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In Vivo Measures of Dopaminergic Radioligands in the Rat Brain: Equilibrium Infusion Studies

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ABSTRACT The application of an equilibrium infusion method for measuring specific in vivo radioligand binding in the conscious rat brain was evaluated for two ligands of the dopaminergic system, (+)-α-[^{11}C]dihydrotetrabenazine (DTBZ) and *d-threo*-[^{11}C]methylphenidate (MePhen). Both radioligands can be successfully utilized to reach equilibrium distributions in rat brain within 1 h; combinations of tritiated and carbon-11-labeled radiotracers can furthermore be used to obtain simultaneous measures of the neuronal membrane dopamine transporter (using [^{3}H]MePhen) and vesicular monoamine transporter (using [^{11}C]DTBZ) in the same animal. These studies provided quantitative measures of distribution volume ratios, which represent specific radioligand binding. Stereospecificity of in vivo binding was demonstrated using equilibrium infusions of the low-affinity isomers of each ligand, (−)-α-[^{11}C]dihydrotetrabenazine (DTBZ) and *l-threo*-[^{11}C]methylphenidate, both of which produced uniform brain distributions and no specific binding. Specific binding of (+)-α-[^{11}C]dihydrotetrabenazine was blocked by co-infusion of tetrabenazine, but was unaffected by administration of methylphenidate, haloperidol, or apomorphine. Specific binding of *d-threo*-[^{11}C]methylphenidate, conversely, was blocked with unlabeled methylphenidate but not affected by tetrabenazine or the dopamine receptor ligands. Equilibrium measures of in vivo radioligand binding, as utilized in this study, offer a quantitative means to evaluate acute and chronic drug effects on in vivo radioligand binding in the rat brain. **Synapse 43: 188–194, 2002.**

INTRODUCTION

In recent years there have been significant advances in our understanding of the structure and functioning of the transporters for neurotransmitters, particularly the neuronal membrane and vesicular transporters for the monoamines (Olivier et al., 2000). Alterations in the number or functioning of these neurotransmitter transporters, and the relationships between them, may be important parts of such diverse human health problems as neurodegeneration, drug abuse, and psychiatric diseases. With the availability of in vitro methods utilizing cloned and expressed transporters and animal models of altered levels of these transporters (Gainetdinov et al., 1999), one can expect more advances to be made in our knowledge of the role of these proteins in normal and diseased brain function. Even more exciting is the potential of taking this basic biochemical knowledge to the living human patient, using in vivo imaging methods such as positron emission tomography (PET) (Brooks, 1997; Stoessl and Ruth, 1998) and single photon emission computed tomography (SPECT) (Kugaya et al., 2000) measures of transporter numbers or functions.

Many research groups have investigated in vivo radioligand methods for studying the neuronal membrane dopamine transporter (DAT) (Carroll et al., 1995). Less effort has been devoted to the vesicular monoamine transporter (VMAT2), partly due to the more limited selection of potential radioligands, but a successful in vivo radioligand for this transporter is also now available (Kilbourn, 1994). The availability of these radioligands provides opportunities to examine neurochemistry in intact animal models, particularly

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in rodents where neurotoxin, surgical, or genetic models of numerous neurological diseases are currently available or actively being developed. In vivo studies of radioligand binding to DAT or VMAT2 would also permit evaluation of recently described mechanisms for phosphorylation-dependent regulation of transporter functions (Pristupa et al., 1998; Reith and Chen, 1997). Methods for using in vivo radiotracers in rodents vary widely, including 1) very simple determinations of radioactivity concentrations at a single time point after administration, 2) construction of tissue time–activity curves using groups of animals at distinct time points, and 3) attempts to define the time-dependent regional brain pharmacokinetics using small animal imaging devices. Whereas the last approach would provide information most like that obtained in humans using PET or SPECT imaging studies, small animal imaging devices with the needed resolution and sensitivity (Hume et al., 1998) are as yet not widely available. Ex vivo measures of regional brain tissue radioactivity concentrations at multiple distinct time points, using groups of animals, combined with pharmacokinetic analysis of the extrapolated time-dependent distributions offers an intermediate method for performing such in vivo animal studies, but that is a time- and resource-intensive process requiring large numbers of animals to accurately define the radiotracer pharmacokinetics. The simplest technique available is the bolus injection of a radiotracer and determination of regional brain distributions at a single time point selected to represent a steady state or transient equilibrium; specific binding is then estimated as the simple ratio of radioactivity in one region over a second region selected as representing nonspecific binding. However, this approach may not be appropriate when radioligands do not reach an equilibrium distribution within the experimental time period (Gatley et al., 2000).

