In vivo $[^{11}\text{C}]$dihydrotetrabenazine binding in rat striatum: sensitivity to dopamine concentrations

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Abstract

Introduction: The sensitivity of the in vivo binding of $[^{11}\text{C}]$dihydrotetrabenazine ($[^{11}\text{C}]$DTBZ) and $[^{11}\text{C}]$methylphenidate ($[^{11}\text{C}]$MPH) to their respective targets — vesicular monoamine transporter type 2 (VMAT2) and neuronal membrane dopamine transporter — after alterations in endogenous levels of dopamine was examined in the rat brain.

Methods: In vivo binding of $[^{11}\text{C}]$DTBZ and $[^{11}\text{C}]$MPH was determined using a bolus+infusion protocol. The in vitro number of VMAT2 binding sites was determined by autoradiography.

Results: Repeated dosing with $\alpha$-methyl-$p$-tyrosine (AMPT) at doses that significantly (−75%) depleted brain tissue dopamine levels resulted in increased (+36%) in vivo $[^{11}\text{C}]$DTBZ binding to VMAT2 in the striatum. The increase in binding could be completely reversed via treatment with L-DOPA/benserazide to restore dopamine levels. There were no changes in the total number of VMAT2 binding sites, as measured using in vitro autoradiography. No changes were observed for in vivo $[^{11}\text{C}]$MPH binding to the dopamine transporter in the striatum following AMPT pretreatment.

Conclusion: These results indicate that large reductions in dopamine concentrations in the rat brain can produce modest but significant changes in the binding of radioligands to VMAT2, which can be reversed by replenishment of dopamine using exogenous L-DOPA.

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1. Introduction

$\text{(+)-}\alpha$-Dihydrotetrabenazine (DTBZ; $\text{(+)-2(R)}$-hydroxy-3 (R)-isobutyl-9,10-dimethoxy-1,3,4,6,7-hexahydro-11b(R)$H$benzo[a]quinolizine) is a ligand that is specific and has a high affinity ($K_d=1\text{ nM}$) for vesicular monoamine transporter type 2 (VMAT2). In radiolabeled forms, it is a useful in vitro and in vivo radioligand for studies of the distribution and concentration of vesicular monoamine transporter binding sites in the mammalian brain. This includes the use of the carbon-11-labeled form for positron emission tomography (PET) imaging of the vesicular monoamine transporter site in the living human brain [1]. Studies of the vesicular monoamine transporter as a marker of monoaminergic nerve terminal densities have been completed in a variety of neurodegenerative and psychiatric diseases [2–9]. $[^{11}\text{C}]$Dihydrotetrabenazine ($[^{11}\text{C}]$DTBZ) has been particularly useful as a marker of dopaminergic terminal densities in the human striatum due to the high proportion (>95%) of dopaminergic terminals versus other monoaminergic terminals in that brain region.

Although studies have supported the conclusion that the total number of VMAT2 in the striatum is not readily, if at all, regulated by a wide variety of acute or chronic pharmacological manipulations of the monoaminergic or cholinergic neurotransmitter system [10–14], the potential effects of acute drug challenges that significantly alter the cytosolic or vesicular concentration of dopamine have been less well examined. In studies of DOPA-responsive...
dystonia, a human neurological disorder resulting from a complete lack of ability to synthesize endogenous dopamine, a modest (20%) but significant increase in $^{11}$C]DTBZ binding in the caudate nucleus and putamen was observed [5]. The authors concluded that the increased $^{11}$C]DTBZ binding reflected, at least partly if not fully, the greater availability of VMAT2 binding sites due to lack of competition from vesicular dopamine. In recent studies in rats, pharmacological manipulations of dopamine levels using dopamine-depleting agents [α-methyl-p-tyrosine (AMPT) or amphetamine] or dopamine-elevating agents (L-DOPA or γ-hydroxybutyrate) produced modest (12–20%) changes in in vivo $^{11}$C]DTBZ binding [16]. Finally, L-DOPA administration to subjects with advanced Parkinson’s disease was shown to produce reductions in $^{11}$C]DTBZ binding [16].

