ELSEVIER

European Journal of Pharmacology 278 (1995) 249–252

ejp

Short communication

Binding of α-dihydrotetrabenazine to the vesicular monoamine transporter is stereospecific

Michael Kilbourn *, Lihsueh Lee, Thierry Vander Borght, Douglas Jewett, Kirk Frey

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

Received 13 February 1995; revised 6 March 1995; accepted 10 March 1995

Abstract

The two enantiomers of α-dihydrotetrabenazine were separated using chiral high performance liquid chromatography. The (+)-isomer showed high affinity in vitro ($K_i = 0.97 \pm 0.48$ nM) for the vesicular monoamine transporter (VMAT2) in rat brain striatum, whereas the (−)-isomer was inactive ($K_i = 2.2 \pm 0.3$ μM). Each isomer was then synthesized in carbon-11 labeled form, and regional brain biodistributions in mice determined after intravenous injection. Only (+)-α-dihydrotetrabenazine showed selective and specific accumulations in regions of dense monoaminergic innervation (e.g., striatum, hypothalamus), which could be blocked by coinjection of unlabeled tetrabenazine. Binding of α-dihydrotetrabenazine to the vesicular monoamine transporter is thus stereospecific.

Keywords: Monoamine transporter; Monoamine; Vesicle; Tetrabenazine

1. Introduction

In monoaminergic neurons, the movement of monoamine neurotransmitters (dopamine, norepinephrine, and serotonin) from the cytosol into the storage vesicle lumen is accomplished via the vesicular monoamine transporter. Molecular biology studies support that a single gene encodes for the vesicular transporter in neurons synthesizing and storing these three monoamines (Schuldiner, 1994). The gene for the human synaptic vesicular transporter has been cloned and sequenced, and the chromosomal location identified. Recently, methods to study the vesicular monoamine transporter using in vitro autoradiography (Henry and Scherman, 1989) or in vivo imaging with positron emission tomography (Kilbourn et al., 1993) have emerged: such studies have employed tetrabenazine or a derivative, α-dihydrotetrabenazine, in tritiated or carbon-11 (β^+, $t_{1/2} = 20.4$ min) labeled forms.

Dihydrotetrabenazine (2-hydroxy-3-isobutyl-9,10-dimethoxy-1,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine), the product of hydride reduction of the 2-keto group of tetrabenazine, contains three asymmetric carbon centers (C-2, C-3 and C-11b: Fig. 1). The two isomers at the C-2 carbon can be easily resolved by column chromatography (DaSilva et al., 1993b), and are termed α- and β-dihydrotetrabenazine. The α- and β-isomers of dihydrotetrabenazine show a small degree of stereoselectivity for in vitro binding to the rat brain vesicular monoamine transporter, with a higher affinity for the α-isomer (6 vs. 20 nM). Surprisingly, the importance of chirality at the C-3 and C-11b positions for the biological activity of these benzoisoquinolines has never been addressed. For α-dihydrotetrabenazine, with two asymmetric centers, there are four possible isomers: extensive NMR studies of tetrabenazine, α-dihydrotetrabenazine and related benzoisoquinolines (Lee and Kilbourn, unpublished observations), however, have established fixed relative configurations at the C-3 and C-11b positions, reducing the possibilities to a single pair of enantiomers, which are shown in Fig. 1. For in vivo radioligands, inclusion of inactive isomers produces increased non-specific binding in target tissues. We

* Corresponding author. Cyclotron/PET Facility, 3480 Kresge III, University of Michigan, Ann Arbor, MI 48109, USA. Tel. 313-763-9246, fax 313-764-0288.

SSDI 0014-2999(95)00162-X

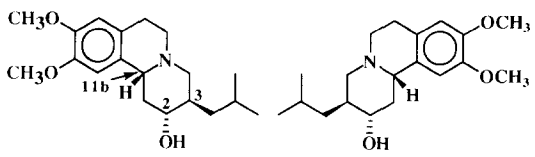

Fig. 1. Enantiomers of α-dihydrotetrabenazine. Chiral carbon centers are present at C-2, C-3 and C-11b.

have therefore undertaken the resolution and biological evaluation, in vitro and in vivo, of these two enantiomers of α-dihydrotetrabenazine.

2. Materials and methods

2.1. Syntheses

(±)-α-Dihydrotetrabenazine was prepared by reduction of tetrabenazine and subsequently demethylated to provide (±)-9-*O*-desmethyl-α-dihydrotetrabenazine (Brossi et al., 1958; Schwartz et al., 1966; DaSilva et al., 1993a,b). (±)-, (+)- and (−)-α-[^{11}C]Dihydrotetrabenazine were prepared by the [^{11}C]methylation of the corresponding 9-*O*-desmethyl precursors (DaSilva et al., 1993a). High specific activity (±)-[^{3}H]methoxytetrabenazine (±)-2-α-[*O*-methyl-^{3}H]methoxy-3-isobutyl-9,10-dimethoxy-1,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine, 82 Ci/mmol) was synthesized by custom *O*-[^{3}H]methylation of (±)-α-dihydrotetrabenazine (Amersham Corp., Arlington Heights, IL, USA).