As an alternative to bolus injections, we and others (Endres and Carson, 1998; Kilbourn and Sherman, 1997) have investigated the application of an infusion to equilibrium approach to radiotracer administrations; regional brain distributions of radioactivity need be determined at only one time point after equilibrium is reached, simplifying the method and minimizing the requirement for large numbers of animals. At equilibrium the relative concentrations of radioactivity between regions of the brain remains constant and the determination of regional brain concentrations and blood concentrations of authentic radiotracer allows simple calculation of the regional distribution volumes (termed DV or V_d) for the radiotracer. Furthermore, the use of a region with minimal specific binding allows calculation of regional distribution volume ratios (DVR) (Carson, 2000; Logan, 2000) and the regional binding potentials (BP = DVR − 1). The infusion protocol in animals thus appears particularly appealing and can provide essentially the same estimates of specific binding generated by imaging studies of radiotracers in the human brain.

We have previously reported the application of the infusion to equilibrium approach to in vivo quantification of (+)-α-[^{3}H]DTBZ binding to the VMAT2 in rat brain (Kilbourn and Sherman, 1997). Although a large number of radioligands have been developed for in vivo studies of the dopamine receptors and the neuronal membrane transporter for dopamine (DAT), the equivalent equilibrium infusion approach in small animals has not been applied to those ligands. We report here the 1) evaluation of infusion to equilibrium methods in conscious rats for the DAT radioligand *d-threo*-[^{3}H]-methylphenidate ([^{3}H]MePhen); 2) comparison of specific binding estimates from bolus infusion, single time point studies with the equilibrium-derived binding potentials for [^{3}H]MePhen and (+)-α-[^{11}C]DTBZ; and 3) development and application of dual equilibrium infusion radiotracer methods for simultaneous measurement of *d-threo*-[^{3}H]MePhen (DAT) and (+)-α-[^{11}C]-DTBZ (VMAT2) radioligand binding.

MATERIALS AND METHODS

Drugs and radiochemicals

d-threo-[^{3}H]Methylphenidate ([^{3}H]MePhen; specific activity 83 Ci/mmol) was prepared (Amersham, Arlington Heights, IL, USA) by a two-step synthesis involving [^{3}H]methylation of a suitably protected ritalinic acid precursor, following the procedure used for synthesis of *d-threo*-[^{11}C]methylphenidate (Ding et al., 1994). The *d-threo*- and *l-threo*-isomers of [^{11}C]methylphenidate (specific activities > 500 Ci/mmol) were prepared by [^{11}C]methylation of the appropriate ritalinic acid precursors followed by deprotection. The (+)- and (−)-isomers of α-[^{11}C]dihydrotetrabenazine (specific activities > 500 Ci/mmol) were prepared by [^{11}C]methylation of the corresponding 9-O-desmethyl precursors, by methods previously described (Jewett et al., 1997). Tetrabenazine was obtained from ICN Biomedicals (Aurora, IL, USA) and methylphenidate hydrochloride, apomorphine, and haloperidol were obtained from Research Biochemicals (Natick, MA, USA). All drugs and radiotracers were administered as solutions in isotonic saline.

