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In vivo binding of (+)-α-[^{3}H]dihydrotetrabenazine to the vesicular monoamine transporter of rat brain: bolus vs. equilibrium studies

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Abstract

The regional rat brain distribution of (+)-α-[^{3}H]dihydrotetrabenazine was determined following (a) infusion to equilibrium between brain and blood or (b) bolus injection. Infusions provide for direct measurement of total distribution volumes. The free plus nonspecific distribution volume for the brain was determined using infusion of very low specific activity (+)-α-[^{3}H]dihydrotetrabenazine; specific distribution volumes, which represent specific radioligand binding, were then calculated as total minus the free + nonspecific distribution volume. Both total and specific distribution volumes correlated very well ($r^2 > 0.99$) with in vitro distributions of the vesicular monoamine transporter binding site. Bolus injection, and measurement of radioactivity at a single time point, also provided regional estimates of radioligand binding which correlated well ($r^2 > 0.98$) with in vitro values. The bolus method shows a small positive bias (+10–15%) in regions of high binding site concentrations. Both infusion and bolus injection methods give acceptable in vivo measures of (+)-α-[^{3}H]dihydrotetrabenazine binding to the vesicular monoamine transporter of rat brain. © 1997 Elsevier Science B.V.

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1. Introduction

(+)-α-[^{3}H]Dihydrotetrabenazine (2-hydroxy-3-isobutyl-9,10-dimethoxy-1,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine) is a specific, high affinity ($K_d = 1.5$ nM) radioligand for the vesicular monoamine transporter type 2 (VMAT2) found in monoaminergic neurons of the mammalian brain, including man. We have developed (+)-α-[^{11}C]dihydrotetrabenazine as one of a series of benzo[*a*]quinolizines (Kilbourn, 1994) suitable for positron emission tomography (PET) imaging of the losses of monoaminergic nerve terminals in neurodegenerative diseases (Frey et al., 1996; Gilman et al., 1996). Radiolabeled (+)-α-dihydrotetrabenazine, in carbon-11 or tritiated forms, can be used in animals to study the in vivo pharmacology and regulation of the vesicular monoamine transporter. In addition, measurements of the vesicular monoamine transporter may provide an excellent means to follow the losses of monoaminergic nerve terminals in animal models of human disease and to evaluate new pharmacotherapies intended to arrest or reverse the degenerative process. We have previously reported numerous regional distribution studies of these radioligands in mouse and rat brain (DaSilva et al., 1994; Kilbourn and Frey, 1996; Kilbourn et al., 1996; Kilbourn, 1996, 1997). All these studies were done using the simple protocol of ex vivo determination of brain radioactivity distribution at a single time point following intravenous administration of radiotracer: in essence, that protocol provides a 'snapshot' of radioligand binding in the brain at that single time point. The bolus injection/single time point protocol can, however, be used to demonstrate stereochemical and pharmacological specificity of the in vivo binding of the radioligand α-(+)-[^{11}C]dihydrotetrabenazine and structurally related benzo[*a*]quinolizines (DaSilva et al., 1994; Kilbourn et al., 1995), as well as a dose–response curve for direct competition with tetrabenazine (Kilbourn, 1997).

The bolus injection/single time point method of determining radiotracer distribution is widely used but is certainly not the only method available for studying in vivo radioligand binding in the rodent brain. Time-dependent radiotracer distributions can be fitted to mathematical mod-

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els, providing estimates of binding parameters such as binding potential or volumes of distribution, or individual binding rate constants (Koeppe et al., 1996, and references therein). Use of discrete time point data for pharmacokinetic modeling requires multiple animals at multiple times, a resource-demanding strategy. Measurements of time-dependent radiotracer distributions in rodent brain can be obtained by external imaging techniques, but such data is limited by the resolution of the instrument, particularly for small regions of the rodent brain (Hume et al., 1996). A strategy which has been the most successful, however, is the ex vivo determinations of regional brain distributions of radiotracers under true equilibrium conditions; the ratios of radiotracer concentrations between tissues and blood at equilibrium represent the total distribution volumes, which have been shown to be appropriate indices of specific binding of radioligands to sites within the brain. As demonstrated for [^{3}H]scopolamine (Frey et al., 1985), [^{18}F]cyclofoxy (Kawai et al., 1991), and [^{123}I]iomazenil (Videbaek et al., 1996), such infusion/equilibrium protocols can be readily implemented in rats.

