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IN VIVO BINDING OF [^{11}C]TETRABENAZINE TO VESICULAR MONOAMINE TRANSPORTERS IN MOUSE BRAIN

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Summary

The time course of regional mouse brain distribution of radioactivity after i.v. injection of a tracer dose of [11C]tetrabenazine ([11C]TBZ) has been determined. Radiotracer uptake into brain is rapid, with 3.2% injected dose in the brain at 2 min. Egress from the brain is also very rapid, with only 0.21% of the injected dose still present in brain at 60 min. Radiotracer washout is slowest from the striatum and hypothalamus, consistent with binding to the higher numbers of vesicular monoamine transporters in those brain regions. The rank order of radioligand binding at 10 min after injection is striatum > hypothalamus > hippocampus > cortex = cerebellum, similar to that found using in vitro assays of the vesicular monoamine transporters. Maximum ratios of striatum/cerebellum and hypothalamus/cerebellum were 2.85 $\pm$ 0.52 and 1.69$\pm$ 0.25, respectively, at 10 min after injection. Co-injection of unlabeled tetrabenazine (10 mg/kg) or pretreatment with reserpine (1 mg/kg i.p., 24 h prior) was used to demonstrate specific binding of radioligand in striatum, hypothalamus, cortex, hippocampus and cerebellum. Distribution of [11C]TBZ was unaffected by pretreatment with the neuronal dopamine uptake inhibitor GBR 12935 (20 mg/kg i.p., 30 min prior). [11C]Tetrabenazine is thus a promising new radioligand for the in vivo study of monoaminergic neurons using Positron Emission Tomography.

In vivo imaging and quantification of neuronal losses in living human brains would be of great utility for the understanding of the development and progression of neurodegeneration in Parkinson's disease. Numerous potential neuronal imaging agents based on the neuronal monoamine reuptake system have been developed, and several of these ([11C]nomifensine (1), [11C]cocaine (2), and [18F]GBR 12909 (3)) are currently being evaluated in human subjects. The imaging of vesicular neurotransmitter uptake sites would form an alternative method for quantitation of neuronal densities, and the feasibility of such imaging using carbon-11 and fluorine-18 labeled inhibitors of vesicular acetylcholine transport has been demonstrated by ourselves (4) and others (5) using derivatives of vesamicol. Tetrabenazine (TBZ) and structurally related benzoquinolines comprise a class of compounds which are high affinity inhibitors (6) for the vesicular monoamine transporters and which exhibit shorter duration of action as compared to reserpine (7). Although the benzoquinolines are not selective for dopaminergic vesicular transporters using in vitro assays, TBZ does deplete dopamine to a greater extent than serotonin and norepinephrine (8,9). [3H]Dihydrotetrabenazine ([3H]TBZOH), a simple reduction product from tetrabenazine, has been recently utilized as an in vitro marker for monoaminergic neurons in brain regions (6,10-15). In 6-hydroxydopamine

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lesioned rats, loss of striatal [3H]TBZOH binding sites correlated well with loss of tyrosine hydroxylase activity (14), and reductions of [3H]TBZOH binding sites in the caudate-putamen of post-mortem Parkinsonian brains are extensive (73-87%) (15). These in vitro results suggest that the binding of radioligands for vesicular monoamine transporters may serve as an excellent measure of losses of dopaminergic neurons in degenerative diseases. To extend this approach to in vivo imaging with Positron Emission Tomography (PET), we have developed a synthesis of [11C]tetrabenazine ([11C]TBZ) and have begun the process of preclinical animal evaluation of this exciting new radioligand. We report here the time course and pharmacological blocking of the specific binding of [11C]tetrabenazine in mouse brain.

Methods

Chemicals

No-carrier-added, high-specific-activity [11C]tetrabenazine was prepared by O-[11C]methylation of 9-desmethyltetrabenazine, by a procedure to be reported elsewhere (16). The product was dissolved in isotonic phosphate buffer (pH 6.0) for injection. Specific activity at time of injection was >1000 Ci/mmol as determined by analytical HPLC.

