Rapid and Differential Losses of In Vivo Dopamine Transporter (DAT) and Vesicular Monoamine Transporter (VMAT2) Radioligand Binding in MPTP-Treated Mice

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ABSTRACT The dose- and time-dependent changes of in vivo radioligand binding to the neuronal membrane dopamine transporter (DAT) and vesicular monoamine transporter type 2 (VMAT2) were examined in mouse brain after MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) administrations. Regional brain distribution studies were done in male C57BL/6 mice using simultaneous injections of d-threo-[3H]methylphenidate (DAT) and (+)-α-[11C]dihydrotetrabenazine (VMAT2). Single (55 mg/kg i.p.) or multiple (4× 10 mg/kg i.p., 1-hour intervals) administration of MPTP caused significant reductions in [3H]methylphenidate and [11C]dihydrotetrabenazine specific striatal binding, measured 14 days later. The single high dose of MPTP produced greater losses of [11C]dihydrotetrabenazine binding than did the multiple MPTP dosing regimen. Using the single high dose of MPTP, changes of in vivo binding of the two radioligands were determined at 1, 3, and 14 days after neurotoxin injection. At 1 day, there are large losses of [3H]methylphenidate binding (DAT) but no changes in [11C]dihydrotetrabenazine binding to the VMAT2 site in the striatum. At 3 and 14 days, there were >50% losses of binding of both radioligands, but significantly (P < 0.001) greater losses of VMAT2 binding of [11C]dihydrotetrabenazine. These studies indicate that the losses of the neuronal membrane and vesicular transporters are not always equal, and do not occur in the same time frame, after administration of the neurotoxin MPTP. Synapse 35:250–255, 2000. © 2000 Wiley-Liss, Inc.

INTRODUCTION

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) intoxication of mice has been repeatedly used as a model of the dopaminergic terminal degeneration in the brains of humans suffering from Parkinson’s disease. Although MPTP lesioning does not completely mimic the time course and biochemical changes seen in a long chronic condition such as Parkinson’s disease, the mouse MPTP model has been an invaluable tool in studies of the mechanisms of and potential treatments for dopaminergic nerve terminal losses (Gerlach and Riederer, 1996).

The most commonly used indicator of dopaminergic terminal toxicity has been the loss of dopamine in the striatum; other biochemical measures of dopaminergic neurons, including aromatic amino acid decarboxylase (AADC), tyrosine hydroxylase (TH), and neuronal membrane dopamine transporters (DAT) have also been widely utilized (Gerlach and Riederer, 1996). Recently, the use of radioligands for the vesicular monoamine transporter type 2 (VMAT2) as in vitro (Scherman et al., 1989; Vander Borght et al., 1995; Wilson et al., 1996) and in vivo (Frey et al., 1996; Kilbourn, 1997) measures of dopaminergic terminals has been proposed, based on the observation that the majority (about 95%) of monoaminergic terminals in the striatum are dopaminergic. In MPTP-treated mice, the vesicular monoamine transporter binding site is lost (Kilbourn et al., 1998), just as are the other specific biochemical measures of presynaptic dopaminergic terminals. Recent studies of degeneration following MPTP administrations have clearly shown that this process, although rapidly initiated, may reach completion only
The potential differences in MPTP-induced losses of DAT and VMAT2 binding sites have not been previously examined. This has relevance to the choice of radioligand for in vivo imaging studies of Parkinson’s and other degenerative diseases; we have recently hypothesized that the vesicular transporter may be a better marker of neuronal losses (Kilbourn et al., 1998) as the concentrations of that transporter may be less likely to be regulated than the DAT, which can clearly be altered by drug treatments (Vander Borght et al., 1995; Wilson et al., 1996) and is hypothesized to undergo compensatory regulation in disease (Zigmond, 1997). In the experiments reported here, the use of MPTP-treated mice provides a model to examine whether the neuronal membrane dopamine and vesicular monoamine transporters, both specific presynaptic markers of dopaminergic terminals in the striatum, are lost at the same rate and extent during MPTP-induced degeneration.

MATERIALS AND METHODS

d-threo- [3H]Methylphenidate (specific activity 83 Ci/mmol) was prepared (Amersham, Arlington Heights, IL) by a two-step synthesis involving [3H]methylolation and subsequent deprotection of the ritalinic acid precursor, following the procedure used for synthesis of [13C]methylphenidate (Ding et al., 1994). (+)-α-[11C]Dihydrotetrabenazine (specific activity >500 Ci/mmol) was prepared by [13C]methylation of the 9-O-desmethyl precursor, as previously described (Jewett et al., 1997). MPTP was obtained from Aldrich Chemical Co.

