ELSEVIER

European Journal of Pharmacology 307 (1996) 227–232

ejp

Striatal concentrations of vesicular monoamine transporters are identical in MPTP-sensitive (C57BL/6) and -insensitive (CD-1) mouse strains

Michael Kilbourn *, Kirk Frey

Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA

Received 18 March 1996; accepted 19 March 1996

Abstract

Sensitivity to the neurotoxic actions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) varies greatly among strains of mice. The numbers of vesicular monoamine transporters was examined in various brain regions of MPTP-sensitive (C57BL/6) and MPTP-insensitive (CD-1) mouse strains. In vivo radioligand binding to the vesicular monoamine transporter was studied using [^{11}C]methoxytetrabenazine, and in vitro B_{max} values determined using [^{3}H]dihydrotetrabenazine autoradiography. Using either technique, no significant differences between the two strains were seen in the striatal binding of these radioligands to the vesicular monoamine transporter. The in vivo binding of radioligands to this transporter in the striatum was also not gender dependent. The relative resistance of CD-1 mice to the neurotoxic effects of peripheral MPTP administration thus does not appear to be a result of enhanced protection by higher levels of vesicular storage in dopaminergic neurons of the striatum.

Keywords: Vesicular monoamine transporter; VMAT2 (vesicular monoamine transporter type 2); MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine); Methoxytetrabenazine; Dihydrotetrabenazine

1. Introduction

Degeneration of dopaminergic neurons following administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to mammals has been utilized as an animal model for Parkinson's disease (Zigmond and Stricker, 1989). This neurotoxin produces many (but not all) of the changes seen in human disease, including reductions of the levels of dopamine and its metabolites, and a loss of dopaminergic neurons in the substantia nigra. Sensitivity to peripheral MPTP exposure varies among species, with primates being the most sensitive and rats being almost completely resistant. In mice, the neurotoxic effects of MPTP are strain dependent; whereas certain pigmented mice, most notably the C57BL/6 strain, are exquisitely sensitive to MPTP other strains including many albino mice (e.g., CD-1, CF-1) are relatively resistant to the neurotoxin. A completely satisfactory explanation for the differential sensitivity of mouse strains to MPTP is still lacking, despite numerous studies of such factors as endogenous dopamine levels (Giovanni et al., 1991; Muthane et al., 1994), monoamine oxidase enzyme levels (both monoamine oxidase A and B) (Giovanni et al., 1991), numbers of neuronal membrane dopamine transporters (Kilbourn et al., 1989), and synaptosomal 1-methyl-4-phenylpyridinium (MPP^+) uptake in vitro (Giovanni et al., 1991). Susceptibility to MPTP among mouse strains does correlate with striatal accumulation of [^{3}H]MPP^+ (Giovanni et al., 1991), but an explanation for the higher levels in the sensitive mouse strains (e.g., C57BL/6) has not been forthcoming; this could be due to differences in neurochemistry, or differences in peripheral metabolism and clearance. Furthermore, rats are resistant to the neurotoxic effects of MPTP despite accumulation of MPP^+ in the striatum.

Recently, potential roles for vesicular uptake and storage of MPP^+ in the mechanism of MPTP neurotoxicity have been proposed (Johannessen, 1991; Edwards, 1993; Schuldiner et al., 1995). The brain vesicular monoamine transporter can clearly transport MPP^+ into the vesicle lumen, providing a mechanism for removal and sequestration of the neurotoxin away from its proposed site of

* Corresponding author. 3480 Kresge III, The University of Michigan, Ann Arbor, MI 48109-0552, USA. Tel.: 313-763-9246; fax: 313-764-0288.