Although previous studies of radioligand binding in the brain have compared the results from infusion/equilibrium methods with the results obtained from compartmental modeling of pharmacokinetic data following bolus injection (Frey et al., 1991; Carson et al., 1993), no previous studies have directly compared equilibrium distributions of radiotracers with the regional distributions obtained from the simple bolus injection/single time point method. The potential utility and limitations of the bolus injection/single time point method are important to know as that simpler approach is more easily applied to large groups of animals, and furthermore may be a more appropriate method for animal models such as genetically-altered, mutant, or neurotoxin-treated mice where infusions are more difficult. To evaluate if the simpler method does indeed provide good estimates of the in vivo regional brain binding of a radioligand, we have compared here the infusion/equilibrium and bolus injection/single time point methods for determining regional binding of α-(+)-[^{3}H]dihydrotetrabenazine to the vesicular monoamine transporter of the rat brain.

2. Materials and methods

2.1. Materials

(+)-α-[^{3}H]Dihydrotetrabenazine (specific activity 82 Ci/mmol) was prepared by [^{3}H]methylation (Amersham, Arlington Heights, IL, USA) of the 9-*O*-desmethyl precursor ((+)-2,9-dihydroxy-3-isobutyl-10-methoxy-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine). (+)-α-[^{11}C]Dihydrotetrabenazine (specific activity > 500 Ci/mmol) was prepared by [^{11}C]methylation of the 9-*O*-desmethyl precursor, as previously described (Jewett et al., 1997). Tetrabenazine was obtained from ICN Biomedicals (Aurora, IL, USA).

2.2. Animal preparation

All studies were done in female Sprague-Dawley rats (150–200 g, Charles River, Portage, MI, USA); two animals were done per experiment. Under sodium pentobarbital anesthesia, the animals were prepared for radiotracer injections by incision of the skin and insertion of a catheter into the femoral vein. The incisions were then closed and the animal placed in a plastic restrainer, and allowed to awaken. All injections were done through the catheters.

2.3. Infusion studies

In one group of animals ($n = 6$), α-(+)-[^{3}H]dihydrotetrabenazine (10 μCi) was infused for one hour at a constant rate of 0.025 ml/min (1.5 ml total volume). For the bolus plus infusion studies ($n = 9$), the total volume of injection solution of α-(+)-[^{3}H]dihydrotetrabenazine (7.6–7.7 μCi) was given as an initial bolus of 1 ml (2/3 of dose) over 1 min, followed by constant infusion of the remaining 0.5 ml over the next 59 min. All animals were killed at 60 min by i.v. injection of an overdose of sodium pentobarbital.

2.4. Bolus injection studies

Bolus injection studies were done using an injection of 7.6–7.7 μCi of α-(+)-[^{3}H]dihydrotetrabenazine in a volume of 0.5 ml, given over a 1 min period. Animals ($n = 4$) were killed 14 min later by i.v. injection of sodium pentobarbital.

2.5. Tetrabenazine blocking studies

For the tetrabenazine blocking studies, tetrabenazine (50 mg/kg total dose) was dissolved in 1.5 ml of saline containing 5% ethanol. For the bolus plus infusion study, α-(+)-[^{3}H]dihydrotetrabenazine (10 μCi) was added to this solution and the combination of hot plus cold ligand infused for 1 h using the 2/3 bolus:1/3 infusion protocol described above. For the bolus study, 50 mg/kg tetrabenazine was administered using the identical infusion protocol, but α-(+)-[^{3}H]dihydrotetrabenazine was administered as a 1 min bolus starting 45 min into the cold drug administration. In both experiments, animals were killed at the end of the 60 min infusion.

2.6. Regional brain distributions

Regional brain dissections were performed according to a slight modification of a literature method (Glowinski and Iversen, 1966). A sample of blood was collected, and then

the brain dissected into samples of striatum, cortex, hippocampus, hypothalamic region, and cerebellum. Tissue samples were then weighed and counted: carbon-11 was determined using an automated γ-counter, and tritium by liquid scintillation counting. Data were calculated as percent injected dose per gram tissue (%ID/g).

2.7. Plasma metabolite studies

For the infusion studies, blood samples were analyzed for metabolites of α-(+)-[^{3}H]dihydrotetrabenazine by a minor modification of the method used in clinical studies (Frey et al., 1996). To each plasma sample was added a small amount of α-(+)-[^{11}C]dihydrotetrabenazine as an internal standard, the plasma diluted with phosphate-buffered saline (pH 7.4) and the mixture applied to a C18 Sep-Pak (Waters Co, Milford, MA). Metabolites were then eluted with a 65:35 mixture of phosphate buffered saline:ethanol, and then authentic radiolabeled α-(+)-dihydrotetrabenazine was eluted with ethanol. The two washes were assayed for carbon-11, and after allowing for decay of that radionuclide, aliquots were assayed for tritium. The percentage of metabolites was calculated using the elution of α-(+)-[^{11}C]dihydrotetrabenazine as an internal correction factor for losses of α-(+)-[^{3}H]dihydrotetrabenazine into the metabolite washings.

2.8. Statistics

Statistics. Differences between groups of animals was assessed using an unpaired *t*-test. Differences between regional values within the brain were determined using a paired *t*-test. In all cases a $P < 0.05$ was considered significant.

