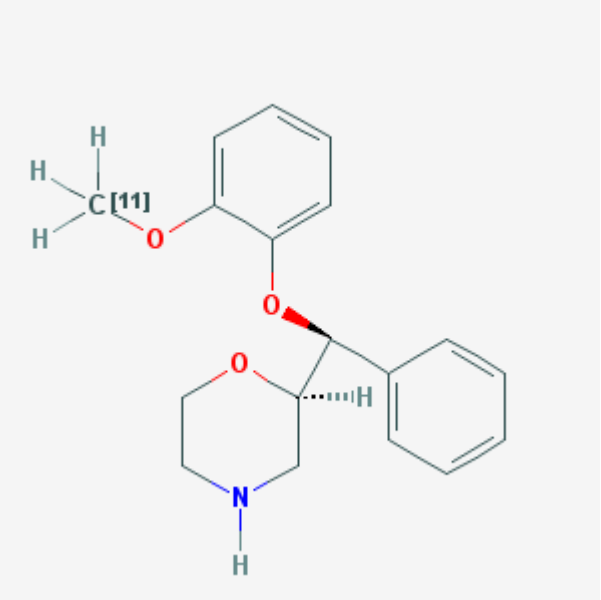
(*S*,*S*)-[^{11}C]Methylreboxetine

(*S*,*S*)-[^{11}C]MRB

The MICAD Research Team

Created: April 19, 2006; Updated: May 8, 2006.

Chemical name:	(*S*,*S*)-[^{11}C]Methylreboxetine
Abbreviated name:	(*S*,*S*)-[^{11}C]MRB
Synonym:	[^{11}C]MeNER, [^{11}C]-*O*-methylreboxetine, (2S, 3S)-[^{11}C]MRB
Agent Category:	Compound
Target:	Brain norepinephrine transporter (NET)
Target Category:	Transporter binding
Method of detection:	PET
Source of signal:	^{11}C
Activation:	No
Studies:	• *In vitro* • Rodents • Non-human primates



Click on the above structure for additional information in PubChem.

Background

[PubMed]

(*S*,*S*)-[^{11}C]Methylreboxetine ((*S*,*S*)-[^{11}C]MRB) is a radioligand developed for positron emission tomography (PET) imaging of the brain adrenergic receptors. It is a derivative of

NLM Citation: The MICAD Research Team. (*S*,*S*)-[^{11}C]Methylreboxetine. 2006 Apr 19 [Updated 2006 May 8]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

reboxetine, a norepinephrine transporter (NET) inhibitor, labeled with ^{11}C, a positron emitter with a physical half-life ($t_{½}$) of 20.4 min (1).

Many diseases affect the sympathetic nervous system (SNS), and imaging of pathologic changes of adrenergic transmission has been an important area of PET research (2, 3). Most postganglionic sympathetic neurons in the autonomic nervous system release the neurotransmitter norepinephrine (NE), which stimulates adrenergic receptors in various effector organs (4). There are different types and subtypes of adrenergic receptors, and they are characterized as α_{1a} to α_{1c}, α_{2a} to α_{2c}, and β_1 to β_3 (5). All of the NE receptors belong to the G-protein-linked receptor superfamily and mediate slow neuromodulatory postsynaptic responses. The NET is a transmembrane protein located in the adrenergic nerve terminals that is responsible for active reuptake (uptake-1) of NE released from neurons (6). NE is stored in the neuronal vesicles and is released on stimulation. Significant expression of NET is found in major organs of the SNS, such as the heart and brain. There is substantial evidence that aberrations in cardiac SNS function contribute to the morbidity and mortality associated with cardiac diseases (7). Brain NETs are involved in various neurologic and psychiatric diseases, including depression, attention deficit hyperactivity disorder, drug addiction, and eating disorders (8). NETs are also the site of action of many antidepressant drugs in the brain (9).

