Nucl. Med. Biol. Vol. 18, No. 7, pp. 803–806, 1991
Int. J. Radiat. Appl. Instrum. Part B

0883-2897/91 $3.00 + 0.00

In Vivo Binding of the Dopamine Uptake Inhibitor [^{18}F]GBR 13119 in MPTP-treated C57BL/6 Mice

MICHAEL R. KILBOURN*, G. KEITH MULHOLLAND, PHIL S. SHERMAN and TERESA PISANI

Division of Nuclear Medicine, Department of Internal Medicine, The University of Michigan Medical School, Ann Arbor, MI 48109, U.S.A.

(Received 12 November 1990)

The *in vivo* regional distribution of [^{18}F]GBR 13119 (1-[(4-[^{18}F]fluorophenyl(phenyl)methoxy)ethyl]-4-(3-phenylpropyl)piperazine), a specific dopamine reuptake inhibitor, was examined in brains of C57BL/6 mice after MPTP treatment. At 2 weeks post MPTP the *in vivo* specific binding of [^{18}F]GBR 13119 in striatum was decreased 63% relative to age and sex-matched controls. Animals studied at 6 and 8 weeks after MPTP treatment showed a gradual recovery of specific [^{18}F]GBR 13119 binding in the striatum. No significant changes were observed in binding of radiotracer to cerebellum or cortex after MPTP treatment, nor were age-related changes observed in control mice. *In vivo* radiotracer studies thus appear useful for following gradual changes in the dopamine uptake system of mouse brain after neurotoxin treatment.

Introduction

Although the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-treated mouse has seen extensive use as a model of Parkinson's disease (review: Kopin and Markey, 1988), it differs from idiopathic parkinsonism in that the onset of neuronal degeneration is very rapid and is followed by slow recovery of the dopaminergic system. The return of endogenous dopamine levels to higher levels is now well documented (Kopin and Markey, 1988; Ricuarte *et al.*, 1987). Similar changes in the neuronal dopamine reuptake system, which is responsible for termination of neurotransmission by removal of dopamine from the synapse, have been reported using *in vitro* methods. After MPTP treatment there is a rapid decline in DA uptake (V_{max}, using [^{3}H]dopamine: Jossan *et al.*, 1989; Ricuarte *et al.*, 1987), and decreased binding of [^{3}H]mazindol to the DA transporter site in striatal synaptosomal preparations (Ricuarte *et al.*, 1987; Sershen *et al.*, 1985; Sundstrum *et al.*, 1988) or striatal tissue by *in vitro* autoradiography (Donnan *et al.*, 1987). The dopamine reuptake system also appears to recover with time, and the number of DA uptake sites as determined by *in vitro* [^{3}H]mazindol binding appears to correlate with endogenous DA levels (Donnan *et al.*, 1987).

We have developed the new radioligands [^{18}F]GBR 12909 (1-[(4-[^{18}F]fluorophenyl(4-fluorophenyl)-methoxy)ethyl]-4-(3-phenylpropyl)piperazine; Haka and Kilbourn, 1990) and [^{18}F]GBR 13119 ((1-[(4-[^{18}F]-fluorophenyl(phenyl)methoxy)ethyl]-4-(3-phenylpropyl)piperazine: Kilbourn and Haka, 1988; Haka *et al.*, 1989) for the *in vivo* study of the DA uptake system, and have recently reported the regional *in vivo* binding and pharmacological specificity of these radioligands in mouse, rat, monkey and human brain (Kilbourn, 1988; Kilbourn *et al.*, 1989a, b; Ciliax *et al.*, 1990; Koeppe *et al.*, 1990). Although the MPTP rodent has been extensively studied using *in vitro* techniques, very few *in vivo* experiments have been reported, mostly due to the lack of suitable radioligands. A thorough understanding of the extent and time course of recovery of the dopaminergic system in MPTP-treated rodents will be important in the interpretation of results of studies into new preventative or therapeutic strategies for neurodegenerative diseases such as Parkinson's disease. The availability of new radioligands such as [^{18}F]GBR 13119 and [^{18}F]GBR 12909, which can be synthesized in high specific activity (>2000 Ci/mmol), makes possible *in vivo* studies of the DA reuptake system in the MPTP mouse model. We report here that [^{18}F]GBR 13119 can be used to demonstrate *in vivo* loss of DA reuptake sites after MPTP treatment, and

*All correspondence should be addressed to: Dr Michael R. Kilbourn, Division of Nuclear Medicine, B1G412 University Hospital, University of Michigan, Ann Arbor, MI 48109, U.S.A.

furthermore that this *in vivo* radiotracer technique has the sensitivity to show the gradual recovery of this neurotransmitter reuptake system.