3. Results

3.1. Plasma metabolite analyses

Analysis of blood samples following the constant infusion or the bolus plus infusion of α-(+)-[^{3}H]dihydrotetrabenazine showed an unmetabolized fraction of $58 \pm 11\%$. Equivalent results were obtained with the blood samples obtained from bolus + infusion of a mixture of tetrabenazine and α-(+)-[^{3}H]dihydrotetrabenazine (unmetabolized fraction: $52 \pm 12\%$).

3.2. Infusion studies

As equilibrium brain distribution studies can be done using either a constant infusion or using a bolus priming dose followed by a constant infusion paradigm, we first compared the ability of these two methods to obtain constant blood levels of α-(+)-[^{3}H]dihydrotetrabenazine. Analysis of blood samples obtained 30 and 60 min after the start of either infusion protocol demonstrated that a constant concentration of authentic radioligand in the blood had been maintained for the last thirty minutes of the infusion (0.0157 ± 0.002 and 0.0168 ± 0.002 μCi/g, respectively, for the constant infusion and the bolus + infusion studies). Whether equilibrium conditions had been achieved earlier in the study, which may have been likely with the bolus + infusion studies, was not determined, as we had decided on a standard one hour infusion protocol for all studies.

For both of the infusion protocols, regional brain distributions obtained at 60 min showed the expected rank order for vesicular monoamine transporter concentrations in the rat brain (striatum > hypothalamus > thalamus > hippocampus > cortex,cerebellum). As there were no statistical differences between the blood (corrected for metabolites) or tissue levels of α-(+)-[^{3}H]dihydrotetrabenazine obtained from these two infusion protocols, and furthermore no differences between ratios of tissue concentrations (e.g., striatum/cerebellum), all of the infusion studies were combined into one group (Table 1) for comparison to the bolus injection group.

Total distribution volumes (DV_{tot}) were calculated by dividing the final regional brain tissue radioactivity concentrations by the metabolite-corrected concentration of radioligand in the blood (Table 1).

Table 1
Regional radioactivity concentrations (percent injected dose per gram tissue, %ID/g) and calculated total distribution volumes (DV_{tot}) for (+)-α-[^{3}H]dihydrotetrabenazine following infusion to equilibrium in rats

Tissue region	%ID/g	$\frac{\%ID/g\ tissue}{\%ID/g\ cerebellum}$	DV_{tot}	$\frac{DV_{tot}, region}{DV_{tot}, cerebellum}$
Striatum	1.15 ± 0.19	3.29 ± 0.55	7.35 ± 2.4	3.56 ± 0.59
Hypothalamus	0.85 ± 0.18	2.39 ± 0.39	5.13 ± 1.6	2.45 ± 0.25
Thalamus	0.49 ± 0.09	1.43 ± 0.28	2.80 ± 0.87	1.27 ± 0.15
Hippocampus	0.41 ± 0.09	1.15 ± 0.17	2.51 ± 0.82	1.20 ± 0.12
Cortex	0.35 ± 0.14	0.98 ± 0.35	2.18 ± 0.76	1.04 ± 0.13
Cerebellum	0.36 ± 0.09	1.00	2.07 ± 0.61	1.00

Total distribution volumes were calculated as the ratio of tissue radioactivity concentration divided by the metabolite corrected blood radioligand concentration. Data are mean $\pm$ S.D. for $n = 15$ animals.

Table 2
Regional tissue radioactivity concentrations (percent injected dose per gram, %ID/g) and tissue concentrations ratios determined 15 min after bolus injection of (+)-α-[^{3}H]dihydrotetrabenazine in rats

Tissue region	%ID/g	%ID/g tissue / %ID/g cerebellum	%ID/g tissue / %ID/g blood
Striatum	1.48 ± 0.19	3.76 ± 0.15 *	4.81 ± 0.98
Hypothalamus	1.18 ± 0.21	2.97 ± 0.34 *	3.79 ± 0.87
Thalamus	0.68 ± 0.12	1.73 ± 0.13	2.21 ± 0.45
Hippocampus	0.57 ± 0.07	1.44 ± 0.04	1.85 ± 0.41
Cortex	0.44 ± 0.05	1.13 ± 0.03	1.44 ± 0.30
Cerebellum	0.32 ± 0.07	1.00	1.28 ± 0.35

Data are mean ± S.D. for $n = 4$ animals.
* $P < 0.02$ vs. ratios from infusion studies (Table 1).

3.3. Bolus studies

The bolus injection/single time point studies also provided a regional brain distribution of radioactivity (Table 2) consistent with the known distribution of vesicular monoamine transporters (Vander Borght et al., 1995b). Absolute tissue radioactivity concentrations in the striatum, hypothalamus, thalamus and hippocampus were significantly ($P < 0.05$) higher than found in the infusion/equilibrium studies. Similarly, region/cerebellum concentration ratios were also consistently higher in the bolus injection/single time point studies, with statistical significance ($P < 0.02$) in the ratios for striatum and hypothalamus.