Radiotracer and drug administration protocols

All studies were done in Sprague-Dawley CD rats (150–200 g; Charles Rivers, Portage, MI, USA). Under sodium pentobarbital anesthesia, the animals were prepared for intravenous radiotracer injections by incision of the skin and insertion of a catheter into one or both femoral veins. The incisions were then closed and the animal placed in a plastic restrainer tube and allowed to awaken; all studies were done using animals

TABLE I. Regional radioactivity concentration ratios for isomers of α-dihydrotetrabenazine (DTBZ) in rat brain

Radioligand	Protocol	*N*	Drug	Striatum	Tissue/cortex		
					Hypothalamus	Hippocampus	Thalamus
(+)-[^{11}C]DTBZ	bol, 30 min	8	None	4.12 ± 0.39	1.93 ± 0.12	0.94 ± 0.08	0.95 ± 0.07
(+)-[^{3}H]DTBZ	bol, 15 min	6	None	3.35 ± 0.09	2.64 ± 0.27	1.28 ± 0.03	1.53 ± 0.11
(+)-[^{11}C]DTBZ[1]	inf, 60 min (control)	11	None	2.79 ± 0.17	1.52 ± 0.22	0.90 ± 0.05	0.87 ± 0.06
(−)-[^{11}C]DTBZ	inf, 60 min	4	None	0.98 ± 0.01*	0.87 ± 0.10*	0.95 ± 0.03	0.92 ± 0.08
(+)-[^{11}C]DTBZ[1]	inf, 60 min	8	MePhen	2.99 ± 0.17	1.52 ± 0.22	0.90 ± 0.05	1.02 ± 0.30
(+)-[^{11}C]DTBZ[1]	inf, 60 min	8	TBZ	0.98 ± 0.11*	0.87 ± 0.10*	0.95 ± 0.03	0.83 ± 0.02
(+)-[^{11}C]DTBZ[1]	inf, 60 min	4	Haloperidol	3.13 ± 0.29	1.29 ± 0.07	0.93 ± 0.04	0.95 ± 0.07
(+)-[^{11}C]DTBZ[1]	inf, 60 min	8	Apomorphine	2.56 ± 0.32	1.14 ± 0.12	0.92 ± 0.10	0.91 ± 0.14

Determined following either bolus injection (bol) or administration using a programmed bolus + constant infusion (inf) protocol. Methylphenidate (MePhen) and tetrabenazine (TBZ) were co-infused with radiotracer and haloperidol and apomorphine administered 30 min prior. Tissue/cortex ratios determined following the infusion protocol represent equilibrium distribution volume ratios (DVR). Data are shown as mean ± SD.
[1]Studies performed using dual radiotracer infusions.
*$P < 0.005$ vs control (+)-[^{11}C]DTBZ infusion study.

in the fully awake but restrained state. Drugs or saline (controls) injections were done via the femoral vein catheter. Radiotracer injections were done through the femoral vein catheters using a Harvard programmable infusion pump. Bolus administrations were done using a 1-min infusion of radiotracer in a volume of 1 mL. Equilibrium infusion studies were performed using a bolus administration of 66.6% of the dose (1 mL) over a 1-min period, followed by constant infusion of the remaining 33.3% (0.5 mL) of the dose over the remaining 59 min (for 1-h infusion studies) or 119 min (2-h infusion studies).

Groups of 2–4 animals were used at a single experimental session and experiments repeated to achieve the group sizes shown in Table I. At designated times after initial bolus or infusion of radiotracers, animals were killed by i.v. injection of an overdose of sodium pentobarbital and the brain rapidly removed and dissected according to a slight modification of a literature method (Glowinski and Iversen, 1966) into samples of striatum, cortex, hippocampus, hypothalamic region, thalamus, pons/medulla, and cerebellum. Tissue samples were then weighed and counted: carbon-11 was determined immediately using an automated γ-counter. Tissue solubolizer was added and after digestion (2–3 days) scintillation fluid was added and the sample counted for tritium (automatic β-counter). Data were calculated as percent injected dose/gram tissue (%ID/g).

For all studies the specific binding was estimated by calculating the concentration ratios of regions of interest to cortex as the reference region. For infusion studies, this measure is termed the distribution volume ratio (DVR).

Statistics

Comparisons between groups were evaluated either with a Student *t*-test or with a repeated measures ANOVA. A level of $P < 0.05$ was considered significant.