Materials and Methods

[^{18}F]GBR 13119 (specific activity > 2000 Ci/mmol) was prepared according to literature methods (Kilbourn and Haka, 1988; Haka *et al.*, 1989). MPTP was prepared by the methylation of 4-hydroxy-4-phenylpiperidine (Aldrich Chem. Co.) followed by acid-catalyzed dehydration. Mice (C57BL/6, male, 25–30 g, 13–15 weeks: Charles River) were injected four times with 10 mg/kg i.p. MPTP at 1 h intervals (Ricuarte *et al.*, 1987). Animals were then used for brain biodistribution studies at 2, 6 and 8 weeks following MPTP treatment; at each time point an equal number of control animals, age- and sex-matched, were also studied. Mice (controls or MPTP-lesioned) were injected via the tail vein with 3–10 μCi of [^{18}F]GBR 13119 (< 1 ng/kg, in isotonic saline). At 60 min the animals were killed by decapitation, and the brain rapidly removed and dissected into regions of interest (striatum, cortex, cerebellum, rest of brain) and a blood sample obtained. The tissue samples were then weighed and counted for fluorine-18. Data were calculated as % injected dose/g (% I.D./g) for all tissues, and analyzed for significance using an unpaired Student's *t*-test.

Results

At 2 weeks after MPTP treatment there is a significant loss of *in vivo* [^{18}F]GBR 13119 binding to mouse striatum (Table 1). The data are presented both as regional brain distributions (% I.D./g) and target to non-target ratios (% I.D. striatum/% I.D. cerebellum; STR/CER). The STR/CER ratios were statistically identical between all control groups demonstrating no age-related dependence of the dopamine uptake system during the time period (13–24 weeks) studied here; this is consistent with the literature (Jonec and Finch, 1975; Strong *et al.*, 1984) and therefore all control animals were combined into a single group. The loss of specific [^{18}F]GBR 13119 binding in the striatum [defined here as % I.D. STR minus % I.D. CER, where cerebellum is assumed to be completely void of dopaminergic terminals (Grigoriadis *et al.*, 1989)] at 2 weeks is approx. 63% (controls, 1.02 ± 0.12; MPTP-treated, 0.382 ± 0.22, $P < 0.006$).

Discussion

[^{18}F]GBR 13119 is a high affinity radioligand for the dopamine uptake site which shows good pharmacological selectivity *in vivo* (Kilbourn, 1988; Kilbourn *et al.*, 1989a, b; Ciliax *et al.*, 1990). The specific binding of this radioligand to sites in the striatum of mice is significantly decreased (63%) at 2 weeks after systemic MPTP treatment (Table 1). This is similar to *in vitro* decreases of 40–80% in [^{3}H]mazindol binding to striatal tissue (Ricuarte *et al.*, 1986; Sershen *et al.*, 1985; Sundstrom *et al.*, 1988) and losses of dopamine of as much as 80% (Nishi *et al.*, 1989; Ricuarte *et al.*, 1987; Sundstrom *et al.*, 1988). No significant changes were observed in [^{18}F]GBR 13119 binding in cortex or cerebellum. Cerebellar radioactivity levels should represent mostly non-specific binding, as no dopamine uptake sites have been observed in the cerebellum using *in vitro* autoradiography ([^{3}H]mazindol: Donnan *et al.*, 1989) or photoaffinity labeling experiments (Grigoriadis *et al.*, 1989). We have consistently observed slightly higher cortical [^{18}F]GBR 13119 levels (cortex/cerebellum values of 1.1–1.3), but this binding (if to DA uptake sites) may not be affected by MPTP treatment.

The time course study of [^{18}F]GBR 13119 binding (Table 1) shows a gradual recovery of the striatal DA uptake system over the 8 week period: full recovery has not been achieved, as STR/CER ratios remain depressed at the end of the study. This is consistent with the demonstration of recovery of dopamine uptake sites *in vitro* using [^{3}H]mazindol binding to mouse brain striatal membranes (Donnan *et al.*, 1987), where control values were reached after 1 year.