Elsevier Science B.V.
PII S0014-2999(96)00252-X

action as an inhibitor of mitochondrial complex I. In such a scheme, vesicular transport provides neuronal protection from the effects of MPTP (Edwards, 1993); neurotoxicity from MPTP might, however, still result from cytosolic MPP^+ which exceeds the capacity of the vesicular storage mechanism. This is consistent with the general actions of MPP^+ as a non-specific toxin when injected directly into the striatum of animals including rats. It has also been proposed that such vesicular storage of MPP^+ provides for a protracted exposure of the neuron to the neurotoxin, since it is available to leak out of the storage vesicle and interact with the mitochondrial complex I site (Johannessen, 1991). A combination of these two alternatives might be even more effective at promoting neurotoxicity: a high but not totally adequate vesicular storage capacity might result in initial damage from cytosolic MPP^+ which cannot be sequestered, and the prolonged leakage of MPP^+ back into the cytosol from a high number of MPP^+-containing vesicles (with such release accentuated by a loss of cellular ATP levels) would provide a continued aggravation of any initial metabolic defect induced in the neuron.

Since vesicular storage may be a factor in the neurotoxicity of MPP^+ it would seem important to determine if there are differences in vesicular transporter numbers and/or function between MPTP-sensitive and -insensitive strains of mice. Higher numbers of vesicular transporters in CD-1 mice might explain MPTP resistance simply as a result of higher storage capacity; alternatively, greater numbers of vesicular transporters in C57BL/6 mice might indicate a greater initial capacity for MPP^+ accumulation, and prolonged storage and exposure. We have recently developed in vitro and in vivo methods for measuring radioligand binding to the tetrabenazine site of the brain vesicular monoamine transporter of rodent, monkey and human brain (Kilbourn, 1994). In vivo uptake of such radioligands correlates well with levels of monoamines and numbers of radioligand binding sites determined in vitro (Kilbourn, 1994), and in vitro or in vivo measures of radioligand binding are sensitive to decreases in brain concentrations of vesicular monoamine transporters (Vander Borght et al., 1995b). Using such assays, we have examined here the hypothesis that the levels of vesicular monoamine transporters in the striatum of CD-1 and C57BL/6 mice, as reflected in the binding of specific radioligands, will be different between these two mouse strains with such disparate sensitivities to MPTP.

2. Materials and methods

2.1. Syntheses

(±)-α-[^{11}C]Methoxytetrabenazine ((±)-(α-2-[^{11}C]-methoxy)-3-isobutyl-9,10-dimethoxy-1,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine) was synthesized by the [^{11}C]methylation of (±)-α-dihydrotetrabenazine (2-α-hydroxy-3-isobutyl-9,10-dimethoxy-1,3,4,6,7-hexahydro-11b*H*-benzo[a]quinolizine) (DaSilva et al., 1993), which was in turn prepared by hydride reduction of tetrabenzine. Specific activity of (±)-α-[^{11}C]methoxytetrabenazine was > 500 Ci/mmol. (±)-α-[^{3}H]Methoxytetrabenazine ((±)-(α-2-[^{3}H]methoxy)-3-isobutyl-9,10-dimethoxy-1,3,4,6,7-hexahydro-11b*H*-benzo[a]quinolizine) was prepared in an analogous fashion by custom tritiation (Amersham, Arlington Heights, IL, USA), and obtained in a specific activity of 82 Ci/mmol. (+)-α-[^{3}H]Dihydrotetrabenazine ((+)-(α-2-hydroxy)-3-isobutyl-9-[^{3}H]methoxy-10-methoxy-1,3,4,6,7-hexahydro-11b*H*-benzo[a]quinolizine) was prepared by custom tritiation ([^{3}H]methylation: Amersham) of (+)-(α-2-hydroxy)-3-isobutyl-9-hydroxy-10-methoxy-1,3,4,6,7-hexahydro-11b*H*-benzo[a]quinolizine), and obtained in a specific activity of 81 Ci/mmol.