Studies were done in male C57BL/6 mice (20–25 g, 7–8 weeks old; Charles Rivers, Portage, MI). Animals were divided into five groups. Groups A (N = 7–8 weeks old; Charles Rivers, Portage, MI). Animals were divided into five groups. Groups A (N = 7), B (N = 8), and C (N = 7) received a single 55 mg/kg i.p. dose of MPTP. Group D (N = 8) received four injections of 10 mg/kg MPTP i.p. at 1-hour intervals. Group E (N = 8) received a single injection of 0.9% saline (control animals). All injections were done at room temperature (23°C) under light diethyl ether anesthesia; for the multiple MPTP administrations animals were allowed to recover between injections. No attempts were made to control for hypothermia in any animals.

Regional brain distribution studies were done at the following times after the last or single MPTP administration: Group A at 24 hours, Group B at 3 days, and Groups C–E at 14 days. Animals were anesthetized (diethyl ether) and a mixture of d-threo- [3H]methylphenidate (5–6 µC) and (+)-α-[11C]dihydrotetrabenazine (175–275 µC) injected via the tail vein. Animals were allowed to awaken, then anesthetized at 20 minutes and killed by decapitation. The brains were quickly removed and dissected into the following regions of interest: striatum, cortex (whole), hippocampus, hypothalamic region, and cerebellum. Tissue samples were rapidly weighed and then counted for carbon-11 in an automatic γ-counter. Tissue solubilizer was added, and after digestion the scintillation fluid was added and the sample counted again for tritium (automatic β-counter). For both isotopes, data were calculated as percent injected dose per gram of tissue (%ID/g). Specific binding in regions of the brain was calculated as (%injected dose/g region)/(% injected dose/g cerebellum)-1.

Comparisons between groups were initially done using unpaired Student’s t-tests. To evaluate the difference between radioligands within a set of animals, a 2-factor repeated measures ANOVA was utilized. In all cases, a P < 0.05 was considered significant.

RESULTS

Radioligand distributions in control mice

The regional brain radioactivity concentrations and specific binding (defined as region/cerebellum—1) for [3H]methylphenidate and [11C]dihydrotetrabenazine are shown in Table I. For both radiotracers, the highest concentrations of radioactivity are seen in the striatum, and the actual concentrations (%ID/g) are quite similar.

MPTP: single vs. multiple doses

The effects of single (55 mg/kg) and multiple (4 × 10 mg/kg, 1-hour interval) injections of MPTP on specific radioligand binding in the striatum, determined 14 days after neurotoxin injection, are shown in Table II. Specific binding of [3H]methylphenidate in striatum was reduced by 51% (single dose) and 32% (multiple dose), with a non-significant trend (P = 0.1) towards a greater loss of binding after the single high dose treatment. Specific binding of [11C]dihydrotetrabenazine in the striatum was reduced 69 and 49% after single and repeated doses of MPTP, respectively, and the greater loss after the single MPTP dose was significant (P < 0.05). For both dose regimens, there was a
greater loss of in vivo specific binding of $[^{11}C]$dihydrotetrabenazine as compared to $[^{3}H]$methylphenidate ($P, 0.0001$).

**Time course of changes after MPTP**

In Figure 1 are shown the losses of in vivo specific binding of $[^{11}C]$dihydrotetrabenazine and $[^{3}H]$methylphenidate in the mouse striatum at 1, 3, and 14 days after a single 55 mg/kg dose of MPTP. At one day, greater than 50% of the in vivo $[^{3}H]$methylphenidate binding to the DAT has been lost (controls, $0.90 \pm 0.21$; MPTP-treated, $0.39 \pm 0.09$, $P < 0.01$), with a slight but non-significant improvement in specific binding at 14 days ($0.47 \pm 0.19$). In contrast, at one day there is no significant change of the in vivo binding of $[^{11}C]$dihydrotetrabenazine to the VMAT2. By three days, and persisting to 14 days, the $[^{11}C]$dihydrotetrabenazine binding has decreased significantly (69% loss: controls, $1.48 \pm 0.29$; MPTP-treated, $0.46 \pm 0.20$, $P < 0.01$), and to a greater extent than the loss of $[^{3}H]$methylphenidate binding ($P < 0.001$). Specific binding of $[^{11}C]$dihydrotetrabenazine did not show any trend towards improvement at 14 days.