2.2. Resolution of α-dihydrotetrabenazine isomers by chiral chromatography

High performance liquid chromatography (HPLC) resolutions of the isomers of (±)-α-dihydrotetrabenazine and (±)-9-*O*-desmethyl-α-dihydrotetrabenazine were done using a semi-preparative Chirex 3014 ((*S*)-val-(*R*)-1-(α-naphthyl)ethylamine) HPLC column (20 × 250 mm: Phenomenex), eluted with 60:30:9.5:0.5 hexane : 1,2-dichloroethane : ethanol : trifluoroacetic acid at a flow rate of 7 ml/min. Isolated products were re-injected until pure by analytical HPLC analysis, determined using a separate, analytical (4.6 × 250 mm) Chirex 3014 column and the above solvent mixture.

2.3. In vitro binding assays

All competition experiments of (±)-[^{3}H]methoxytetrabenazine binding by unlabeled α-dihydrotetrabenazine isomers were conducted in triplicate using intact brain cryostat sections of male Sprague-Dawley rats (200–220 g, Charles River Laboratories, Portage, MI, USA). The binding of (±)-α-[^{3}H]methoxytetrabenazine was assayed under minor modifications of the conditions utilized for α-(±)-[^{3}H]dihydrotetrabenazine (Scherman et al., 1988). Adjacent coronal sections (20 μm) at the level of the striatum were mounted on subbed microscope slides, and prewashed for 5 min at 25° C in 300 mM sucrose, 50 mM Tris, 1 mM EDTA, pH 8.0 (Sucrose buffer) to remove endogenous competing substances. Sections were then incubated for 1 h in the presence of 10 nM (±)-[^{3}H]methoxytetrabenazine and varying concentration of α-dihydrotetrabenazine isomers ranging from 0 to 4 μM. Following the incubation period, slices were washed 3 × 3 min in fresh Sucrose buffer, then wiped from the slides with glass fiber filters (GF/C filters, Whatman, Hillsboro, OR, USA) and counted by liquid scintillation spectrometry.

The apparent K_i of α-dihydrotetrabenazine isomers for rat brain [^{3}H]methoxytetrabenazine binding were calculated by logit plot and using a nonlinear curve fitting program (Munson and Rodbard, 1980) (LIGAND, Elsevier-Biosoft, Cambridge, UK), using 3.94 nM as the K_d of [^{3}H]methoxytetrabenazine.

2.4. In vivo radiotracer distribution studies

Female CD-1 mice (20–25 g, Charles River) were anesthetized with diethyl ether and injected via the tail vein with 100–300 μCi of radiotracer ((+)-, (−)- or (±)-[^{11}C]dihydrotetrabenazine). Animals were allowed to awaken. At 15 min they were anesthetized with ether, decapitated, a blood sample obtained, and the brain rapidly removed and dissected into samples of the striatum, cortex, cerebellum, thalamus, hippocampus, and hypothalamus. Tissue samples were rapidly counted (automated gamma counter) and weighed. Data were calculated as percent injected dose/gram.

For the pharmacological blocking study of (+)-α-[^{11}C]dihydrotetrabenazine uptake, animals were injected with unlabeled tetrabenazine (10 mg/kg i.p.) 10 min prior to the radiotracer study.

Numbers of animals used were as follows: (+)-α-[^{11}C]dihydrotetrabenazine, $n = 13$; (−)-α-[^{11}C]dihydrotetrabenazine, $n = 15$; (±)-α-[^{11}C]dihydrotetrabenazine, $n = 10$; (+)-α-[^{11}C]dihydrotetrabenazine with tetrabenazine pretreatment, $n = 4$.

2.5. Statistical analyses

Comparison of groups of animals was done using both a Student's unpaired *t*-test, and an analysis of variance (ANOVA) test. A $P < 0.05$ was considered significant.

3. Results

3.1. Synthesis and resolution of isomers of α-dihydrotetrabenazine

Racemic α-dihydrotetrabenazine could be resolved into two isomers using chiral high pressure liquid chro-

matography, with the (−)-isomer eluting first (14.4 min) and the (+)-isomer second (15.6 min). Chiral purity of the resolved isomers was > 99% as determined using an analytical chiral high performance liquid chromatography column. Compounds were assigned as the (+)- and (−)-isomers of α-dihydrotetrabenazine based on rotations of polarized light. Racemic 9-*O*-desmethyl-α-dihydrotetrabenazine, synthesized by a one-step demethylation of racemic α-dihydrotetrabenazine, was similarly separated into the (−)- and (+)-isomers by chiral HPLC (retention times 23.2 and 25.4 min, respectively).