Depletion of dopamine in the brains of animals and humans is readily accomplished by the administration of AMPT, an inhibitor of tyrosine hydroxylase, which is the rate-limiting step in the enzymatic synthesis of catecholamines. We report here that severe depletion of brain dopamine levels by AMPT does increase the in vivo binding of the VMAT2 radioligand (+)–$^{11}$C]DTBZ, and the increase can be blocked by restoration of dopamine levels. Parts of this work have been previously presented in abstract form [17].

2. Materials and methods

2.1. Materials

α-Methyl-DL-tyrosine methyl ester hydrochloride (AMPT), DL-serine 2-[2,3,4-trihydroxybenzyl]-hydrazide hydrochloride (benserazide) and L-3,4-dihydroxyphenyl alanine methyl ester (methyl L-DOPA) hydrochloride were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Sterile sodium phosphate (45 mM) was obtained from Hospira, Inc. (Lake Forest, IL, USA). (+)–α-[11C]DTBZ and d-threo-[11C]methylphenidate ([11C]MPH) (specific activity, 20,000–80,000 GBq/mmol at the end of syntheses) were prepared by $^{11}$C]methylation of the appropriate desmethyl precursor using published methods [18]. $^{3}$H]Dihydrotetrabenazine (2927 GBq/mmol) was obtained from Amersham Biosciences (Piscataway, NJ, USA).

2.2. Animals

Unless otherwise noted, studies were performed on mature male CD-1 rats (Charles River Laboratories, Inc., Wilmington, MA, USA) weighing between 250 and 300 g. All experiments were approved by the Institutional Animal Care and Use Committees of the University of Michigan and Columbia University and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. AMPT-induced dopamine depletion studies

Male Lewis rats (Charles River Laboratories, Inc.) weighing between 250 and 300 g at the start of the experiments were used. Dopamine depletion was effected by systemic administration of AMPT (250 mg/kg/day ip, in isotonic saline) for 3 days. Control rodents received intraperitoneal injections of saline alone. On the third day, 2 h after the last injection, animals were euthanized by CO$_2$ asphyxiation. Brain tissue was harvested by blunt dissection, excess fluids were blotted and then the tissue was weighed and snap frozen in liquid nitrogen. Frozen specimens were then stored at −80°C until further use. Frozen specimens were pulverized in a liquid-nitrogen-cooled mortar and then homogenized in 10 ml of 0.01 N HCl with 1 mM EDTA and 4 mM sodium metabisulfate. One milliliter of homogenate was spun at 20,000×g for 30 min at 4°C for clarification. One hundred microliters (1/10) of the supernatant of the extract was then analyzed for dopamine content by ELISA (Rocky Mountain Diagnostics, Colorado Springs, CO, USA), in accordance with the manufacturer’s instructions. The total dopamine content of the brain tissue was calculated and normalized to the total wet weight of the brain.

2.4. AMPT blocking studies

AMPT (100 mg/kg) was dissolved in 37°C sterile saline and administered intraperitoneally to male CD-1 rats (one injection per day for 3 days prior to radiotracer injection). The third injection of AMPT was administered 2 h prior to the injection of radiotracer. In studies where both AMPT and L-DOPA were administered, the L-DOPA (100 mg/kg)/benserazide (25 mg/kg) dose was given intravenously 1 h prior to the injection of the radiotracer. L-DOPA/benserazide were dissolved in 0.1% ascorbic acid in sterile saline, and sterile sodium phosphate was added to adjust the pH to approximately pH 6 for intravenous injection.

2.5. Ex vivo dissection studies

Regional brain distributions of $^{11}$C]DTBZ and $^{11}$C]MPH were determined following the administration of the radioligands using a bolus+constant infusion technique that has been used for more than 10 years [19,20]. Under diethyl ether anesthesia, catheters were inserted in the tail vein for infusion, and the rats were restrained in plastic tubes and allowed to awaken. The experiment was performed simultaneously on four to six animals. Injected doses of radiopharmaceuticals ranged from 68 to 133 mBq, with specific activities of 11,100–18,500 GBq/mmol at the time of administration. All animals were euthanized at 60 min by an intravenous injection of sodium pentobarbital (50 mg/kg), and brains were rapidly dissected into regions of interest (striatum, cortex, hypothalamus and cerebellum), which were weighed and counted for carbon-11 radioactivity. Data were calculated as percent injected dose per gram of tissue and converted into distribution volume ratios (DVRs) [where
DVR=(percent injected dose in the striatum)/(percent injected dose in the cerebellum)], and specific binding was calculated as binding potential (BP=DVR−1).