**DISCUSSION**

In this experiment, the dose- and time-dependent changes of in vivo radioligand binding to the dopamine neuronal transporter (DAT) and vesicular monoamine transporter (VMAT2) in the mouse brain were determined following treatments with one or multiple doses of MPTP. These studies have demonstrated that losses of in vivo radioligand binding to the two transporters does not occur at the same rate or to the same extent.

**DAT and VMAT2 radioligand binding in vivo: control animals**

The regional distributions for both $[^{3}H]$methylphenidate and $[^{11}C]$dihydrotetrabenazine are consistent with in vitro $B_{\text{max}}$ values for in vitro radioligand binding to the respective transporter (DAT and VMAT2) and are very similar to the in vivo regional distributions previously reported using these radioligands in the rodent brain (Gatley et al., 1995; Kilbourn, 1997; Kilbourn et al., 1995, 1998). The regional distributions of the two ligands do not correlate with each other ($r^2 = 0.59$ for all regions, $r^2 = 0.2$ without striatum). For $[^{11}C]$dihydrotetrabenazine, there is intermediate specific binding to monoaminergic terminals (dopaminergic, serotonergic, and adrenergic) in the hypothalamus, which can be competed for by cold VMAT2 antagonists (Kilbourn and Sherman, 1997), and insignificant specific binding in the cortex. For $[^{3}H]$methylphenidate, low but discern-
able levels of residual radioactivity are observed in cortex and hippocampus and very low levels in the hypothalamus; in the rat, however, none of the $[^3]H$methylphenidate uptake in these regions can be blocked by administration of cold methylphenidate (Kilbourn and Sherman, unpublished results), and thus likely do not represent significant specific binding.

**Single vs. multiple doses of MPTP**

A single high dose of MPTP (55 mg/kg) was apparently more neurotoxic than multiple low doses (4 x 10 mg/kg, 1-hour intervals), as demonstrated by greater losses of both $[^{11}C]$dihydrotetrabenazine and $[^3]H$methylphenidate binding (Table II). Although many investigators have utilized multiple injections of MPTP to create dopaminergic lesions, there is ample literature showing that a single high dose of MPTP is neurotoxic and produces significant loss of striatal dopamine concentrations and dopamine uptake (Freyaldenhoven et al., 1995; Kilbourn et al., 1997; Hoskins and Davies, 1989; Jossan et al., 1989; Pileblad and Carlsson, 1988; Pileblad et al., 1985; Vaglini et al., 1996). We have previously shown that the multiple injection protocol (4 x 10 mg/kg) reduced in vivo radioligand binding to the dopamine transporter (using $[^{18}F]$GBR 13119 as radioligand; Kilbourn et al., 1991) as well as the VMAT2 (using $[^3]H$dihydrotetrabenazine as radioligand; Kilbourn et al., 1998) in mouse striatum. It was not clear, however, if the simpler single injection protocol would produce equivalent decrements of in vivo radioligand binding. Surprisingly, and quite unexpectedly, the single 55 mg/kg MPTP dose produced clearly larger losses of VMAT2 radioligand binding, and a trend towards greater loss of DAT radioligand binding.

Comparisons of results previously obtained from MPTP administrations, including both single-dose and multiple dose regimens, are quite difficult as there have been no consistent protocols regarding neurotoxin doses, intervals between MPTP administrations, intervals from administration to analysis, mouse strains and sources, and biochemical measures (dopamine levels, dopamine transporter, vesicular monoamine transporter, TH enzyme activity). To minimize such difficulties in comparing studies, we utilized here a single group of C57BL/6 mice of identical sex and age, from a single supplier, with neurotoxin injections (single vs. multi-dose protocols) done on consecutive days. This should have minimized any potential differences between the groups, and the greater losses of $[^3]H$dihydrotetrabenazine binding to the VMAT2 site can be attributed to the MPTP dose regimen alone.

**Time course of losses of radioligand binding**

There was a difference in both the rate and extent of loss of in vivo radioligand binding to the DAT and the VMAT2 following the single MPTP dose. At 24 hours the in vivo binding of $[^3]H$methylphenidate to the DAT had decreased by 50%, but $[^{11}C]$dihydrotetrabenazine binding to the VMAT2 remained unchanged. At 3 days, and persisting at 14 days, both radioligands showed losses in vivo binding, with a greater loss of $[^{11}C]$dihydrotetrabenazine binding to the VMAT2.