2.2. In vivo radiotracer distribution studies

Studies used male and female mice (20–25 g) of the CD-1 and C57BL/6 strains obtained from Charles Rivers Laboratories (Portage, MI, USA). All animals were approximately age-matched (7–9 weeks old). Mice were anesthetized with diethyl ether and injected via the tail vein with 100–300 μCi of (±)-α-[^{11}C]methoxytetrabenazine (female mice) or 10 μCi of (±)-α-[^{3}H]methoxytetrabenazine (male mice) in saline, and then allowed to awaken. At 15 min post-injection, mice were re-anesthetized, decapitated, and brains quickly removed and dissected into samples of the striatum, cortex (whole), cerebellum, thalamus, hippocampus, and hypothalamic region. In the studies using carbon-11, tissue samples were rapidly counted (automated gamma counter) and weighed. In studies with (±)-α-[^{3}H]methoxytetrabenazine, tissue samples were assayed for tritium following oxidation. Data were calculated as percentages injected dose per gram of tissue. Tissue concentration ratios were calculated as percentages injected dose/gram per region divided by percentages injected dose/gram for the entire brain.

2.3. In vitro binding autoradiography

Male C57BL/6 ($N = 4$) and CD-1 ($N = 6$) mice, which were age-matched to the groups used for the in vivo studies, were anesthetized, killed by decapitation, and the brains quickly dissected and frozen in crushed dry ice. Frozen brains were mounted on microtome chucks, covered with frozen section embedding medium (M-1 embedding medium, Lipshaw, Detroit, MI), and stored at −70°C. Brains were sectioned saggitally in a cryostat microtome at −18°C. Brain sections at 100 μM intervals were thaw-mounted on gelatin-subbed microscope slides, allowed to air-dry, and stored at −70°C until used in binding assays.

Binding of (+)-α-[^{3}H]dihydrotetrabanazine was assessed by minor modifications of prior methods used for (±)-α-[^{3}H]methoxytetrabenazine binding assays (Vander

Borght et al., 1995b). Sections were pre-incubated in potassium phosphate buffer (137 mM KCl, 3 mM NaCl, 8 mM K_2HPO_4, 1.5 mM NaH_2PO_4, 1 mM EDTA; pH 8.0) at 25°C. Slides were next incubated in buffer contining 10 nm (+)-α-[^{3}H]dihydrotetrabenazine for 30 min and then washed twice for 2 min each in fresh buffer at 4°C and dipped briefly in distilled water to remove excess buffer salts. Sections were air-dried and apposed to tritium-sensitive X-ray film (Hyperfilm, Amersham) for two weeks. Autoradiograms were analyzed by video-assisted densitometry (MCID M2 system, Imaging Research, St. Catherines, Ontario, Canada), using calibrated plastic standards to convert film density to apparent tissue radioactivity levels as described previously (Vander Borght et al., 1995b).

For each brain, (+)-α-[^{3}H]dihydrotetrabanazine binding was determined in 20 sections containing the striatum, and the data averaged to provide a binding site density per striatum for each individual animal.

2.4. Statistical analysis

Group comparisons were made with unpaired Student's *t*-tests using a significance threshold of 0.05.

3. Results

In the in vivo assays of regional brain uptake and binding of labeled (±)-α-methoxytetrabenazine (using ^{11}C or ^{3}H), there were no significant differences observed in either the absolute concentrations of radioactivity or tissue concentration ratios between the two mouse strains in either the male or female mice (Tables 1 and 2). Within each strain, the only significant difference between male and female mice was in hypothalamic uptake and retention of radioligand, resulting in significant increases ($P < 0.05$) in the hypothalamus/whole brain ratios for male mice of both CD-1 and C57BL/6 strains.

Table 1
In vivo regional brain distribution of radiolabeled methoxytetrabenazine in female mice of the CD-1 and C57BL/6 strains

Region	Female CD-1 ($N = 84$)	Female C57BL/6 ($N = 22$)
[^{11}C]Methoxytetrabenazine, %ID/g		
Striatum	6.77±2.08	8.93±2.5
Cortex	2.48±0.77	3.56±1.12
Hypothalamus	4.08±1.02	5.20±1.99
Cerebellum	1.99±0.52	2.90±0.77
Brain	2.83±0.77	3.89±1.12
Blood	1.65±0.31	1.83±0.28
Concentration ratio		
Striatum/brain	2.41±0.35	2.30±0.30
Hypothalamus/brain	1.46±0.18	1.30±0.29

Data shown as percentage injected dose per gram (%ID/g) and as region/whole brain concentration ratios. Data represent means±S.D.