3.4. Tetrabenazine blocking studies

Infusion of low specific activity (0.003 Ci/mmol) α-(+)-[^{3}H]dihydrotetrabenazine resulted in a relatively uniform brain distribution of radioactivity, consistent with an effective full pharmacological block of the binding site at the vesicular monoamine transporter (Table 3). A lower radiotracer concentration was found in the cerebellum, and this was significantly lower than all other regions except the thalamus.

The ratios of regional radioactivity concentrations to the metabolite-corrected blood concentration represent the free + nonspecific distribution volume (DV_{f+ns}) for α-(+)-[^{3}H]dihydrotetrabenazine in the rat brain. Using these values, it was then possible to calculate a specific distribution volume (DVsp) for each brain region by subtracting the free + nonspecific distribution volume from the total distribution volume (DV_{tot}) obtained from the infusion studies at high specific activity. The regional specific distribution volumes (Table 4) show highest specific binding of the radioligand in the striatum and the hypothalamus, with little or no specific binding in the cortex and cerebellum.

Infusion of tetrabenazine prior to and during the bolus injection of α-(+)-[^{3}H]dihydrotetrabenazine also produced a uniform brain distribution of radioactivity (Table 3). Levels of radioactivity in the blood and regions of low

Table 3
Regional rat brain radioactivity distributions (% injected dose per gram tissue, %ID/g) and calculated free plus nonspecific distribution volumes (DV_{f+ns}) determined for co-infusion of (+)-α-[^{3}H]dihydrotetrabenazine with 50 mg/kg tetrabenazine, using the infusion to equilibrium method (left column)

Tissue region	Infusion		Bolus
	% ID/g	DV_{n+s}	% ID/g
Striatum	0.246 ± 0.03 *	1.91 ± 0.29	0.494 ± 0.49 **
Hypothalamus	0.261 ± 0.04 *	1.92 ± 0.22	0.487 ± 0.066 **
Thalamus	0.228 ± 0.03 *	1.80 ± 0.30	0.436 ± 0.058 **
Hippocampus	0.254 ± 0.04 *	1.91 ± 0.29	0.489 ± 0.036 **
Cortex	0.249 ± 0.03 *	1.84 ± 0.12	0.511 ± 0.061 **
Cerebellum	0.218 ± 0.03 *	1.61 ± 0.19	0.443 ± 0.047 **
Blood	0.239 ± 0.110	—	0.400 ± 0.040 **

For comparison are shown the regional rat brain radioactivity distributions (%ID/g) following infusion of 50 mg/kg tetrabenazine and bolus injection of (+)-α-[^{3}H]dihydrotetrabenazine (right column). Data for individual tissues represent the mean ± S.D. ($n = 3$ animals).
* $P < 0.05$ vs. control infusion studies (Table 1).
** $P < 0.05$ vs. control bolus studies (Table 2).

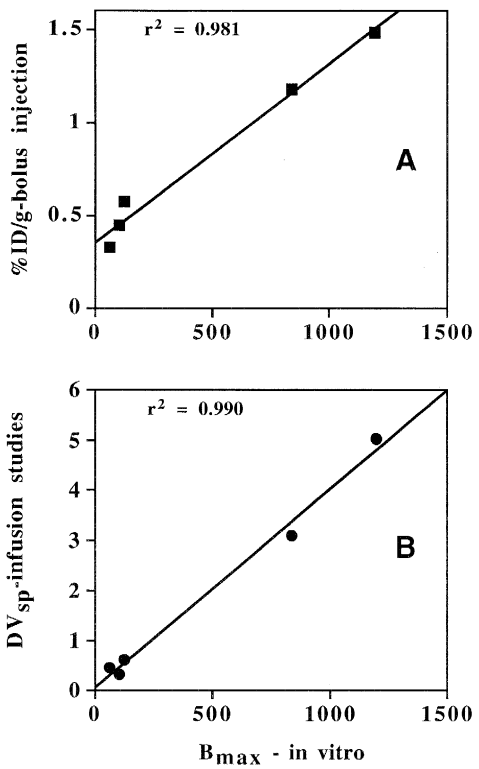

Fig. 1. Correlation of regional values for in vitro binding of [^{3}H]methoxytetrabenazine to the vesicular monoamine transporter (Vander Borght et al., 1995a,b) with (panel A) in vivo regional values for radiotracer concentrations measured after bolus injection of α-(+)-[^{3}H]dihydrotetrabenazine and (panel B) in vivo specific distribution volumes for α-(+)-[^{3}H]dihydrotetrabenazine determined using an infusion to equilibrium protocol. Tissue regions analyzed were (in ascending order of in vitro B_{max} values): cerebellum, cortex, hippocampus, hypothalamus and striatum.