RESULTS

Single radiotracer studies

(+)-α-[^{11}C]dihydrotetrabenazine ((+)-α-[^{11}C]dtbz)

The regional brain distribution of radioactivity after bolus and equilibrium administration of (+)-α-[^{3}H]-DTBZ has been previously reported (Kilbourn and Sherman, 1997). In this study, we compared the regional brain distribution of the carbon-11-labeled tracer at a longer time point after bolus administration (30 min) with that obtained using infusion to equilibrium. Use of the longer time point for sampling after bolus injection produces an even larger bias in the estimates of specific binding, as the striatum/cortex ratio significantly exceeds the value obtained at equilibrium (Table I) and is higher than obtained at 15 min (Kilbourn and Sherman, 1997). Using either the 30-min bolus sampling point or infusion to equilibrium method; however, highest concentrations of the radioligand can be clearly demonstrated in both the striatum and the hypothalamic region.

(−)-α-[^{11}C]dihydrotetrabenazine ((−)-α-[^{11}C]dtbz)

The bolus + infusion administration of (−)-α-[^{11}C]DTBZ, the inactive isomer (K_i = 4 micromole: Kilbourn et al., 1995) produced a nearly uniform brain distribution of radioactivity, with no evidence for specific binding in any region (Table I).

d-threo-[^{3}H]methylphenidate

The regional brain distribution of *d-threo*-[^{3}H]MePhen was determined at 30 min following bolus administration and after 30 or 60 min of a bolus + infusion protocol (Table II). In a separate experiment, the regional distribution of radioactivity was also determined using infusion of the carbon-11-labeled radiotracer. Distribution volume ratios for the infusion studies were equivalent for all three groups, with specific binding only detected in the striatum; in contrast to the results with DTBZ, the bolus administration of *d-threo*-[^{3}H]MePhen and sampling at 30 min provided a striatum/cortex ratio more similar to that obtained using

TABLE II. Regional radioactivity concentration ratios for isomers of methylphenidate (MePhen) in rat brain

Radioligand	Protocol	*N*	Drug	Striatum	Tissue/cortex		
					Hypothalamus	Hippocampus	Thalamus
d-threo-[^{3}H]MePhen	bol, 30 min	8	None	1.76 ± 0.17	0.86 ± 0.05	0.99 ± 0.08	—
d-threo-[^{3}H]MePhen	inf, 30 min	8	None	1.61 ± 0.06	0.91 ± 0.07	1.00 ± 0.05	0.77 ± 0.06
d-threo-[^{3}H]MePhen[1]	inf, 60 min (control)	7	None	1.54 ± 0.13	0.85 ± 0.13	1.01 ± 0.16	0.64 ± 0.08
d-threo-[^{11}C]MePhen	inf, 60 min	4	None	1.54 ± 0.06	0.87 ± 0.02	0.96 ± 0.02	0.73 ± 0.03
l-threo-[^{11}C]MePhen	inf, 60 min	4	None	1.04 ± 0.01*	0.95 ± 0.03	1.03 ± 0.01	0.87 ± 0.01
d-threo-[^{3}H]MePhen[1]	inf, 60 min	8	MePhen	0.99 ± 0.04*	0.87 ± 0.08	1.01 ± 0.06	0.62 ± 0.04
d-threo-[^{3}H]MePhen[1]	inf, 60 min	8	TBZ	1.53 ± 0.14	0.78 ± 0.06	0.96 ± 0.03	0.75 ± 0.05
d-threo-[^{3}H]MePhen[1]	inf, 60 min	4	Haloperidol	1.65 ± 0.13	0.84 ± 0.08	1.10 ± 0.03	0.83 ± 0.03
d-threo-[^{3}H]MePhen[1]	inf, 60 min	8	Apomorphine	1.61 ± 0.13	0.85 ± 0.04	1.01 ± 0.03	0.71 ± 0.03