Table 2
In vivo regional brain distribution of [^{3}H]methoxytetrabenazine in male mice of the CD-1 and C57BL/6 strains

Region	Male CD-1 ($N = 6$)	Male C57BL/6 ($N = 6$)
[^{3}H]Methoxytetrabenazine, %ID/g		
Striatum	6.56±2.5	5.49±1.03
Cortex	3.11±0.53	2.68±0.23
Hypothalamus	6.12±1.36	5.25±0.72
Cerebellum	2.42±0.33	2.16±0.26
Brain	2.82±0.95	2.58±0.18
Blood	1.15±0.29	1.04±0.20
Tissue concentration ratio		
Striatum/brain	2.31±0.79	2.13±0.36
Hypothalamus/brain	2.17±0.34	2.03±0.20

Data shown as percentage injected dose per gram (%ID/g) and as region/whole brain concentration ratios. Data represent means±S.D.
* $P < 0.05$ vs. female of same strain (see Table 1).

The in vitro autoradiographic assays revealed no significant differences between the two mouse strains in the striatal concentrations of (+)-α-[^{3}H]dihydrotetrabenazine binding sites (Table 3).

4. Discussion

The CD-1 and C57BL/6 mouse strains examined here have been previously reported not to show any significant differences in the brain levels of dopamine (Giovanni et al., 1991; Muthane et al., 1994). In this study, we have found that there are also equivalent numbers of vesicular monoamine transporters as measured using both in vitro and in vivo radioligand binding assays. The resistance of CD-1 mice to the actions of MPTP is thus not, apparently, a simple result of higher striatal levels of vesicular transporters and a greater potential ability to sequester MPP^+.

Two recent studies (Takada et al., 1993; Muthane et al., 1994) have reported that C57BL/6 mice show a significantly lower (−20 to −33%) number of dopaminergic neurons in the substantia nigra pars compacta as compared to CD-1 mice. This would suggest that there are increased

Table 3
Striatal B_{max} values for (+)-α-[^{3}H]dihydrotetrabenazine binding in male CD-1 and C57BL/6 mice, as determined using in vitro autoradiography

	CD-1	C57BL/6	
	3.18	3.02	
	3.28	2.94	
	3.44	3.25	
	3.14	3.26	
	3.34		
	2.92		
Mean	3.22±0.18	3.12±0.16	($P = 0.40$)

Data for individual mice represent the average concentrations (pmol/μg protein) determined for 20 slices through the striatum.

numbers of vesicular transporters and higher dopamine concentrations per neuron in the C57BL/6 mouse strain. Could this explain the differences in sensitivity to MPTP between these two mouse strains? From the work of Giovanni et al. (1991), the C57BL/6 mouse is able to concentrate MPP^+ to a nearly five-fold higher level than the CD-1 mouse; unfortunately, the intracellular location of the MPP^+ was not identified. This higher MPP^+ accumulation would not appear to be the result of increased monoamine oxidase enzymatic activity, total numbers of neuronal membrane dopamine transporters, or increased neuronal membrane transport of MPP^+ in the C57BL/6 mouse strain (Kilbourn et al., 1989; Giovanni et al., 1991). Provided the ratio of the numbers of vesicular transporters to the numbers of storage vesicle is relatively constant among different mouse strains, and the capacity of the individual vesicles for MPP^+ are similar, then the striatal tissue of the C57BL/6 mouse has essentially the same total capacity for vesicular storage of MPP^+ although the amount of neurotoxin stored per neuron would be significantly higher in the C57BL/6 mouse. If the storage capacity is similar, but MPP^+ accumulation in C57BL/6 striatum is five-fold higher, then this suggests that a significant proportion of the MPP^+ accumulated by the neurons in the C57BL/6 mouse striatum might not be stored in the vesicles. This would explain the higher neurotoxicity, as this cytosolic MPP^+ would have increased access to the mitochondrial complex I site, with subsequent inhibition of energy metabolism. The effects of MPP^+ might be further accentuated in the C57BL/6 mouse striatum by a higher stored neurotoxin level per neuron due to either a higher vesicle/neuron ratio or a greater sequestration of MPP^+ per vesicle; either mechanism of increased MPP^+ storage might result in enhanced toxicity due to prolonged leakage back into the cytosol.