Molecular probes with structures closely related to NE can be used to assess the integrity of presynaptic sympathetic nerve terminals in various diseases. *In vivo* NE synthesis is similar to dopamine synthesis, and dopamine is converted to NE by the enzyme dopamine-β-hydroxylase (5). [^{123}I]-*meta*-Iodobenzylguanidine, [^{11}C]*meta*-hydroxyephedrine, [^{11}C]norepinephrine, and many other radioligands have been developed and used for peripheral neuronal imaging (10). However, this class of tracers is not suitable for the study of brain NET system because they are not able to cross the blood-brain barrier (11). In the brain, NET levels are relatively lower than those of other receptors, such as dopamine transporters (DATs) and serotonin transporters (9). Several NET reuptake inhibitors, for example, [^{11}C]desipramine, have been tested, but they showed high nonspecific binding. Reboxetine ((*RS*)-2-[(*RS*)-2-ethoxyphenoxy)benzyl]morpholine) is a specific NET inhibitor with a high affinity and selectivity (IC_{50} DAT/NET = 4,000). It has been developed in Europe for the treatment of depressive illness. Reboxetine is available as a racemic mixture of the (*R,R*) and (*S,S*) enantiomers. The (S,S) enantiomer has been found to be more potent, with a IC_{50} of 3.6 nM for inhibiting NE uptake in rat hypothalamic synaptosomes. Among the different reboxetine derivatives that have been tested, (*S,S*)-MRB has an IC_{50} of 2.5 nM ((*R,R*)-MRB has an IC_{50} of 85 nM) and is considered a promising candidate to be developed as a PET ligand for studying the brain NET system.

Synthesis

[PubMed]

Based on the report of Melloni et al. (12) on the synthesis and activity of reboxetine, Wilson et al. (9) first published the radiosynthesis of (*S*,*S*)-[^{11}C]MRB by ^{11}C methylation of the normethyl precursor, (*S*,*S*)-2-(morpholin-2-yl-phenyl-methoxy)-phenol, in a high-performance liquid chromatography (HPLC) sample loop method. [^{11}C]Iodomethane was reacted with the precursor, which had been treated with methanolic tetrabutylammonium hydroxide. Radiochemical yields were 25-40% (uncorrected, based on [^{11}C]iodomethane trapped), and the synthesis time was 25 min from the end of bombardment (EOB). After HPLC purification and filtration through a 0.22 µm filter, the final product was >98% radiochemically pure and >97% optically pure. The specific activity was 35-65 GBq (0.95-1.76 Ci)/µmol.

Lin and Ding (13) developed a nine-step synthetic procedure to prepare the racemic precursor, (2*S*,3*S*)/(2*R*,3*R*)-2-[α-(2-hydroxphenoxy)benzyl]-morpholine ((2*S*,3*R*)-desethylreboxetine). This procedure started with catechol monoprotected with a methanesulfonyl group. The protecting group was removed by use of excess hydride reducing agent at the end of the procedure. The racemic precursor was resolved by chiral HPLC into >98% enantiomerically pure individual precursors. Selective ^{11}C O-methylation was carried out by reacting (2*S*, 3*S*)-desethylreboxetine in dimethylformamide and excess sodium hydroxide with [^{11}C]methyl iodide at 100 °C for 10 min. At the end of the reaction, the product was purified by HPLC. Radiochemical yields were 61-74% (decay-corrected from [^{11}C]methyl iodide), and the synthesis time was 40 min from EOB. Radiochemical purity was >96%, and the specific activity was 62.9-85.1 GBq (1.7-2.3 Ci)/µmol at EOB.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Wilson et al. (9) measured the partition coefficient (P) of (*S*,*S*)-[^{11}C]MRB and determined that the log P between 1-octanol and 0.02 M phosphate buffer at pH 7.4 was 2.35 (± 0.06; n = 12). *In vitro* incubation of slide-mounted rat brain coronal sections with 10 nM (*S*,*S*)-[^{11}C]MRB for 10 min produced a regional distribution pattern that was similar to patterns of [^{3}H]nisoxetine, an NET uptake inhibitor. High amounts of radioactivity were found in the anteroventral nucleus of the thalamus, the bed nucleus of the stria terminalis, the paraventricular nucleus of the hypothalamus, and the locus coeruleus and subcoeruleus areas. Co-incubation with 100 µM desipramine (NE reuptake inhibitor) abolished binding in all studies.

Ding et al. (11) reported that the log P of (*S*,*S*)-[^{11}C]MRB was 1.17 (between octanol and 0.1 M phosphate buffer). Human plasma protein binding of (*S*,*S*)-[^{11}C]MRB was determined to be 14%.