Determined following either bolus injection (bol) or administration using a programmed bolus + constant infusion (inf) protocol. Methylphenidate (MePhen) and tetrabenazine (TBZ) were co-infused with radiotracer and haloperidol and apomorphine administered 30 min prior. Tissue/cortex ratios determined following the infusion protocol represent equilibrium distribution volume ratios (DVR). Data are shown as mean ± SD.
[1]Studies performed using dual radiotracer infusions.
*$P < 0.005$ vs control *d-threo*-[^{3}H]MePhen infusion study.

the constant infusion protocol. The regional distributions of radioactivity were identical after 30 and 60 min of infusion, supporting that equilibrium distribution of the radiotracer had been reached by 60 min (or likely earlier).

l-threo-[^{3}H]methylphenidate

Equilibrium infusion of the low-affinity *l-threo*-[^{11}C]MePhen gave a uniform distribution of radioactivity with no evidence of specific binding in the striatum (Table II).

Dual radiotracer studies

Regional distribution volume ratios for (+)-α-[^{11}C]DTBZ and *d-threo*-[^{3}H]MePhen were next determined using a dual-radiotracer infusion protocol. In control studies ($n = 4$), tissue concentrations and distribution volume ratios for both radiotracers were essentially identical to those obtained in the prior single radiotracer studies.

Significant and essentially complete reductions in striatal and hypothalamic DVR were obtained for (+)-α-[^{11}C]DTBZ with coadministration of tetrabenazine (Table I), with tissue distribution volume ratios identical to those found with the inactive (−)-isomer. Prior administration of tetrabenazine, however, had no effect on the equilibrium distribution of *d-threo*-[^{3}H]MePhen (Table II). Pretreatment of rats with methylphenidate produced the opposite results, no effect on equilibrium (+)-α-[^{11}C]DTBZ distribution (Table I) but a significant reduction of *d-threo*-[^{3}H]MePhen binding in the striatum (Table II).

Using the dual radiotracer infusion protocol, no significant effects of a dopamine receptor antagonist (haloperidol) or agonist (apomorphine) on the equilibrium distribution of *d-threo*-[^{3}H]MePhen and (+)-α-[^{11}C]-DTBZ could be demonstrated.

DISCUSSION

The in vivo infusion to equilibrium method for determination of regional specific binding in rat brain has been evaluated for two radioligands currently in clinical use for the study of the dopaminergic system: *d-threo*-[^{11}C]methylphenidate (MePhen) and (+)-α-[^{11}C]dihydrotetrabenazine (DTBZ). In prior bolus studies in animals and humans, it was apparent that these ligands show readily reversible binding brain with pharmacokinetics suitable for an infusion to equilibrium study design and such infusion protocols have been reported in rats and humans for (+)-α-[^{11}C/^{3}H]DTBZ (Kilbourn and Sherman, 1997; Koeppe et al., 1997). In this study, we have demonstrated that *d-threo*-[^{11}C]MePhen is also a very good radioligand for this approach to quantification of in vivo regional binding in rodent brain. As there are reported significant effects of common anesthetics on in vivo radiotracer distributions all studies were performed in the awake rat brain.

Regional brain distributions of radioligands

Following either bolus administration or use of a bolus + infusion protocol, both radioligands produced a heterogeneous regional brain distribution of radioactivity, with highest concentrations found in the striatum; this is consistent with the known distribution of the DAT and the VMAT2 and similar to previous bolus studies using (+)-α-[^{3}H]DTBZ (Kilbourn and Sherman, 1997) and *d-threo*-[^{3}H]MePhen (Ding et al., 1994; Gatley et al., 1995). For (+)-α-[^{11}C]DTBZ, there is an intermediate concentration of radioactivity in the hypothalamic region, reflecting the binding of the radioligand to the VMAT2 in the serotonergic and adrenergic neurons located in that region of the brain. For *d-threo*-[^{3}H]MePhen, which is more specific for the dopaminergic neurons, no significant concentration of radioligand is noted in any other brain region than the striatum. With both radioligands, equilibrium infusion of the low-affinity stereoisomer ((−)-α-[^{11}C]DTBZ), $K_i = 4$ mmol; *l-threo*-[^{11}C]MePhen, $IC_{50} = 540$ nM (Gatley et al., 1996)) produced uniform brain distributions and tissue concentration ratios near unity.