Tetrabenazine was obtained from Fluka, and prepared for injection by dissolution in 4% ethanol: isotonic phosphate buffer, pH 4.5. Reserpine was obtained from Sigma Chem. Co. and prepared for injection as a solution containing 250 mg reserpine, 2 ml benzyl alcohol, 250 mg citric acid, 10 ml Tween-80 and 100 ml sterile water. GBR 12935 methanesulfonate (obtained from Gist-Brocades, Inc.) was dissolved in 5% ethanol : isotonic saline solution.

Regional Brain and Whole Organ Distributions

In vivo tissue distribution studies were performed in female CD-1 mice, 20-27 g (Charles River). For each data point 4 animals were used. Animals were anesthetized with diethyl ether and [11C]TBZ (0.02 - 0.57 mCi in 0.05 to 0.1 ml) injected via the tail vein. The animals were allowed to recover, then sacrificed by decapitation at 2,5,10,20,30,40 or 60 min after injection. A blood sample was collected, and the brain rapidly removed and dissected into samples of striatum, cortex, hippocampus, hypothalamus and cerebellum. The heart, lungs, adrenals and rest of brain were also removed. All samples were counted for carbon-11 (Packard 5780 gamma-counter) and then weighed. Data were calculated as % injected dose per gram (%ID/g) of tissue, and %ID/organ for brain, heart, adrenals and lungs.

Pharmacological Competition Studies

Pharmacological competition studies were done using co-injection of tetrabenazine (10 mg/kg, i.v) and [11C]TBZ, or by pretreatment of the animals with reserpine (1 mg/kg, i.p., 24 h prior) or GBR 12935 (20 mg/kg i.p., 30 min prior) followed by injection of [11C]TBZ. Experiments were done using groups of four animals per study (total N = 12 for TBZ). For all competition studies, control animals (total N = 32) were also studied using an injection of high specific activity [11C]TBZ. Regional brain distributions and organ distributions were determined as described above.

Data were analyzed for significance using an unpaired Student's t-test.

Results

The time course of regional brain distribution of [11C]TBZ following i.v. injection is shown in Fig. 1. In all brain areas, radioactivity levels were highest at the earliest time point measured (2 min) and declined rapidly in all regions over the 60 min period. Radiotracer washout was slowest from the striatum and hypothalamus (Fig. 1A), consistent with specific binding to the highest numbers of vesicular monoamine transporters in those brain regions. The time-tissue activity curves for hippocampus, cortex and cerebellum were very similar, and after about 10 minutes paralleled the blood curve although they remained slightly higher (Fig. 1B). At the 10 minute point, hippocampus, hypothalamus and thalamus showed higher radiotracer concentrations ($p < 0.05$) than did cerebellum. Maximum tissue contrasts (striatum/cerebellum, STR/CER; and hypothalamus/cerebellum, HYPO/CER) were observed at 10 min after injection, therefore this time point was chosen for pharmacological competition studies.

TABLE I

Effect of Treatment With Tetrabenazine, Reserpine and GBR 12935 on Regional Brain Distribution of [^{11}C]TBZ in Mice, 10 min After Injection[a]