Table 4
Calculated specific distribution volumes ($DV_{sp} = DV_{tot} - DV_{f+ns}$) and ratios of specific distribution volumes to the cerebellum for in vivo binding of (+)-α-[^{3}H]dihydrotetrabenazine in rat brain

Region	DV_{tot}	DV_{sp}	B_{max} (fmol/mg protein)	Region DV_{sp} / Cerebellar DV_{sp}	Region B_{max} / Cerebellar B_{max}
Striatum	7.35 ± 2.4	5.44	1199	11.82	20
Hypothalamus	5.13 ± 1.6	3.21	838	6.97	13.9
Thalamus	2.80 ± 0.87	1.00	—	2.17	—
Hippocampus	2.51 ± 0.82	0.60	124	1.30	2.06
Cortex	2.18 ± 0.76	0.34	103	0.74	1.71
Cerebellum	2.07 ± 0.61	0.46	60	1.0	1.0

For comparison, the in vitro concentrations of vesicular monoamine transporter binding sites and ratios to cerebellum are shown (in vitro data from Vander Borght et al., 1995a,b). Distribution volume data is presented as means ± S.D. ($n = 15$).

binding site densities (cortex and cerebellum) were significantly higher than observed after the bolus injection of α-(+)-[^{3}H]dihydrotetrabenazine, and also higher than the levels observed in the hot plus cold infusion study.

3.5. Correlation of in vivo and in vitro radioligand binding

Both the bolus studies and the infusion studies provided regional brain distributions of radiotracer which showed the correct rank order for binding site densities for the vesicular monoamine transporter. The correlation between the in vivo determined values for total distribution volumes, representing total binding, with the regional values for radioligand binding in vitro to the vesicular transporter (Vander Borght et al., 1995a) is shown in Fig. 1. Both total ($r^2 = 0.991$, correlation not shown) and specific binding ($r^2 = 0.990$, Fig. 1) correlate well with the in vitro density of vesicular monoamine transporter binding sites. The regional distribution of brain radioactivity obtained after bolus injection of radiotracer in rats also correlates well ($r^2 = 0.981$) with the in vitro concentrations (Fig. 1).

4. Discussion

4.1. Infusion / equilibrium studies

As a readily reversible radioligand, α-(+)-[^{3}H]dihydrotetrabenazine is quite suitable for use in an infusion to equilibrium protocol. In humans studies, constant blood levels of radioligand can be obtained in 30 min, with equilibrium brain tissue concentrations reached after 45 min (Koeppe et al., 1997). As determined here, a constant blood concentration of this radioligand in the rat can also be obtained by thirty minutes (if not sooner). Sampling of blood and brain tissues at sixty minutes, then, provide good estimates of the equilibrium distribution of α-(+)-[^{3}H]dihydrotetrabenazine in the rat brain. Additional evidence for equilibrium of brain tissue concentrations comes from separate studies in two rats where the same total amount of α-(+)-[^{3}H]dihydrotetrabenazine was administered as an initial bolus followed by a constant infusion for 119 min; the concentration ratios between regions determined at this longer period (2 h total) were equivalent to those at one hour of infusion (e.g., striatum/cerebellum = 4.43 and 3.25; hypothalamus/cerebellum = 2.23 and 1.92; and hippocampus/cerebellum = 1.23 and 1.33; compare with mean values for 60 min study in Table 1).

Application of the infusion/equilibrium method to high specific activity α-(+)-[^{3}H]dihydrotetrabenazine in the rat brain provides regional estimates of total distribution volumes (Table 1). In these rat studies we were also able to determine the free + nonspecific distribution volume for α-(+)-[^{3}H]dihydrotetrabenazine, through the infusion of radioligand with very low specific activity (0.003 Ci/mmol). This is an experiment we have not, for ethical reasons, been able to perform in humans. Although the free + nonspecific binding of α-(+)-[^{3}H]dihydrotetrabenazine was uniform through most of the brain, a lower value was observed for the cerebellum; regional differences in free + nonspecific distributions have been noted for other radioligands such as [^{18}F]cyclofoxy (Carson et al., 1993), and an assumption of uniform free + nonspecific distribution for the whole brain could therefore lead to biased calculations of regional specific binding parameters.

The difference between the calculated values for total distribution volume and free + nonspecific distribution volume, in any brain region, provides the specific distribution volume DV_{sp}, a value which represents the specific binding of α-(+)-[^{3}H]dihydrotetrabenazine to the vesicular monoamine transporter. The regional in vivo specific binding of α-(+)-[^{3}H]dihydrotetrabenazine correlates well (Fig. 1) with the regional concentrations of vesicular monoamine transporters determined using in vitro autoradiographic assays of the rat brain. It should be noted that the dynamic range for the in vivo values is a little more than half that found in vitro: the striatum to cerebellum ratio of distribution volumes is almost 12, whereas the ratio of in vitro B_{max} values is 20 (Table 4).