Differential losses of dopaminergic markers after MPTP treatment have been reported numerous times, but this is the first study demonstrating a differential loss of the DAT and VMAT2. For applications of such radioligands as markers of dopaminergic terminals, it thus has to be considered that they are not equivalent and potential different regulatory compensation of one (or both) of these binding sites needs to be considered. There is clear evidence that the DAT binding site can be regulated by dopaminergic drugs (Vander Borght et al., 1995; Wilson et al., 1996a); whether the DAT is regulated after a neurotoxic insult, as part of a compensatory mechanism of the remaining dopaminergic terminals, is presently not fully established. In contrast, the VMAT2 site may be more robust and not readily regulated (Vander Borght et al., 1995), and a number of investigators have utilized VMAT2 radioligand binding as in vitro and in vivo measure of dopaminergic terminal density in the rodent and human brain (Frey et al., 1996; Scherman et al., 1989; Wilson et al., 1996a–c).

The early loss of the DAT site, with retention of the VMAT2, would be consistent with rapid biochemical changes of the dopaminergic terminal in response to the neurotoxic insult, but without actual degeneration of the terminal structure. A previous study of the biochemical and morphological changes following MPTP administration described a degenerative process beginning as early as 12 hours after MPTP administration but continuing for up to 4 days (Jackson-Lewis et al., 1995). At some point, however, between 1 and three days the terminals have suffered sufficient damage and show losses of the vesicular structures, with concomitant loss of the VMAT2 binding site, as demonstrated in this study. This time sequence is very similar to the results recently reported following methamphetamine administration to mice, where losses (-77%) of DAT radioligand binding ($[^3]H$WIN 35,428 determined in vitro) in the striatum were observed as early as one day after neurotoxin injection, but significant losses of in vitro $[^3]H$dihydrotetrabenazine binding to the VMAT2 were not seen until 3 days later (Hogan and Sonsalla, 1998) and these losses of both transporters persist at later time points (Frey et al., 1997; Hogan and Sonsalla, 1998).

The greater loss of in vivo VMAT2 radioligand binding, as compared to DAT radioligand binding, was not expected. Other studies have noted a dissociation between various dopaminergic markers (DAT, TH, DA concentrations) after MPTP lesioning of dopaminergic nerve terminals of mouse brain (Donnan et al., 1987). However, due to the wide variation in experimental
design (discussed above), comparison of our results with these earlier findings is impossible. In human autopsy samples from both patients with Parkinson’s disease as well as methamphetamine users, differential losses of DAT and VMAT2 radioligand binding have also been observed (Wilson 1996a–c); in those cases, greater losses of DAT than VMAT2 radioligand binding have been reported, which may perhaps reflects long-term compensatory mechanisms involving the DAT.

**Regional specificity of changes**

The MPTP-induced losses of in vivo radioligand binding to both the DAT and the VMAT2 were observed only in the striatum. No significant losses of [11C]dihydrotetrabenazine binding were observed in the hypothalamus, a region with clearly an intermediate concentration of VMAT2 binding sites. Finally, no significant MPTP-induced changes were observed in the cerebellum for either radioligand, an important observation as that region was chosen as the denominator for estimation of specific binding.

**Recovery of in vivo radioligand binding**

There have been reports of recovery of dopaminergic markers after MPTP administration, presumably by sprouting of terminals (Donnan et al., 1987; Mitsumoto et al., 1998; Ricuarte et al., 1987; Saitoh et al., 1987); in some cases these changes have been seen as early as the first 2 weeks after MPTP administration. In this study, we did not observe any significant improvement of in vivo DAT or VMAT2 radioligand binding during the first 2-week period after a single high-dose MPTP administration (Fig. 1).

**Dual in vivo radiotracer studies: applications**

The simultaneous, dual radiotracer in vivo technique described here can be used to follow differential losses of both the dopamine transporter (DAT) and the vesicular monoamine transporter type 2 (VMAT2) binding sites following MPTP administrations. A single high MPTP dose (55 mg/kg i.p.) produced significant losses (>50%) of both [3H]methylphenidate (DAT) and [11C]dihydrotetrabenazine (VMAT2) binding to the mouse striatum in vivo; losses of radioligand binding only to the DAT occurred at one day after MPTP administration, whereas greater losses of VMAT2 radioligand binding were observed at 3 and 14 days. This dual radiotracer in vivo technique will next be applied to (1) studies of differential long-term recovery of these transporters after MPTP treatment and (2) evaluation of which type of radioligand (DAT or VMAT2) provides better measures of prevention or amelioration of degenerative effects of such neurotoxin treatments.

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