	Control N = 32	TBZ[b] N = 12	Reserpine[c] N = 4	GB 12935[d] N = 4
Region				
striatum	4.96 ± 1.0*	1.54 ± 0.37***	2.80 ± 0.25***	5.44 ± 0.57
cortex	1.91 ± 0.34	1.50 ± 0.35***	1.95 ± 0.36	2.01 ± 0.19
hippocampus	2.01 ± 0.36**	1.50 ± 0.36***	1.65 ± 0.40***	2.17 ± 0.16
hypothalamus	2.96 ± 0.61*	1.40 ± 0.33***	2.16 ± 1.48***	3.20 ± 0.41
cerebellum	1.76 ± 0.36	1.52 ± 0.37****	3.44 ± 2.38***	1.76 ± 0.22
thalamus	2.06 ± 0.59**	N.D.	N.D.	N.D.
pons-medulla	1.96 ± 0.30	1.77 ± 0.25	N.D.	N.D.
blood	1.59 ± 0.26	1.44± 0.27	1.67 ± 0.24	1.72 ± 0.17
Region Ratios				
STR/CER	2.85 ± 0.52	1.02 ± 0.06***	1.40 ± 1.10***	3.10 ± 0.20
HYPO/CER	1.69 ± 0.25	0.93 ± 0.07***	0.71 ± 0.98***	1.82 ± 0.19
STR/CTX	2.63 ± 0.52	1.03 ± 0.05***	1.49 ± 0.38***	2.71 ± 0.18
HYPO/CTX	1.55 ± 0.20	0.94 ± 0.09***	0.65 ± 0.38***	1.59 ± 0.19
Organ				
brain	2.18 ± 0.41	1.53 ± 0.36***	2.02 ± 0.33	N.D.
heart	2.54 ± 0.17	2.52 ± 0.67	3.05 ± 0.29	N.D.
adrenal	7.30 ± 1.01	6.42 ± 1.74	7.29 ± 4.89	N.D.
lung	2.44 ± 0.33	2.86 ± 0.54	3.31 ± 1.36	N.D.

a Results are expressed as % injected dose per gram of tissue (%ID/g) ± standard deviation
b Animals were coinjected with 10 mg/kg of unlabeled TBZ (i.v.).
c Animals were pretreated with 1 mg/kg of reserpine, i.p., .24 h prior to radiotracer injection.
d Animals were pretreated with 20 mg/kg GBR 12935, i.p., 30 min prior to radiotracer injection.
* $p < 0.001$ compared to control cerebellum.
** $p < 0.05$ compared to control cerebellum.
*** $p < 0.001$ compared to controls.
**** $p < 0.06$ compared to control.
N.D. Not determined.