4.2. Bolus injection / single time point studies

The bolus injection/single time point method of radiotracer distribution measurement also provided a regional distribution of radioactivity which correlated very well

($r^2 = 0.981$, Fig. 1) with in vitro values for radioligand binding to the vesicular monoamine transporter. However, higher absolute tissue levels of radioactivity are found in the bolus injection/single time point method (Table 2), as well as greater contrast between radioligand concentrations in regions of high and low binding site densities (striatum/cerebellum and hypothalamus/cerebellum ratios). The apparent bias in these ratios for the bolus injection/single time point method data is about 10–15%, and is a result of over-estimation of radioligand binding in the striatum and hypothalamus. These results are consistent with our observations using α-(+)-[^{11}C]dihydrotetrabenazine in humans, and with the results using radiolabeled cyclofoxy in rat and primate brain (Kawai et al., 1991; Carson et al., 1993). As previously discussed (Carson et al., 1993) achieving a transient equilibrium following a bolus injection of radiotracer (a condition reasonably met by α-(+)-[^{3}H]dihydrotetrabenazine and related radiolabeled benzo[*a*]quinolizines, where relatively constant regional ratios of tissue concentrations are maintained from 10–30 min in rodent brain) still leads to biased measures of radioligand binding in regions with high concentrations of binding sites. These observations apply to every reversible radioligand, but the magnitude of the bias is dependent on the rates of tissue and plasma clearances of a particular radiotracer, and the bias is higher in regions of high binding site concentrations (which have the slowest clearance). This bias was demonstrated for positron emission tomographic imaging of [^{18}F]cyclofoxy in primate brain by comparison of the distribution volumes obtained from equilibrium studies with the results from bolus injection and pharmacokinetic modeling (Carson et al., 1993). We have shown here that a bias of similar direction (positive) and magnitude (10–15%) can be obtained for in vivo studies of vesicular monoamine transporters in rat brain using a simple bolus injection/single time point method and radiolabeled α-(+)-dihydrotetrabenazine.

The regional brain concentrations of radioligand following bolus injection do correlate well with the in vitro values for vesicular monoamine transporter binding (Fig. 1). The dynamic range for this correlation (striatum/cerebellum ratio is less than four), however, is much smaller than that obtained from the equilibrium method.

4.3. In vivo studies of the vesicular monoamine transporter

What does this mean for application of radiolabeled benzo[*a*]quinolizines for in vivo studies of vesicular monoamine transporters in the brains of rodents? Use of the infusion to equilibrium method would still be the best choice, as that protocol provides excellent measures of the concentrations of vesicular monoamine transporters by a method which is insensitive to changes in blood flow or peripheral pharmacokinetics of the radiotracer. We observed considerable variability (30–35%) in the estimates of total binding (DV_{tot}) in the separate brain regions, but normalization of the data as ratios to the cerebellum provided ratios ($DV_{region}/DV_{cerebellum}$) with very acceptable variances (10–16%). Both the total and specific distribution volumes, which correspond to total and specific in vivo binding of α-(+)-[^{11}C]dihydrotetrabenazine, correlate with the concentrations of vesicular monoamine transporter binding sites determined in vitro.

As an alternative, however, the measurement of radiotracer distributions at a single time point following bolus injection appears to also be an appropriate method for determining regional concentrations of the vesicular monoamine transporter binding site. In particular, the use of cerebellum as a reference region for non-specific binding appears quite acceptable, as there is very little specific binding in the cerebellum (as shown in the infusion studies), and the region/cerebellum ratios of radiotracer concentrations have low variability (3–11%). Use of the bolus injection method does have certain limitations, however, which need to be recognized and appreciated.

In regions of high binding densities there will be an over-estimation of the numbers of sites present: this bias, on the order of 10–15%, is less in regions of low binding site densities. Experimental protocols used to examine decreases in vesicular monoamine transporters, such as neurotoxin administrations, will perhaps suffer from reduced sensitivity to initial small changes in binding site concentrations; since the positive bias in the in vivo measure of radioligand binding decreases as the numbers of binding sites within a region grows smaller, more accurate and sensitive measures will be possible with larger lesions. Conversely, experimental protocols designed to increase numbers of binding sites through physiological or pharmacological upregulation may produce artificially large positive changes, as the bias of the in vivo measure of binding in the regions of high binding site number will increase up to the point the radiotracer accumulation becomes delivery limited. This would limit the quantitative ability of this method for measuring the extent of upregulation, but increases the sensitivity of the method to qualitatively detect positive changes in binding site numbers.

