2 days, and 3 days post-treatment. DAT sites were labeled with [125 I]RTI-121. (B) METH induced a progressive loss of [125 I]RTI-121 binding sites (mean $\pm$ SEM). In the dorsal-medial (DM) region, METH induced a loss of 0%, 36.43%, 60.62%, and 65.13% of DAT levels at 16 h, 1 day, 2 days, and 3 days post-treatment, respectively. In the ventral-medial (VM) region, METH induced a loss

of 0.45%, 27.43%, 50.51%, and 60.16% of DAT levels at 16 h, 1 day, 2 days, and 3 days post-treatment, respectively. In the dorsal-lateral (DL) region, METH induced a loss of 6.35%, 27.20%, 70.76%, and 70.29% of DAT levels at 16 h, 1 day, 2 days, and 3 days post-treatment, respectively. In the ventral-lateral (VL) region, METH induced a loss of 4.16%, 21.33%, 63.42%, and 65.5% of DAT levels at 16 h, 1 day, 2 days, and 3 days post-treatment, respectively. Regional comparisons showed that the lateral aspect of the striatum displayed a greater density of DAT sites than the medial aspect. The DL region displayed the highest vulnerability to METH-induced DAT depletion. $^{\dagger}P < 0.0001$, $^{\dagger\dagger}P < 0.0005$, $^{\dagger\dagger\dagger}P < 0.005$ compared to the DM region within each experimental group. $^!P < 0.0001$, $^{!!}P < 0.0005$, $^{!!!}P < 0.001$, $^{!!!!}P < 0.005$, $^{!!!!!}P < 0.01$, $^{!!!!!!}P < 0.05$ compared to the VM region within each experimental group. $^aP < 0.0001$, $^{aa}P < 0.0005$, $^{aaa}P < 0.001$, $^{aaaa}P < 0.05$ compared to the corresponding region of saline at 1 day. $^bP < 0.0001$ compared to the corresponding region of saline at 2 days. $^cP < 0.0001$ compared to the corresponding region of saline at 3 days. $^{\#}P < 0.0001$, $^{##}P < 0.005$, $^{###}P < 0.05$ compared to the corresponding region of METH 30 mg/kg at 16 h. $^*P < 0.0001$, $^{**}P < 0.001$, $^{***}P < 0.05$ compared to the corresponding region of METH at 1 day. $n = 8$ animals per experimental group.

Fig. 5.

Assessment of the induction of glial fibrillary acidic protein (GFAP) by METH in the striatum. The animals received a single injection of METH (30 mg/kg, i.p.) and were sacrificed at various times after METH. Coronal sections of brain tissue through the striatum were processed for immunohistofluorescence with an antibody against GFAP conjugated to the chromophore Cy3. (A) Epifluorescent images of GFAP-stained mouse striata at 16 h, 1 day, 2 days, and 3 days post-METH treatment. Scale bar = 20 μ m. (B) Number of METH-induced reactive astrocytes (mean $\pm$ SEM) in the striatum. * P < 0.0001, ** P < 0.005, *** P < 0.01, **** P < 0.05 compared to the corresponding region of saline at 2 days. † P < 0.0001, †† P < 0.005, ††† P < 0.05 compared to the corresponding region of saline at 3 days. † P < 0.0001, †† P < 0.005, ††† P < 0.01, †††† P < 0.05 compared to the corresponding region of METH at 16 h. # P < 0.0001, ## P < 0.005, ### P < 0.05 compared to the corresponding region of METH at 1 day. ^a P < 0.0001, ^{aa} P < 0.0005 compared to the corresponding region of METH at 2 days. There is no significant difference between the DM, VM, DL, and VL regions of the striatum at any time point. n = 6 animals per experimental group.