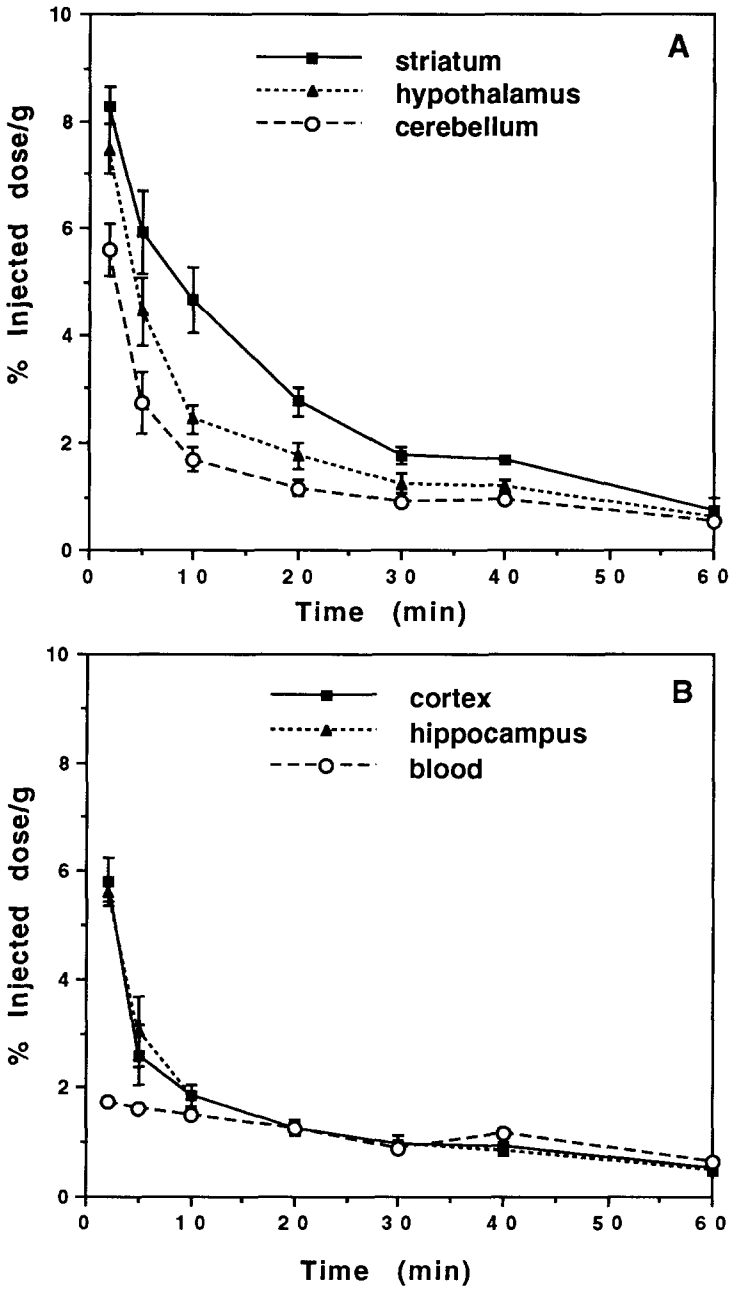

FIG. 1

Time course of regional brain uptake of [11C]TBZ following i.v. injection. Values shown are means + S.D. (N = 4).

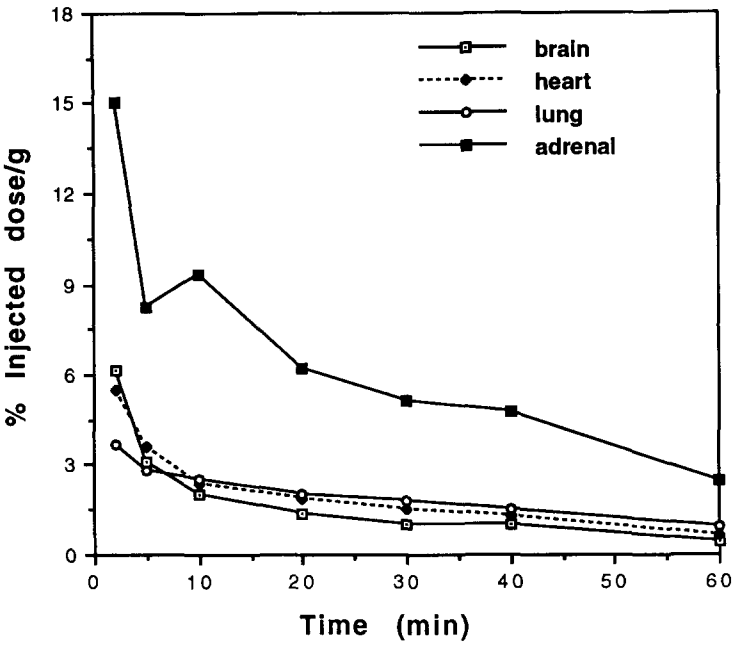

FIG. 2.
Time course of [11C]TBZ in brain, heart, lung and adrenals after i.v. injection.

The effects of co-injection of 10 mg/kg unlabeled TBZ, pretreatment with 1 mg/kg reserpine, or pretreatment with 10 mg/kg GBR 12935 on the regional brain distribution of [11C]TBZ are summarized in Table I. Specific binding of [11C]TBZ in striatum, hypothalamus, cortex, hippocampus and cerebellum was significantly blocked by coinjection of TBZ. Statistically significant reductions of radioligand concentrations in striatum, hippocampus and hypothalamus were observed after reserpine pretreatment. In the reserpine treated animals there was also a variable increase in radioactivity concentration in the cerebellum as compared to controls. Treatment with reserpine or TBZ did not affect blood radioactivity levels. As expected, pretreatment with 20 mg/kg GBR 12935, a high affinity inhibitor of the neuronal dopamine transporter, had no effect on the levels of [11C]TBZ in any brain region or the blood (Table I).

The time course of [11C]TBZ uptake and clearance in brain, heart and adrenals, tissues with potential [11C]TBZ binding sites, and lung, a tissue with no specific sites but an affinity for lipophilic amines, is shown in Fig. 2. Very high initial uptake of radiotracer into the adrenals was observed. Except for the brain, none of these tissues showed any specific binding at the ten min post-injection point which could be blocked with TBZ or reserpine treatment (Table I).