2.6. In vitro autoradiographic binding studies

In vitro binding of [3H]DTBZ was performed using a previously reported autoradiographic assay [21]. Rats were euthanized via carbon dioxide asphyxiation and decapitated. Brains were removed and frozen in crushed dry ice, coated with frozen tissue-embedding medium (Lipshaw, Inc., Detroit, MI, USA) to prevent desiccation and maintained at −80°C until sectioning. Pairs of adjacent sagittal brain sections (20 μm thick) were cut on a cryostat (−18°C), thaw-mounted onto polylysine-subbed microscope slides and allowed to air dry. Sections were preincubated in 137 mM KCl, 3 mM NaCl, 8 mM K2HPO4, 1.5 mM NaH2PO4 and 1 mM EDTA (pH 8.0) at 25°C then incubated in 10 nM [3H] dihydrotetrabenazine with and without 10 μM tetrabenazine (to identify nonspecific binding) for 30 min at 25°C. Slides were washed twice in cold buffer and dipped in distilled water before air drying. The slides were then apposed to tritium-sensitive film (Hyperfilm; Amersham Biosciences) for 4 weeks. Calibrated plastic radioactive standards were included with each cassette of slides to allow for variation in exposure. Autoradiograms were analyzed by computer-assisted video densitometry (MCID; Imaging Research, St. Catherines, ON, Canada). Regions of interest were drawn on the striatum and on a section of the occipital–frontal cortex. Imaging plate densities were converted into apparent tissue radioactivity based on radioactive standards and the specific activity of the [3H]DTBZ used. Radioactive densities were calculated as femtomoles per microgram of protein and were averaged across adjacent sections.

2.7. Statistics

Statistical significance between groups was performed using unpaired Student’s t test. P<.5 was considered significant.

3. Results

3.1. AMPT depletion of brain tissue dopamine

The 3-day treatment of rats with 250 mg/kg intraperitoneal doses of AMPT produced a 75% reduction (Fig. 1) in whole-brain dopamine tissue concentrations (P<.01 vs. controls).

3.2. AMPT effects on in vivo [11C]DTBZ binding in rat brains

The mean and variance of [11C]DTBZ binding potential in the control group (2.70±0.28; n=12) were essentially identical to those of a larger group of controls previously determined using carbon-11 or tritium-labeled dihydrotetrabenazine (2.61±0.24; n=35) [20]. Pretreatment of rats with multiple doses of AMPT to deplete brain tissue dopamine levels resulted in a significant 26% increase in DTBZ binding potential in the striatum (Fig. 2). The differences in DTBZ binding potential were entirely due to increases in the striatum (% injected dose/g tissue in controls=0.87±0.24; % injected dose/g tissue in drug-treated rats=1.08±0.33; P<.01) and not due to alterations in radiotracer uptake into nonspecific regions of the brain such as the cerebellum or cortex, as equilibrium concentrations in these tissues were identical in control (% injected dose/g tissue in cerebellum=0.23±0.06; % injected dose/g tissue in cortex=0.27±0.07) and AMPT-treated animals (% injected dose/g tissue in cerebellum=0.24±0.08; % injected dose/g tissue in cortex=0.29±0.09). The treatment with AMPT also did not change radiotracer localization in the hypothalamus [DTBZ binding potential values: AMPT-treated, 1.09±0.09 (n=4); controls, 1.20±0.34 (n=23)]. The administration of L-DOPA and benserazide after the third dose of AMPT, but 1 h before the start of the [11C]DTBZ infusion, resulted in striatal DTBZ binding potential values that were significantly reduced (~44%) from the AMPT-treated group. In a group of animals treated only with L-DOPA+benserazide 1 h prior to [11C]DTBZ infusion, DTBZ binding potential values were also lower (~26%) than those in control untreated animals. However, this was the result of a nonsignificant decrease (~7%) in radioligand concentration in the striatum (controls, 0.87±0.24; DOPA-treated, 0.81±0.13; P=0.49), coupled with a larger but again nonsignificant increase (+17%) in radioligand concentration in the cerebellum (controls,
0.23±0.06; DOPA-treated, 0.27±0.06; \( P = 0.14 \), and the combination of these two nonsignificant tissue concentrations resulted in the observed change in their ratio.