The bolus injection/single time point method will also be sensitive to experimental variables that change the rate of delivery and clearance of radiotracer to the brain tissues: this would include alterations in regional cerebral blood flow, or changes in plasma clearance rates for authentic radiotracer. Thus, drugs or other experimental paradigms which are clearly known to alter cerebral blood flow on a regional basis, or which significantly alter the pharmacokinetics of the radiotracer in the blood, are better studied using the infusion to equilibrium protocol. As an example, co-administration of tetrabenazine along with bolus injection of radiolabeled α-(+)-dihydrotetrabenazine produces a uniform brain distribution of radioactivity due to blocking of the specific binding to the vesicular monoamine transporter, but absolute tissue levels in regions of low binding site concentrations (e.g., cortex,

cerebellum) determined at equilibrium are actually higher than in the unblocked state due to an increased concentration of radiotracer in the blood (Table 3).

4.4. In vivo radiotracer studies: What method to use?

These experiments with (+)-α-[^{3}H]dihydrotetrabenazine in rat brain raise some interesting questions regarding what are the proper methods to select for in vivo radiotracer studies of brain biochemistry (e.g., enzymes, transporters and receptors). Despite advances in the design and implementation of positron emission tomography scanners for imaging the time-dependent distribution of radiotracers in rat brain, measurement of radiotracer distributions in small regions of the rodent brain or in regions of close proximity (but with disparate concentrations) remain very difficult. The most accurate method available still remains the ex vivo dissection and either counting of tissue samples or autoradiography. The best experimental protocol to employ would thus be the infusion to equilibrium; if desired, such equilibrium studies can be designed to provide estimates of such parameters as the K_d and B_{max} of radioligand binding (Carson et al., 1993; Videbaek et al., 1996). However, the vast majority of in vivo radiotracer studies utilize a single bolus radiotracer administration followed by single time point determinations of the regional brain distribution. Such studies are often deemed successful when (1) an in vivo regional brain distribution is obtained which correlates well with the in vitro measured values for the same binding site or enzyme, and (2) such in vivo localization of radiotracer is completely blocked by the appropriate pharmacological or physiological interventions. Are these sufficient criteria for judging the bolus injection/single time point method? For radiotracers which do not approach a transient equilibrium within the time frame of the experiment, measured levels of radioactivity may also be partially dependent on the delivery of the radiotracer to the tissues. In addition, even for a radiotracer which exhibits a reasonable transient equilibrium, there may be an over-estimation of binding sites in regions of high density leading to biased ratios between regions of high and low density of binding sites: the extent of this over-estimation depends on the rates of tissue and plasma clearances of radiotracer (Carson et al., 1993) and this bias needs to be determined for each and every radiotracer. The sensitivity of a bolus injection/single time point protocol, that is the ability to detect gradual changes in numbers of in vivo binding sites, needs to be separately established for a radiotracer using an animal model of varying concentrations of such sites: this can be accomplished using a dose–response curve, a drug washout study, or graded lesions. Unfortunately, without this latter test the simple in vitro–in vivo correlations of regional binding of a radiotracer can be misleading, as good correlations can be obtained even with (a) partial delivery dependence of the radiotracer and (b) regional bias in the localization of radiotracer. For α-(+)-[^{3}H]dihydrotetrabenazine the bias of the bolus injection/single time point method is acceptably small, and the sensitivity of the in vivo measure to varying concentrations of vesicular monoamine transporter binding sites has been established using a drug washout study (Kilbourn, 1997).

5. Conclusions

Use of an infusion to equilibrium protocol and (+)-α-[^{3}H]dihydrotetrabenazine provides excellent in vivo measures of the vesicular monoamine transporter in the rat brain. The much simpler experimental protocol of bolus administration of radioligand coupled with tissue measurement at a single time point also provides good estimates of the vesicular transporter binding site, with a small positive bias in regions of high binding site concentrations. Both methods are appropriate for studies of the changes of the vesicular monoamine transporter in rodent models of neurodegenerative diseases.

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References

Carson, R.E., Channing, M.A., Blasberg, R.G., Dunn, B.B., Cohen, R.M., Rice, K.C., Herscovitch, P., 1993. Comparison of bolus and infusion methods for receptor quantitation: Application to [^{18}F]cyclofoxy and positron emission tomography. J. Cereb. Blood Flow Metab. 13, 24–42.

DaSilva, J.N., Kilbourn, M.R., Carey, J.E., Sherman, P., Pisani, T., 1994. Characterization of [^{11}C]tetrabenazine as an in vivo radioligand for the vesicular monoamine transporter. Nucl. Med. Biol. 21, 151–156.

Frey, K.A., Ciliax, B., Agranoff, B.W., 1991. Quantitative in vivo receptor binding IV: Detection of muscarinic receptor down-regulation by equilibrium and by tracer kinetic methods. Neurochem. Res. 16, 1017–1023.