The calculation of distribution volume ratios from the infusion studies requires reaching an equilibrium of tissue radioactivity concentrations and selection of an appropriate brain region for use as an estimate of nonspecific binding. We had previously demonstrated that α-(+)-[^{3}H]DTBZ reached equilibrium by 60 min of infusion (Kilbourn and Sherman, 1997). In this study the equilibrium for *d-threo*-[^{3}H]MePhen was also clearly reached within this time frame, as the values for striatum/cortex (and striatum/cerebellum, data not shown) at 30 min were indistinguishable from those obtained at 60 min. For both (+)-α-[^{11}C]DTBZ and *d-threo*-[^{3}H]MePhen we have evaluated using either the cerebellum or the cortex and the region of nonspecific binding. For both radioligands there is minimal specific binding in either region and as the cortex is the brain region we and others have utilized for in vivo imaging studies of these radioligands in human brain (Frey et al., 1996; Lee et al., 2000), it was chosen in this study. Using the same reference region for both radiotracers minimizes the experimental errors introduced in tissue dissection and counting from a dual-radiotracer infusion protocol. (+)-α-[^{11}C]DTBZ shows higher specific binding in the striatum as compared to [^{3}H]MePhen, but both radiotracers have binding potentials (BP = DVR − 1) in the striatum which are within the range suggested as suitable for measurements of changes of in vivo radioligand binding due to physiological or pharmacological challenges (Endres and Carson, 1998).

The use of a bolus injection of radiotracer, followed by determination of regional brain concentrations of radioactivity at a single time point, certainly offers a simpler experimental protocol for estimating specific binding. Most often, ratios between brain regions are calculated at 1) the point of greatest difference between the region of interest and a region purportedly void of specific binding sites, or 2) at a time point occurring within a time span when the ratios between tissues are fairly steady. The estimated specific binding for (+)-α-[^{11}C]DTBZ and *d-threo*-[^{3}H]MePhen determined here following bolus injection can be compared to the equivalent values determined by infusion studies (Tables I, II). The choice of a time point for tissue analysis is crucial. For *d-threo*-[^{3}H]MePhen the distribution volume ratio estimated at 30 min after the bolus injection is similar to that determined using the equilibrium infusion method, but the choice of time for measurement remains critical as the striatum/cerebellum ratio clearly changes with time after a bolus administration (Gatley et al., 1995; Kilbourn and Sherman, unpublished data). For (+)-α-[^{11}C]DTBZ, however, the specific binding estimate following the bolus injection was higher than determined using the equilibrium approach and the difference is consistently seen with sacrifice times chosen at both the peak striatal uptake (15 min, DVR = 3.76 ± 0.15; Kilbourn and Sherman, 1997) and a later time point (30 min, this study). Thus, the use of the bolus injection with a single time point measurement of concentrations should be made with caution and with an understanding of these possible limitations.

Pharmacological specificity

Using the dual infusion of *d-threo*-[^{3}H]MePhen and (+)-α-[^{11}C]DTBZ, the effects of three types of dopaminergic drugs were examined. Co-injection of tetrabenazine, the specific and high-affinity inhibitor of the VMAT2, completely eliminated specific binding of (+)-α-[^{11}C]DTBZ in the striatum and hypothalamus, but did not affect the in vivo binding of *d-threo*-[^{3}H]MePhen. Conversely, coadministration of methylphenidate essentially completely reduced the specific binding of *d-threo*-[^{3}H]MePhen, but had no effect on (+)-α-[^{11}C]DTBZ binding. These results were expected, as each of these two drugs have been shown to be specific for one type of transporter.

Administration of the dopamine receptor antagonist (haloperidol) or agonist (apomorphine) had no significant effect on either (+)-α-[^{11}C]DTBZ or d-threo-[^{3}H]MePhen specific binding in any region of the rat brain. These studies with a dopaminergic receptor ligand also demonstrate the lack of effect of changes in blood flow on the equilibrium radiotracer measures, since such drugs are known to significantly alter regional cerebral blood flow rates in the rat brain (Ingvar et al., 1983; McCulloch and Teasdale, 1979).