3.3. AMPT effects on in vitro \([3H]DTBZ\) binding in rat brains

By the in vitro autoradiographic assay performed on rat brain slices, the binding of \([3H]DTBZ\) in control rats and the binding of \([3H]DTBZ\) in AMPT-treated rats were found to be identical in both the cortex and the striatum (Fig. 3).

3.4. AMPT effects on in vivo \([11C]MPH\) binding in rat brains

In rats treated with three injections of AMPT using the same protocol as for the \([11C]DTBZ\) studies, regional brain uptake and binding potential values for \([11C]MPH\) were identical in AMPT-treated (1.05±0.23; \( n = 6 \)) and control (1.07±0.12; \( n = 9 \)) animals for in vivo binding to the neuronal membrane dopamine transporter.

4. Discussion

This study was undertaken to determine whether pharmacological treatments known to alter cytosolic and vesicular concentrations of dopamine would affect the in vivo binding of \([11C]DTBZ\), a radioligand for VMAT2 in the rat brain, or \([11C]MPH\), a radioligand for the neuronal membrane dopamine transporter. Studies were performed using a well-established quantitative in vivo radioligand methodology that provides reproducible estimates of in vivo specific binding (DVR values) that are insensitive to a wide range of injected mass amounts [19,20].

The repeated treatment of rats with AMPT, an inhibitor of the first enzymatic step in the biosynthesis of dopamine, produced the expected significant reductions (−75%) in brain tissue dopamine levels (Fig. 1). The dose of AMPT used in this experiment was the highest dose tolerated by the animals and has been reported to produce maximal inhibition (87%) of dopamine biosynthesis in the rat brain [22]. Application of a multiday repeated administration of AMPT has been previously reported to produce a lasting and significant (78%) reduction in brain tissue dopamine levels [23].

The AMPT dose protocol employed here also resulted in an increase in the in vivo specific binding of \([11C]DTBZ\) in the striatum, a region of the brain with a high concentration of VMAT2 in dopaminergic terminals (Fig. 2). The increased specific binding, measured as the binding potential, was entirely attributable to increases in \([11C]DTBZ\) binding in the striatum, as there were no observed changes for radioligand uptake and retention in regions of nonspecific binding (cortex and cerebellum). As further support for the finding that changes in striatal radioligand binding reflect alterations in dopamine, no changes were observed for the hypothalamus — a region of the brain containing intermediate concentrations of VMAT2, but where serotonin and norepinephrine are the predominant endogenous monoamine neurotransmitters and where treatments with AMPT do not affect the biosynthesis of serotonin.

The changes in the in vivo binding of \([11C]DTBZ\) are not the result of a rapid up-regulation of the concentrations of vesicular monoamine transporters, as studies of the in vitro binding of \([3H]DTBZ\) in AMPT-treated animals demonstrated no differences from controls (Fig. 3). This result is
consistent with the experience of Gatley et al. [24], who demonstrated that de novo synthesis of vesicular monoamine transporter molecules is quite slow following irreversible inhibition by reserpine, supporting a slow turnover of these proteins in neurons.

The AMPT-induced increase in $[^{11}\text{C}]\text{DTBZ}$ binding in vivo in the striatum can be completely reversed by administration of L-DOPA, the availability of which bypasses the enzymatic step blocked by AMPT. This precursor to dopamine was administered for a sufficient time after the last dose of AMPT to avoid potential complications from the competition between AMPT and L-DOPA for the large amino acid transporter, which both compounds share as the mechanism for transport across the blood–brain barrier. The reversal of the striatal binding potential value for the AMPT+DOPA group was due to reductions in the striatal concentrations of the radioligand (−38%; $P<0.01$ vs. AMPT group), with only small and nonsignificant increases (7–8%) in radioligand uptake and retention in the cortex or cerebellum ($P>0.5$ for both).