Discussion

Tetrabenazine is a specific, high affinity (K_i approx. 3 nM) inhibitor of monoamine uptake into vesicles of presynaptic neurons (6,10-13). Previous in vitro and in vivo studies of vesicular monoamine transporters have utilized low specific activity [3H]reserpine (7.9-14 Ci/mmol) (12,17,18) or [3H]dihydrotetrabenazine (10-15 Ci/mmol) (6,10-12,18). The use of the carbon-11 labeled tetrabenazine, with a specific activity of greater than 1000 Ci/mmol, allows injections of truly tracer amounts of the radioligand. We report here the first in vivo study of vesicular monoamine transporters using a high specific activity radioligand, [11C]tetrabenazine, as a potential probe of monoaminergic neuronal losses in neurodegenerative diseases.

[11C]Tetrabenazine shows rapid brain uptake and the clearance kinetics are similar to that previously reported using pharmacological doses of unlabeled tetrabenazine (19). Regional pharmacokinetics, however, show a significantly slower clearance of the radioligand from striatum and hypothalamus (Fig. 1A), such that at 10 min after injection there is a clear preferential retention of the radioligand in these regions (STR/CER = 2.93 ± 0.19, HYPO/CER = 1.65 ± 0.08). The rank order of regional brain distribution of [11C]tetrabenazine was striatum > hypothalamus > hippocampus > cortex = cerebellum, and was consistent with that observed using in vitro assays and [3H]dihydrotetrabenazine (11,12). For the calculation of target to non-target ratios, we have used cerebellum as only an approximation of a non-target tissue, as it is clear from the literature and this work that specific binding sites for tetrabenazine are present in cerebellum (but are the lowest of the tissue studied here). As is commonly observed with in vivo studies, the ratios between target and non-target regions (e.g., striatum/cerebellum) do not reach that obtained with the in vitro assays. Although the rank order of [11C]TBZ binding is also consistent with the greater effect of TBZ on the dopaminergic system, such effects cannot at this time be simply ascribed to higher binding of TBZ in the striatum. Our rank order of regional tissue binding of [11C]TBZ is also similar to that obtained with [3H]reserpine (17), but [11C]tetrabenazine has a much higher brain uptake, most likely due to the high lipophilicity and high molecular weight of reserpine which may limit its availability to the brain and its passage through the blood-brain-barrier. In that regard, although higher target/nontarget ratios might be obtained with a radiolabeled derivative of reserpine, this would be offset by the long time necessary for washout of non-specific binding and the low count rate due to limited radiotracer accumulation in brain, even if labeled with a long half-life radionuclide.

That the retention of [11C]TBZ in mouse brain is due to specific binding to monoaminergic vesicular sites (predominantly dopaminergic in striatum, serotonergic + noradrenergic in others) is supported by the competition studies, where virtually all of the retention of radioligand in the various brain regions is blocked by co-injection of unlabeled TBZ (e.g., STR/CER = 1.04 ± .08). Pretreatment with reserpine blocks specific binding in striatum (e.g., STR/CER 1.40 ±1.10) and in hypothalamus (HYPO/CER = 0.71± 0.98). Pretreatment with reserpine also causes a variable increase in cerebellar uptake of [11C]TBZ and similar variable changes in radioligand uptake may have occurred in other brain regions; for that reason, interpretation of radiotracer distribution changes in other tissues is difficult, even though statistical changes are also seen in hippocampus and cerebellum but not cortex. By using cortex as a "non-target" tissue which does not show the variability of the cerebellum, significant reductions of radioligand binding in striatum and hypothalamus are still clearly evident. Changes in the blood-brain-barrier (BBB) permeability of radioligands after reserpine pretreatments have been noted in other studies (20,21). Such changes in radiotracer delivery complicate interpretation of studies in reserpine-treated animals, but the decrease in radiotracer concentrations in target regions as observed here would be the opposite of the higher tissue concentrations to be expected if radiotracer retention was due solely to increased delivery to the brain. Reserpine and tetrabenazine are mutually antagonistic in both in vitro and in vivo assays of vesicular monoamine transporters (13). The monoamine transporter of vesicles is believed to possess two binding sites, one (R1) of high affinity for reserpine and monoamines but TBZ resistant, and the second (TBZ site) of high affinity for TBZ but with lower affinity for reserpine and monoamines (18,22). The blocking of [11C]TBZ binding by TBZ and reserpine is consistent with this model of the vesicular transporter. It is interesting that reserpine did not appear to fully block [11C]TBZ binding, but whether this is dose-related, due to variability in BBB permeability, or due to existence of transporters with possibly only one binding site cannot be determined by this study. As expected, pretreatment of animals with the *neuronal* dopamine uptake inhibitor GBR 12935 (23) does not affect [11C]TBZ distribution in the brain.