In other brain regions the in vivo binding of methoxytetrabenazine to the vesicular monoamine transporter was not significantly different between the two mouse strains. Although the effects of MPTP on nigral cells has been well characterized it is also neurotoxic to cerebellar Purkinje cells in the mouse, and furthermore MPTP is more toxic to these cerebellar cells in the C57BL/6 mouse strain as compared to CD-1 mice (Takada et al., 1993). As with cell counts in the substantia nigra pars compacta, the number of Purkinje cells in the cerebellum is lower in the C57BL/6 mouse strain (Takada et al., 1993). In combination with our findings of equivalent methoxytetrabenazine binding in C57BL/6 and CD-1 mouse cerebellum, this would suggest an increased concentration of vesicular transporters per Purkinje cell in the C57BL/6, similar to that observed per dopaminergic neuron of the substantia nigra of that mouse strain. Whether the cerebellar Purkinje cells of C57BL/6 mice accumulate more MPP^+, as observed in the striatum, has not been determined; studies of MPP^+ uptake using crude cerebellar synaptosomes have shown variable results (Del Zompo et al., 1993), but specific uptake into the Purkinje cells has not been examined. However, it is conceivable that there is a similar underlying biochemical sensitivity of monoaminergic neurons in both the substantia nigra and the cerebellum.

During the course of these studies, interesting questions were raised as to the possible effect of gender on our results. Although the majority of studies of MPTP toxicity have been done in male mice, there are several studies which demonstrate that MPTP is quite neurotoxic to female mice of the C57BL/6 strain (Unzeta et al., 1994; Jossan et al., 1989; Dluzen et al., 1996) but less so to females of the CD-1 strain (Dluzen et al., 1996). The long-term effects of MPTP have, in fact, been reported to be greater in female than male C57BL/6 mice (Jossan et al., 1989). Possible sex differences in vesicular monoamine transporter numbers within any region of the rat or mouse brain have not previously been examined. However, it is well established that there are differences in catecholamine metabolism, neuronal dopamine transporters and dopamine receptors in male versus female rat or mouse striatum, and that each of these biochemical measures can be regulated by the hormonal status of the animal (Dluzen et al., 1996). We therefore examined in vivo binding of our radioligands in both male and female mice of both strains. With the exception of the hypothalamus, there were no significant differences between male and female mice of either strain. Our total data base for radioligand binding (using (±)-[^{11}C]tetrabenazine, (±)-α-[^{11}C]methoxytetrabenazine, and (±)-α-[^{11}C]dihydrotetrabenazine) in female CD-1 mice exceed 150 individual animals, used in small groups (6–12 per group) over a period of 4 years, with no control at any time for the estrous cycles of the animals (although all were approximately age-matched). The low variance in the in vivo data for radioligand binding in both striatum and hypothalamus (coefficient of variations of $< 15\%$ for region/whole brain ratios) support little if any hormonal effects on available radioligand binding sites in these brain regions of female mice. This would be consistent with our proposal of the vesicular transporters as unregulated synaptic components; we have recently demonstrated that the neuronal dopamine transporter, and the dopamine D_2 receptor, are modulated by chronic dopaminergic drug treatments, but the vesicular monoamine transporter binding site is not affected (Vander Borght et al., 1995a). Thus, hormonal modulation of endogenous dopamine levels should also not affect vesicular monoamine transporter levels, although they will do so with the neuronal dopamine transporter. The possible differences in hypothalamic binding of radioligands to the vesicular monoamine transporter is intriguing and worthy of further examination.