Treatment of control animals with a high dose of L-DOPA, which should increase the tissue concentrations of dopamine in the rat brain, produced a significant (−26%) decrease in the in vivo binding potential for $[^{11}\text{C}]\text{DTBZ}$. Although this result is consistent with the experience of De La Fuentes-Fernandez et al. [16] with L-DOPA treatment in Parkinson’s disease, the significance here was the result of a very small and nonsignificant decrease in radioligand concentration in the striatum, coupled with a larger but again nonsignificant increase in radioligand concentration in the cerebellum, and the combination of these two nonsignificant tissue concentrations resulted in the observed change in their ratio. As the cerebellum was used in this study as an estimate of nonspecific radiotracer distribution in the rat brain, ratios calculated from data where cerebellar concentrations are altered by drug treatment may no longer be valid estimates of specific binding. Further studies are needed to confirm that increasing dopamine concentrations can affect the in vivo binding of VMAT2 radioligands in the rat brain.

Finally, the effects of AMPT are not observed with the neuronal membrane dopamine transporter radioligand $[^{11}\text{C}]\text{MPH}$. This is consistent with the study of DOPA-responsive dystonia patients, where no changes in $[^{11}\text{C}]\text{MPH}$ binding were observed, as well as with previous animal studies that have reported the insensitivity of $[^{11}\text{C}]\text{MPH}$ to synaptic dopamine levels [25,26], although the literature is inconsistent on the sensitivity of other radioligands to this transporter [27,28].

Taken in composite, the in vivo and in vitro studies reported here support the finding that pharmacological (AMPT) treatments that dramatically decrease rat brain dopamine levels can produce modest changes in the in vivo binding of $^{+}[^{11}\text{C}]\text{DTBZ}$ to VMAT2 sites of dopaminergic terminals in the striatum. The converse effect of increasing dopamine levels through administration of L-DOPA needs further evaluation to verify possible changes in radioligand binding. The changes in dopamine levels produced by the pharmacological treatments utilized here are quite large (decreases of 75% or more). Although there is no consistency in the reported changes in brain dopamine levels after exogenous L-DOPA administration due to widely varying doses, routes of administration, timing of measurements and methodologies for measuring dopamine in different compartments (e.g., whole tissue, extracellular, cytosolic, releasable or vesicular) [29–31], the magnitude of such changes is, in general, of the same magnitude (increases of about 100%) as the decreases produced by the AMPT depletion protocols. Thus, for both conditions of large decreases or increases in dopamine levels, the percent change in the in vivo vesicular monoamine transporter binding of $[^{11}\text{C}]\text{DTBZ}$ is only a fraction of the alteration in the endogenous neurotransmitter level. Further studies will be needed to determine any dose–response relationship and, in particular, whether changes in $[^{11}\text{C}]\text{DTBZ}$ binding are observed with smaller and more physiologically relevant changes in dopamine concentrations.

The experimental results obtained here confirm and significantly extend the observations originally reported by Tong et al. [15] regarding the sensitivity of $[^{11}\text{C}]\text{DTBZ}$ to endogenous dopamine levels. In this study, different drug (AMPT and L-DOPA) doses were employed, and a different in vivo radioligand binding assay was performed, but the results were very similar. Importantly and not shown in the Tong et al. study, the changes in endogenous dopamine levels were shown to be very high (decreased by 75%), tissue concentrations of VMAT2 were not altered by AMPT treatment and the increased in vivo $[^{11}\text{C}]\text{DTBZ}$ binding could be reversed in AMPT-treated animals by an intervening treatment with L-DOPA to restore tissue dopamine levels.

The results of our experiments are relevant to the use of $[^{11}\text{C}]\text{DTBZ}$ and $[^{11}\text{C}]\text{MPH}$ as potential quantitative imaging agents for dopaminergic nerve terminals in human PET imaging studies. In the rat, large changes in brain dopamine levels produce small but measurable changes in $[^{11}\text{C}]\text{DTBZ}$ binding to the vesicular monoamine transporter. The study of De La Fuente-Fernandez et al. [5] on DOPA-responsive dystonia demonstrated changes of similar magnitude (20%) for in vivo $[^{11}\text{C}]\text{DTBZ}$ binding in that severe example of dopamine deficiency. There is, however, no change in the total number of transporters in the rat striatum, consistent with prior studies showing that the vesicular monoamine transporter of the rat brain is not readily regulated by acute or chronic dopaminergic drug treatments [10–14]. The lack of change in $[^{11}\text{C}]\text{MPH}$ binding after AMPT treatment is consistent with prior experiments using this radioligand, but generalization of these findings to all radioligands for the neuronal membrane dopamine transporter may be unwarranted, and such dopamine sensitivity should be evaluated for each and every radioligand.
References