As part of this study we also examined [11C]tetrabenazine pharmacokinetics in peripheral tissues which should have vesicular binding sites, such as heart and adrenals, and a non-specific organ, the lung. Radioactivity uptake and washout from heart and adrenals was very rapid and specific binding of the radioligand in these tissues could not be demonstrated at the 10 min time point. Although consistent with the lower peripheral actions of tetrabenazine compared to reserpine, we have examined specific binding only at the ten min time point, and thus we cannot rule out a pharmacological effect during the initial high uptake of TBZ into these tissues due to

binding to noradrenergic neurons (heart) or chromaffin granules (adrenals). The low lung uptake is in agreement with the relatively moderate lipophilicity (log P = 2.68) of tetrabenazine (6), and is different than other more lipophilic amines that have been prepared as in vivo radioligands.

[11C]Tetrabenazine shows regional brain kinetics which are substantially different than many of the radioligands previously prepared for quantifying neuronal densities in vivo. Dopamine uptake inhibitors such as [11C]nomifensine (1) and [18F]GBR 12909 (3) exhibit a slow washout of radioactivity from all brain regions, and a relatively slow development of adequate target-to-nontarget ratios. [11C]MABV ([11C]N-methylaminobenzovesamicol), developed for in vivo study of the vesicular acetylcholine uptake sites, shows rapid brain penetration and moderately slow clearance from non-target tissues, but little egress from striatum of mice (4). In many ways, [11C]TBZ more closely resembles [11C]cocaine, which shows rapid brain uptake and rapid brain clearance, with early (t=10-20 min) delineation of striatal tissues (2).

Throughout this study we have not analyzed blood or tissue samples for possible radioactive metabolites of [11C]tetrabenazine. The metabolism of tetrabenazine in rabbit, dog and man has been published (24); numerous conjugated and unconjugated metabolites were observed in the urine, although the rate of formation and proportions of such metabolites were not presented. Analyses of blood and brain samples obtained from rodents, and blood samples from humans, show the presence of the metabolite dihydrotetrabenazine (TBZOH) following administration of a therapeutic dose of TBZ (25). TBZOH is known to be an inhibitor of monoamine vesicular transport with nearly the same K_i as TBZ, and furthermore TBZOH can clearly cross the blood-brain-barrier and exert pharmacological effects (25). For future quantitative pharmacokinetic analyses of this binding site, the rate and proportion of metabolite formation following tracer dose injection of [11C]TBZ will have to be determined. Use of TBZ itself, however, did allow us to avail ourselves of the large previous literature on the pharmacology and pharmacokinetics of TBZ.

In conclusion, we have performed the first in vivo study of the regional distribution and pharmacokinetics of a tracer dose of [11C]tetrabenazine following i.v. injection. The regional distribution is consistent with binding to monoaminergic nerve terminals, and the kinetics of uptake and egress from brain regions are as expected for a short-acting drug. The BBB permeability of this drug, and the kinetics of regional brain accumulations, are supportive of further development of labeled benzoquinolines as potential neuronal imaging agents. As tetrabenazine is a well understood and clinically used drug with low toxicity (7), the use of [11C]tetrabenazine or a close structural analog as an in vivo imaging agent for PET should provide an important new method for assessment of the loss of neurons in Parkinson's and other neurodegenerative diseases.

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