The lack of effect of methylphenidate or dopamine receptor ligands on the in vivo binding of (+)-α-[^{11}C]DTBZ agrees with prior studies which have failed to demonstrate regulation of the VMAT2 binding site in the dopaminergic neurons of the rat striatum (by such drugs as dopamine receptor ligands, dopamine reuptake inhibitors, L-DOPA, or monoamine oxidase A inhibitors) (Wilson and Kish, 1996; Naudon et al., 1994; Vander Borght et al., 1995). Our results do not match those of Fleckenstein and co-workers, who recently reported a modest (20%) cocaine-induced increase in binding of [^{3}H]DTBZ in purified rat brain synaptic vesicles (Brown et al., 2000), an effect also observed for dopamine transporter inhibitors such as amfonelic acid and GBR 12935. Methylphenidate, used in this experiment, is an effective inhibitor of the DAT and produces rapid changes in dopamine levels in the rat brain (Gatley et al., 1999), but in our experiments it did not alter in vivo radioligand binding to the VMAT2. The explanation for these divergent results may be due simply to different drug effects (methylphenidate vs. cocaine) or the difference between in vitro studies (Brown et al., 2000) and our in vivo studies; even for in vitro assays, divergent results using radioligand binding of [^{3}H]DTBZ have been reported, depending on the tissue preparation (Hogan et al., 2000).

Since TBZ rapidly and effectively depletes brain tissues of dopamine (Kuszenski, 1977), as evidenced by increased in vivo [^{11}C]RAC binding (Dewey et al., 1993), the lack of effect of TBZ on *d-threo*-[^{3}H]MePhen binding would support the prior conclusions (Gatley et al., 1995) that this radioligand is not sensitive in vivo to acute changes of endogenous levels of dopamine. The lack of acute effects of dopaminergic drugs (other than methylphenidate itself) on in vivo binding of this radioligand also suggests that occupation of dopamine receptors does not induce rapid (<1 h) changes in the binding of radioligands to the transporter; such lack of effects of dopamine receptor agonists and antagonists have been reported with other DAT radioligands such as [^{11}C]CFT and [^{125}I]RTI-55 (Cline et al., 1992; Scheffel et al., 1996). However, it has been recently reported that stimulants such as amphetamine, and more importantly dopamine itself, induce a rapid internalization of transporter protein in hDAT-expressing cell lines (Saunders et al., 2000), resulting in a rapid loss of DA uptake. As haloperidol and tetrabenazine should have had significant effects on dopamine release and turnover, the in vivo and in vitro data may not be in agreement. It is not known if the radioligand employed in our in vivo studies, *d-threo*-[^{3}H]MePhen, is capable of binding to transporters which have been trafficked to cytosolic sites; in that case, the in vivo measures would simply be insensitive to the differential location of DAT at cell surface vs. internal sites. Further studies in this area are needed to understand the potential for in vivo radioligands to measure the proportions of transporters in different physical environments.

Finally, these studies provide some insight into the importance of specific activity for in vivo radiotracer studies and the potential for application of this technique to studies utilizing small animal PET imaging devices. Most of the equilibrium infusion studies performed here utilized 4–6 mCi of total carbon-11-labeled radiotracer. For methylphenidate, using a specific activity of approximately 500 Ci/mmol at time of beginning the infusion and a molecular weight of 233 g/mol, this calculates to a total injected mass of nearly 2 μg for a 4 mCi infusion. Infusion studies with the tritiated radioligand employed a total of 10 μCi per animal; at a specific activity of 83 Ci/mmol, that is an injected dose of 0.027 μg. Despite a mass dose difference of 75, the carbon-11 and tritium-labeled radioligands provided equivalent in vivo regional brain distributions and estimates of specific binding. This provides encouragement that sufficient amounts of carbon-11-labeled tracers should be tolerated in future in vivo imaging studies in rats and the use of the equilibrium infusions provide an excellent approach to quantification of specific binding.

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