(+)-, (−)- and (±)-α-[^{11}C]Dihydrotetrabenazine were synthesized by *O*-[^{11}C]methylation of the corresponding 9-desmethyl precursors (DaSilva et al., 1993b). Products were obtained with radiochemical purities > 95% and specific activities > 1000 Ci/mmol.

3.2. *In vitro binding affinities of α-dihydrotetrabenazine isomers*

The two resolved isomers of α-dihydrotetrabenazine were examined for their ability to inhibit the binding of 10 nM (±)-[^{3}H]methoxytetrabenazine, a high affinity ($K_d = 3.94 \pm 0.39$ nM) radioligand for the vesicular monoamine transporter (Vander Borght et al., 1994). The autoradiographic binding assays gave a K_i value of 0.97 ± 0.48 nM for (+)-α-dihydrotetrabenazine, whereas the (−)-isomer was much less effective with a $K_i = 2.2 \pm 0.3$ μM.

3.3. *In vivo brain distributions of α-dihydrotetrabenazine isomers*

In vivo, the highest brain uptake and regional contrast was shown by (+)-α-[^{11}C]dihydrotetrabenazine, with a low and uniform brain distribution shown for the (−)-isomer (Fig. 2A). The racemic mixture showed intermediate values. Differences of radiotracer uptake between groups ((+) vs. (−), (+) vs. (±), and (±) vs. (−)) were significant ($P < 0.001$) in all brain regions examined. Brain retention of (+)-α-[^{11}C]dihydrotetrabenazine was also significantly reduced ($P < 0.001$) in all regions following pretreatment with unlabeled tetrabenazine.

4. Discussion

This represents the first report of the stereospecificity of in vivo binding of a ligand to the vesicular monoamine transporter. In vitro, (+)-α-[^{11}C]dihydrotetrabenazine exhibits a K_i for [^{3}H]methoxytetrabenazine binding to rat brain striatal vesicular monoamine transporters that is > 2000-fold higher than the (−)-isomer. In vivo in mouse brain, (+)-α-[^{11}C]dihydrotetrabenazine shows a regional distribution that correlates ($r = 0.99$; Fig. 2B) with the published distribution of [^{3}H]dihydrotetrabenazine binding sites in mouse brain (Henry and Scherman, 1989). The in vivo retention of (+)-α-[^{11}C]dihydrotetrabenazine is largely due