Frey, K.A., Ehrenkaufer, R.L., Agranoff, B.W., 1985. Quantitative in vivo receptor binding. II. Autoradiographic imaging of muscarinic cholinergic receptors. J. Neurosci. 5, 2407–2414.

Frey, K.A., Koeppe, R.A., Kilbourn, M.R., Vander Borght, T.M., Albin, R.L., Gilman, S., Kuhl, D.E., 1996. Presynaptic monoamine vesicles in Parkinson's disease and normal aging. Ann. Neurol. 40, 873–884.

Gilman, S., Frey, K.A., Koeppe, R.A., Junck, L., Little, R., Vander Borght, T.M., Lohman, M., Martorello, S., Lee, L.C., Jewett, D.M., Kilbourn, M.R., 1996. Decreased striatal monoaminergic terminals in OPCA and MSA demonstrated with PET. Ann. Neurol. 40, 885–892.

Glowinski, J., Iversen, L.L., 1966. Regional studies of catecholamines in the rat brain. The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine

and [^{3}H]DOPA in various regions of the brain. J. Neurochem. 13, 655–669.

Hume, S.P., Lammertsma, A.A., Myers, R., Rajeswaren, S., Bloomfield, P.M., Ashworth, S., Fricker, R.A., Torres, E.M., Watson, I., Jones, T., 1996. The potential of high resolution positron emission tomography to monitor dopaminergic function in rat models of disease. J. Neurosci. Methods 67, 103–112.

Jewett, D.M., Kilbourn, M.R., Lee, L.C., 1997. A simple synthesis of [^{11}C]dihydrotetrabenazine. Appl. Radiat. Isot. 24, 197–199.

Kawai, R., Carson, R.E., Dunn, B., Newman, A.H., Rice, K.C., Blasberg, R.G., 1991. Regional brain measurement of B_{max} and K_D with the opiate antagonist cyclofoxy: Equilibrium studies in the conscious rat. J. Cereb. Blood Flow Metab. 11, 529–544.

Kilbourn, M.R., 1994. PET radioligands for vesicular neurotransmitter transporters. Med. Chem. Res. 5, 113–126.

Kilbourn, M.R. (1996). In vitro–in vivo correlations: An example from vesicular monoamine transporters. In: Myers, R., Cunningham, V., Bailey, D., Jones, T. (Eds.), Quantification of Brain Function Using PET. Academic Press, London, p. 3.

Kilbourn, M.R., 1997. Time-dependent recovery of in vivo binding sites after drug dosing: A method for radiotracer evaluation. Nucl. Med. Biol. 24, 115.

Kilbourn, M., Frey, K.A., 1996. Striatal concentrations of vesicular monoamine transporters are identical in MPTP-sensitive (C57Bl/6) and -insensitive (CD-1) mouse strains. Eur. J. Pharmacol. 307, 227–232.

Kilbourn, M.R., Lee, L., Vander Borght, T., Jewett, D., Frey, K., 1995. Binding of α-dihydrotetrabenazine to the vesicular monoamine transporter is stereospecific. Eur. J. Pharmacol. 278, 249–252.

Kilbourn, M.R., Frey, K.A., Vander Borght, T., Sherman, P.S., 1996. Effects of dopaminergic drug treatments on in vivo radioligand binding to brain vesicular monoamine transporters. Nucl. Med. Biol. 23, 467–471.

Koeppe, R.A., Frey, K.A., Vander Borght, T.M., Karlamangla, A., Jewett, D.M., Lee, L.C., Kilbourn, M.R., Kuhl, D.E., 1996. Kinetic evaluation of [^{11}C]dihydrotetrabenazine by dynamic PET: Measurement of the vesicular monoamine transporter. J. Cereb. Blood Flow Metab. 16, 1288–1299.

Koeppe, R.A., Frey, K.A., Jewett, D.M., Kilbourn, M.R., Kuhl, D.E., 1997. Equilibrium vs compartmental analysis for assessment of the vesicular monoamine transporter using (+)-α-[C-11]dihydrotetrabenazine (DTBZ) and PET. J. Nucl. Med. 37, 109P.

Vander Borght, T.M., Sima, A.A.F., Kilbourn, M.R., Desmond, T.J., Frey, K.A., 1995a. [^{3}H]Methoxytetrabenazine: A high specific activity ligand for estimating monoaminergic neuronal integrity. Neuroscience 68, 955–962.

Vander Borght, T.M., Kilbourn, M.R., Desmond, T.J., Kuhl, D.E., Frey, K.A., 1995b. The vesicular monoamine transporter is not regulated by dopaminergic drug treatments. Eur. J. Pharmacol. 294, 577–583.

Videbaek, C., Andersen, J.V., Dalgaard, L., Foged, C., Paulson, O.B., Lassen, A.N., 1996. Neuroreceptor quantification in vivo by the steady state principle and [^{123}I]iomazenil in rats. Eur. J. Pharmacol. 281, 117–122.