Does vesicular storage function as a general mechanism for protection against neurotoxins? We have not observed a difference in the numbers of vesicular monoamine transporters in MPTP-sensitive vs. -insensitive mouse strains; furthermore, rats (which are MPTP-resistant) have very nearly the same numbers of vesicular transporters per

volume in the striatum as mice. Numbers of these transporters clearly do not explain the high concentrations of MPP^+ in the C57BL/6 mouse striatum, nor why that mouse strain is sensitive but the rat is not. A more general role of vesicles as 'safe depots' for neurotoxins is also unclear; although inhibitors of vesicular monoamine storage (reserpine, tetrabenazine) enhance MPTP neurotoxicity in striatal dopaminergic neurons (Reinhard et al., 1988), they do not do so for a second neurotoxin, 6-hydroxy-DOPA (Cohen and Evans, 1992). Sequestration and retention of MPP^+ within the total pool of storage vesicles also appears inconsistent with the rapid pharmacokinetics of MPP + in the mouse brain. Following peripheral administration, MPP^+ is rapidly formed within the mouse brain but clears quite rapidly ($t_{1/2}$ of 1–2 h: Zuddas et al., 1989), presumably through the choroid plexus and into the cerebrospinal fluid (Zuddas et al., 1989); MPP^+, being a cation, is not freely permeable back through the blood brain barrier. Movement of vesicularly stored MPP^+ into the extracellular fluid of the brain would require exocytosis, yet it has been proposed that neurotransmitter storage vesicles are found in active (recycling) and inactive pools, with the latter tethered to the cytoskeleton and generally not routinely utilized in neurotransmission (Kelly, 1993). It is possible that only the active, cycling pool of vesicles may be more active in transport and storage of MPP^+, and perhaps there are strain differences in the size and dynamics of this pool. Differences in the populations of releasable and storage pools of dopaminergic vesicles in mouse strains has been previously postulated (Sanghera et al., 1990). This rapid uptake/exocytosis of neurotoxins would provide a *third* alternative function of monoamine vesicular transporters, such that they function in neuroprotection by rapid collection and extrusion of toxins, in a manner analagous to the multidrug resistance transporters; it should be noted that the vesicular monoamine transporters have been suggested to be a part of a superfamily of toxin extruding antiporters which include structurally related p-glycoproteins (Schuldiner et al., 1995). It is also possible that the dopaminergic neuron of the C57BL/6 mouse has fewer monoamine transporters per vesicle, which (since the numbers of transporters are equivalent) would result in perhaps a greater total vesicular capacity for MPTP in the C57BL/6 striatum: our experiments with these radioligands cannot provide information on that possibility. The pharmacokinetics of MPP^+ in primates is strikingly different, with significant long-term retention within catecholaminergic neurons; this could represent accumulation in the static pool of vesicles, from where the neurotoxin continuously 'leaks' and provides the greater toxicity in these species. Thus, the role of vesicular storage in neurotoxic effects of MPTP appears complicated, and can be viewed as capable of blunting or enhancing neurotoxin effects.

In conclusion, we have found that the relative MPTP sensitivity of two mouse strains cannot be explained as a simple difference in the concentrations of striatal vesicular monoamine transporters (and, by inference, numbers of vesicles), although the number of such transporters per neuron may be higher in the MPTP-sensitive C57BL/6 strain. The reasons for the exquisite sensitivity of C57BL/6 mice to the effects of MPTP administration thus continue to be unclear. That uncertainty, as well as the larger question of the role of vesicular transporters in the actions of endogenous or exogenous neurotoxins, are both worthy of further investigation.

Acknowledgements

This work was supported by grants from the National Institutes of Health (MH 47611 and NS15655) and the Department of Energy (DOE-DE-FG021ER60561). The authors thanks the cyclotron and radiochemistry staff for the production of [^{11}C]methoxytetrabenazine, Phillip Sherman for animal injections and tissue dissections, and Timothy Desmond for the autoradiographic assays.