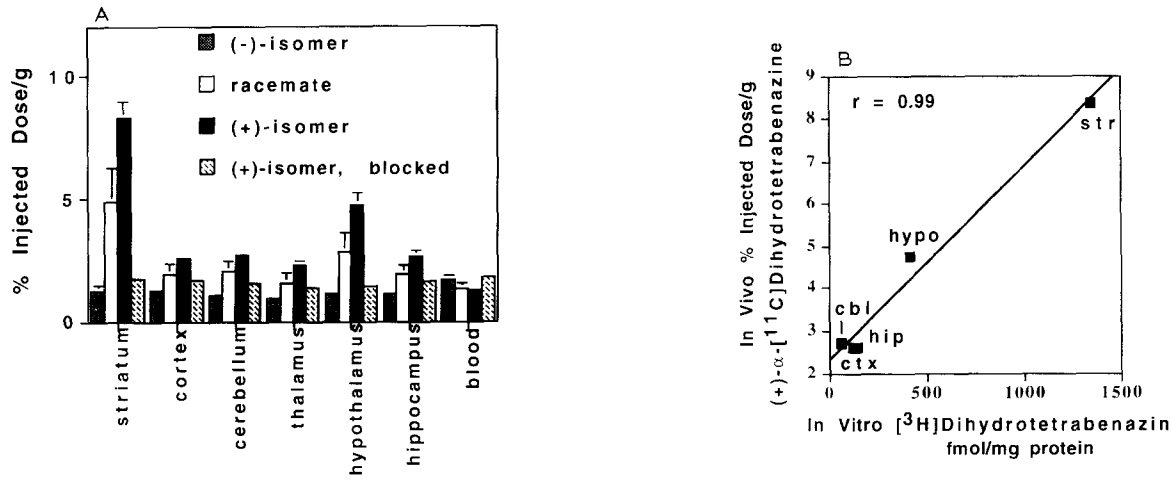

Fig. 2. (A) Regional in vivo mouse brain distribution of isomers of α-[^{11}C]dihydrotetrabenazine. Data shown are tissue radioactivity concentrations (percent injected dose per gram) determined at 15 min after injection; error bars represent one S.D. Data for (+)-α-[^{11}C]dihydrotetrabenazine are shown with and without prior i.p. injection of 10 mg/kg unlabeled tetrabenazine. Radiotracer uptakes of (+)-, (±)- and (−)-α-[^{11}C]dihydrotetrabenazine were significantly ($P < 0.001$) different from each other in all brain regions. Concentrations of radioactivity after (+)-α-[^{11}C]dihydrotetrabenazine injection were significantly reduced ($P < 0.001$) in all regions after tetrabenazine pretreatment (blocked group). (B) Correlation of regional in vitro mouse brain binding of (±)-α-[^{3}H]dihydrotetrabenazine (data from Henry and Scherman (1989)) with the in vivo regional mouse brain distribution of (+)-α-[^{11}C]dihydrotetrabenazine determined 15 min after i.v. injection. Abbreviations: str = striatum, hypo = hypothalamus, cbl = cerebellum, hip = hippocampus, and ctx = cortex.

to binding to the vesicular monoamine transporter, as pretreatment of animals with tetrabenazine results in a uniform low concentration of radioactivity in all brain regions (Fig. 2) with no statistical difference between regions of high and low concentrations of the transporters (striatum vs. cortex, $P = 0.374$). (−)-α-[^{11}C]Dihydrotetrabenazine shows a low and uniform brain distribution which is unaffected by pretreatment with tetrabenazine (data not shown). Finally, comparison of data from positron emission tomography (PET) studies of (±)-α-[^{11}C]dihydrotetrabenazine and (+)-α-[^{11}C]dihydrotetrabenazine in human brains demonstrates the superior striatal uptake and specific to nonspecific binding ratio for the resolved isomer, (+)-α-[^{11}C]dihydrotetrabenazine (Kilbourn, Frey and Koeppe, unpublished results).

That the monoamine vesicular transporter shows stereoselectivity for binding of α-dihydrotetrabenazine is perhaps not surprising, given that most if not all of the receptors and neuronal membrane transporters for the monoamines show stereoselective or stereospecific binding of ligands. Only a few compounds are known as high affinity inhibitors of vesicular monoamine transport: isomeric forms of reserpine and ketanserin, two other inhibitors, have not been evaluated for in vitro binding affinities for the vesicular monoamine transporter. It is not possible at this time to determine if the high stereospecificity of (+)-α-dihydrotetrabenazine binding is inherent in the tertiary structure of the transporter itself, or is a result of the rigid tricyclic nature of the benzoisoquinoline structure. It is interesting, however, that the vesicular monoamine transporter shows such stereospecificity in ligand binding, given its promiscuous nature in transporting a variety on different biogenic amines, including dopamine which is achiral.

In conclusion, the binding of α-dihydrotetrabenazine to the brain vesicular monoamine transporter is stereospecific, with both the in vitro and in vivo biological activity due only to the (+)-isomer.

Acknowledgements

This work was supported by National Institutes of Health grants MH 47611, NS 15655, AG 08671, and T-32-CA09015 (to L.C.L.), and Department of Energy grant DOE-DE-FG021-87ER60561.

References

Brossi, A., L.H. Chopard-dit-Jean and O. Schnider, 1958, Syntheseversuche in der Emetin-Reihe, Helv. Chim. Acta 41, 1793.

DaSilva, J.N., M.R. Kilbourn and T.J. Mangner, 1993a, Synthesis of [^{11}C]tetrabenazine, a vesicular monoamine uptake inhibitor, for PET imaging studies, Appl. Radiat. Isot. 44, 673.

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

Henry, J.-P. and D. Scherman, 1989, Radioligands of the vesicular monoamine transporter and their use as markers of monoamine storage vesicles, Biochem. Pharmacol. 38, 2395.

Kilbourn, M.R., J.N. DaSilva, K.A. Frey, R.A. Koeppe and D.E. Kuhl, 1993, In vivo imaging of vesicular monoamine transporters in human brain using [^{11}C]tetrabenazine and positron emission tomography, J. Neurochem. 60, 2315.

Munson, P.J. and D. Rodbard, 1980, LIGAND: a versatile computerized approach for characterization of ligand-binding system, Anal. Biochem. 107, 220.

Scherman, D., R. Raisman, A. Ploska and Y. Agid, 1988, [^{3}H]Dihydrotetrabenazine, a new in vitro monoaminergic probe for human brain, J. Neurochem. 50, 1131.

Schuldiner, S., 1994, A molecular glimpse of vesicular monoamine transporters, J. Neurochem. 62, 2067.

Schwartz, D.E., H. Bruderer, J. Rieder and A. Brossi, 1966, Metabolic studies of tetrabenazine, Biochem. Pharmacol. 15, 645.

Vander Borght, T.M., T.J. Desmond, M.R. Kilbourn, R.L. Albin, R.A. Koeppe, D.E. Kuhl and K.A. Frey, 1994, Evaluation of methoxytetrabenazine as an in vivo tracer of monoamine nerve terminal integrity, J. Nucl. Med. 35, 121P.