[^{18}F]GBR 13119 would thus appear a suitable marker for dopaminergic terminals in the striatum of mice, and the loss and subsequent recovery of DA uptake sites after MPTP treatment have for the first time been demonstrated using *in vivo* radiotracer techniques. The recovery of DA reuptake sites with time is consistent with the proposals of regrowth of dopaminergic terminals from areas which are less sensitive to the toxin (ventral tegmental area, substantia nigra) (Donnan *et al.*, 1987; Kopin and Markey, 1988). The availability of such high specific activity, specific radioligands for *in vivo* studies eliminates possible problems with using less specific radioligands such as [^{3}H]mazindol or [^{3}H]nomifensine, which bind to both dopamine and norepinephrine reuptake sites. The use of [^{18}F]GBR 13119 may also avoid difficulties with the use of [^{3}H]GBR 12935 and *in vitro* analysis methods, in which a second binding

Table 1. Time course of changes in regional brain distribution of [^{18}F]GBR 13119 in control and MPTP-lesioned C57BL/6 mice. Values shown are mean ± SD

	% Injected dose/g			
	Striatum	Cerebellum	Cortex	Striatum/cerebellum
Control	1.47 ± 0.13	0.45 ± 0.04	0.76 ± 0.06	3.1 ± 0.16 (n = 16)
2 weeks post MPTP	0.86 ± 0.17**	0.48 ± 0.11	0.64 ± 0.14	1.87 ± 0.3* (n = 4)
6 weeks post MPTP	1.25 ± 0.07	0.48 ± 0.12	0.66 ± 0.02	2.3 ± 0.20** (n = 4)
8 weeks post MPTP	1.28 ± 0.31	0.47 ± 0.06	0.66 ± 0.05	2.7 ± 0.14** (n = 7)

*$P < 0.01$; **$P < 0.05$; Student's *t*-test.

site for this class of compounds has been described: originally termed a "piperazine acceptor site" (Andersen, 1987, 1989; Andersen *et al.*, 1987) it has been more recently identified as the cytochrome *P*450IID1 enzyme present in brain tissue (Niznik *et al.*, 1990). Importance of this second binding site to our *in vivo* results obtained with [18F]GBR 13119 remains unclear (Kilbourn *et al.*, 1989b), although it probably contributes to overall radioligand binding in the brain, and has been recently proposed as the explanation for the mismatch between losses of DA uptake sites measured with this type of radioligand (40–60%, including present work) and the much greater losses of endogenous dopamine (>95%) (Seeman and Niznik, 1990).

The study of the time course of [18F]GBR 13119 binding in recovering MPTP-treated mice also demonstrates that such *in vivo* radiotracer techniques may have the sensitivity needed to measure gradual changes in the dopaminergic system, presumably representing changes in the number of dopaminergic terminals. In this study, specific binding was estimated using simple target to non-target ratios: application to human studies using PET may require more complex pharmacokinetic modeling approaches (Koeppe *et al.*, 1990). Such studies may be of considerable value in the study of new pharmacological approaches to prevent or treat degeneration of this important neuronal system (Zigmond and Stricker, 1989), whether the degeneration is of natural cause (Parkinson's disease) or neurotoxin produced (MPTP, methamphetamine; Wagner *et al.*, 1980).

Acknowledgements—This work was supported by National Institutes of Health Grant NS15655 and Department of Energy Grant DE-AC02-76EV02031. The authors thank the cyclotron staff for the production of the radionuclide.

References

Andersen P. H. (1987) Biochemical and pharmacological characterization of [3H]GBR 12935 binding *in vitro* to rat striatal membranes. *J. Neurochem.* **48,** 1887–1896.

Andersen P. H. (1989) The dopamine uptake inhibitor GBR 12909: selectivity and molecular mechanism of action. *Eur. J. Pharmacol.* **166,** 493–504.

Andersen P. H., Jansen J. A. and Nielsen E. B. (1987) [3H]GBR 12935 binding *in vivo* in mouse brain: labelling of a piperazine acceptor site. *Eur. J. Pharmacol.* **144,** 1–6.

Ciliax B. J., Kilbourn M. R., Haka M. S. and Penney J. B. (1990) Imaging the dopamine uptake site with *ex vivo* [18F]GBR 13119 binding autoradiography in rat brain. *J. Neurochem.* **55,** 619–623.

Donnan G. A., Kaczmarczyk S. J., McKenzie J. S., Rowe P. J., Kalnins R. M. and Mendelsohn F. A. O. (1987) Regional and temporal effects of 1-methyl-1,2,3,6-tetrahydropyridine on dopamine uptake sites in mouse brain. *J. Neurol. Sci.* **81,** 261–271.

Grigoriadis D. E., Wilson A. A., Lew R., Sharkey J. S. and Kuhar M. J. (1989) Dopamine transport sites selectively labeled by a novel photoaffinity probe: 125I-DEEP. *J. Neurosci.* **9,** 2664–2670.

Haka M. S. and Kilbourn M. R. (1990) Synthesis of [18F]GBR 12909, a dopamine reuptake inhibitor. *J. Labelled Compd. Radiopharm.* **28,** 793–800.