References

Cohen, G. and J. Evans, Vesicular binding of 6-hydroxydopamine does not explain the resistance of dopamine nerve terminals to 6-hydroxydopa, Neurodegeneration 1, 241.

DaSilva, J.N., M.R. Kilbourn and T.J. Mangner, 1993, Synthesis of a [^{11}C]methoxy derivative of α-dihydrotetrabenazine: a radioligand for studying the vesicular monoamine transporter, Nucl. Med. Biol. 44, 1487.

Del Zompo, M., M.P. Piccardi, S. Ruiu, M. Quatru, G.L. Gessa and A. Vaccari, 1993, Selective MPP^+ uptake into synaptic dopamine vesicles: possible involvement in MPTP neurotoxicity, Br. J. Pharmacol. 109, 411.

Dluzen, D.E., J.L. McDermott and B. Liu, 1996, Estrogen alters MPTP-induced neurotoxicity in female mice: effects on striatal dopamine concentrations and release, J. Neurochem. 66, 658.

Edwards, R.H., 1993, Neural degeneration and the transport of neurotransmitters, Ann. Neurol. 34, 638.

Giovanni, A., B. Sieber, R. Heikkila and P.K. Sonsalla, 1991, Correlation between the neostriatal content of the 1-methyl-4-phenylpyridinium species and the dopaminergic neurotoxicity following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration to several strains of mice, J. Pharmacol. Exp. Ther. 257, 691.

Johannessen, J.N., 1991, A model of chronic neurotoxicity: long-term retention of the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) within catecholaminergic neurons, NeuroTox. 12, 285.

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

Kelly, R.B., 1993, Storage and release of neurotransmitters, Neuron 10, 43.

Kilbourn, M.R., 1994, PET radioligands for vesicular neurotransmitter transporters, Med. Chem. Res. 5, 113.

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

Muthane, U., K.A. Ramsay, H. Jiang, V. Jackson-Lewis, D. Donaldson, S. Fernaldo, M. Ferreira and S. Predborski, 1994, Differences in

nigral neuron number and sensitivity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in C57/bl and CD-1 mice, Exp. Neurol. 126, 195.

Reinhard, J.F., Jr., A.J. Daniels and O.H. Viveros, 1988, Potentiation by reserpine and tetrabenazine of brain cetecholamine depletions by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) in the mouse: evidence for subcellular sequestration as basis for cellular resistance to the toxicant, Neurosci. Lett. 90, 349.

Sanghera, M.K., F. Crespi, K.F. Martin, D.J. Heal, W.R. Buckett and C.D. Marsden, 1990, Biochemical and in vivo voltammetric evidence for differences in striatal dopamine levels in inbred strains of mice, Neuroscience 39, 649.

Schuldiner, S., A. Shirvan and M. Linial, 1995, Vesicular neurotransmitter transporters: from bacteria to humans, Physiol. Rev. 75, 369.

Takada, M., T. Sugimoto and T. Hattori, 1993, MPTP neurotoxicity to cerebellar Purkinje cells in mice, Neurosci. Lett. 150, 49.

Unzeta, M., S. Baron, V. Perez, S. Ambrosio and N. Maly, 1994, Sex-related effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment may be related to differences in monoamine oxidase B, Neurosci. Lett. 176, 235.

Vander Borght, T.M., M.R. Kilbourn, T.J. Desmond, D.E. Kuhl and K.A. Frey, 1995a, The vesicular monoamine transporter is not regulated by dopaminergic drug treatments, Eur. J. Pharmacol. 294, 577.

Vander Borght, T.M., A.A.F. Sima, M.R. Kilbourn, T.J. Desmond and K.A. Frey, 1995b, [^{3}H]Methoxytetrabenazine: a high specific activity ligand for estimating monoaminergic neuronal integrity, Neuroscience 68, 955.

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

Zuddas, A., G.U. Corsini, S. Schinelli, J.L. Barker, I.J. Kopin and U. di Porzio, 1989, Acetaldehyde directly enhances MPP^+ neurotoxicity and delays its elimination from the striatum, Brain Res. 501, 11.