Haka M. S., Kilbourn M. R., Watkins G. L. and Toorongian S. A. (1989) Aryltrimethylammonium trifluoromethanesulfonates as precursors to aryl [18F]fluorides: improved synthesis of [18F]GBR 13119. *J. Labelled Compd. Radiopharm.* **27,** 823–833.

Jonec V. and Finch C. E. (1975) Ageing and dopamine uptake by subcellular fractions of the C57BL/6 male mouse brain. *Brain Res.* **91,** 197–215.

Jossan S. S., Sakurai E. and Oreland L. (1989) MPTP toxicity in relation to age, dopamine uptake and MAO-B activity in two rodent species. *Pharmacol. Toxicol.* **64,** 314–318.

Kilbourn M. R. (1988) *in vivo* binding of [18F]GBR 13119 to the brain dopamine uptake system. *Life Sci.* **42,** 1347–1353.

Kilbourn M. R. and Haka M. S. (1988) Synthesis of [18F]GBR 13119, a presynaptic dopamine uptake antagonist. *Appl. Radiat. Isot.* **39,** 279–282.

Kilbourn M. R., Carey J. E., Koeppe R. A., Haka M. S., Hutchins G. D., Sherman P. S. and Kuhl D. E. (1989a) Biodistribution, dosimetry, metabolism and monkey PET studies of [18F]GBR 13119: imaging the dopamine uptake system *in vivo*. *Nucl. Med. Biol.* **16,** 331–334.

Kilbourn M. R., Haka M. S., Mulholland G. K., Sherman P. S. and Pisani T. (1989b) Regional brain distribution of [18F]GBR 13119, a dopamine uptake inhibitor, in CD-1 and C57BL/6 mice. *Eur. J. Pharmacol.* **166,** 331–334.

Koeppe R. A., Kilbourn M. R., Frey K. A., Penney J. B., Haka M. S. and Kuhl D. E. (1990) Imaging and kinetic modelling of [F-18]GBR 12909, a dopamine uptake inhibitor. *J. Nucl. Med.* **31,** 720.

Kopin I. J. and Markey S. P. (1988) MPTP toxicity: implications for research in Parkinson's disease. *Annu. Rev. Neurosci.* **11,** 81–96.

Nishi K., Kondo T. and Narabayashi H. (1989) Difference in recovery patterns of striatal dopamine content, tyrosine hydroxylase activity and total biopterin content after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration: a comparison of young and older mice. *Brain Res.* **489,** 157–162.

Niznik H. B., Tyndale R. F., Sallee F. R., Gonzalez F. J., Hardwick J. P., Inaba T. and Kalow W. (1990) The dopamine transporter and cytochrome P450IID1 (debrisoquine 4-hydroxylase) in brain: resolution and identification of two distinct [3H]GBR 12935 binding proteins. *Archs Biochem. Biophys.* **276,** 424–432.

Ricuarte G. A., Irwin I., Forno L. S., Delanney L. E., Langston E. and Langston J. W. (1987) Ageing and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induces degeneration of dopaminergic neurons in the substantia nigra. *Brain Res.* **403,** 43–51.

Ricuarte G. A., Langston J. W., Delanney L. E., Irwin I., Peroutka S. J. and Forno L. S. (1986) Fate of nigrostriatal neurons in young mature mice given 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: a neurochemical and morphological reassessment. *Brain Res.* **376,** 117–124.

Seeman P. and Niznik H. B. (1990) Dopamine receptors and transporters in Parkinson's disease and schizophrenia. *FASEB J.* **4,** 2737–2744.

Sershen H., Mason M. F., Hashim A. and Lajtha A. (1985) Effect of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on age-related changes in dopamine turnover and transporter function in the mouse striatum. *Eur. J. Pharmacol.* **113,** 135–136.

Strong R., Samorajski T. and Gottesfeld Z. (1984) High-affinity uptake of neurotransmitters in rat striatum: effects of ageing. *J. Neurochem.* **43,** 1766–1768.

Sunstrom E., Luthman J., Goldstein M. and Jonsson G. (1988) Time course of MPTP-induced degeneration of the nigrostriatal dopamine system in C57BL/6 mice. *Brain Res. Bull.* **21,** 257–263.

Wagner G. C., Ricuarte G. A., Seiden L. S., Schuster C. R., Miller R. J. and Westley J. (1980) Long-lasting depletions

of striatal dopamine and loss of dopamine uptake sites following repeated administration of methamphetamine. *Brain Res.* **181,** 151–160.

Zigmond M. J. and Stricker E. M. (1989) Animal models of Parkinsonism using selective neurotoxins: clinical and basic implications. *Int. Rev. Neurobiol.* **31,** 1.