Animal Studies

Rodents

[PubMed]

Biodistribution and metabolism studies of (*S*,*S*)-[^{11}C]MRB in rats were conducted by Wilson et al. (9). Each rat received (*S*,*S*)-[^{11}C]MRB (8-33 MBq (0.22-0.89 mCi)/1-2 nmole) by i.v. injection. Rats were killed by decapitation at various time intervals after injection. Brain uptake was moderate (0.53% of injected dose (ID) at 5 min) with a slow washout of radioactivity in NET-rich brain regions (hypothalamus and cortex) and a faster washout in NET-poor regions (striatum). A hypothalamus/striatum ratio of 2.5 was reached at 60 min. Pretreatment of rats with reboxetine or desipramine resulted in no specific retention of radioactivity in the hypothalamus or other NET-rich regions. HPLC analysis of serum and brain samples showed that (*S*,*S*)-[^{11}C]MRB was rapidly metabolized in the blood. Only 50% of (*S*,*S*)-[^{11}C]MRB remained unchanged at 30 min and 20% after 60 min. In the brain, >95% of radioactivity was unchanged (*S*,*S*)-[^{11}C]MRB. All radioactive metabolites were found to be hydrophilic.

Ding et al. (11) studied (*S*,*S*)-[^{11}C]MRB metabolism in mice by radio-HPLC of brain tissue obtained at 10 min after injection. They determined that >95% of radioactivity was unmetabolized (*S*,*S*)-[^{11}C]MRB.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

Ding et al. (11, 14) evaluated (*S*,*S*)-[^{11}C]MRB as a radioligand for PET imaging studies of brain NET systems in baboons. The average specific activity at the time of injection was ~18.5-55.5 GBq (0.5-1.5 Ci)/μmol. The radiochemical purity was >98%, and enantiomeric purity was 99%. A dose of (*S*,*S*)-[^{11}C]MRB (74-111 MBq (2-3mCi)) was administered i.v. to three adult female baboons. Two doses were used when a comparison or blocking study was conducted. The first dose was used for the baseline PET study, and the second dose was administered 2-3 h after the first dose for the pretreatment study with desipramine. Imaging studies showed moderate brain uptake with a peak brain uptake of 3.2-4% ID. Clearance of radioactivity in NET-rich regions (thalamus and cerebellum) was slow, whereas washout was faster in striatum and cortical regions. The $t_{½}$ for clearance from peak uptake in the thalamus was ~250 min. The ratio of the distribution volume (DV) in the NET-rich regions to the DV in the NET-poor regions was 1.8-2.2. Pretreatment with nisoxetine (1 mg/kg) markedly reduced the uptakes by ~50% in the thalamus and ~40% in the cerebellum, but not in the striatum. This showed the saturable and specific binding of

(*S*,*S*)-[^{11}C]MRB to NETs in the brain. However, there was also a relatively high striatum uptake because of possible nonspecific binding. Pretreatment with desipramine also led to reduced heart radioactivity uptake. In comparison, (*R*,*R*)-[^{11}C]MRB showed similar radioactivity uptakes and similar clearance kinetics in all baboon brain regions, and there was no nisoxetine blocking effect on binding. HPLC analysis of baboon plasma showed that the fractions of unchanged (*S*,*S*)-[^{11}C]MRB were 98, 59, 41, 39, and 33% at 1, 5, 10, 30, and 60 min after administration, respectively. Only polar metabolites were detected in the plasma samples.

Human Studies

[PubMed]

No publication is currently available.

References

1. Rosenspire KC, Haka MS, Van Dort ME, Jewett DM, Gildersleeve DL, Schwaiger M, Wieland DM. Synthesis and preliminary evaluation of carbon-11-meta-hydroxyephedrine: a false transmitter agent for heart neuronal imaging. J Nucl Med. 1990;31(8):1328–1334. PubMed PMID: 2384800.
2. Konishi J., Dwamena B.A., Gross M.D., Shapiro B., Misaki T., Fukunaga M., Sisson J.C., Oei H.-Y., De Jong M., Krenning E.P. *Endocrinology, in Molecular Nuclear Medicine*. L.E. Feinendegen, W.W. Shreeve, W.C. Eckelman, Y.-W. Bahk, and H.N. Wagner Jr., Editor. 2003, Springer: New York. p. 357-409.
3. Antoni G., Kihlberg T., Langstrom B. *Aspects on the synthesis of 11C-Labelled compounds*, in Handbook of Radiopharmaceuticals, M.J. Welch, and C.S. Redvanly, Editor. 2003, John Wiley & Sons Ltd.: West Sussex, England. p. 141-194.
4. Sunderland P.M. *Pathophysiology. The Biologic basis for disease in adults and children*, K.L. McCance, and S. E. Huether, Editor. 1994, Mosby-Year Books, Inc.: St, Louis. p. 397-436.
5. Frey K.A. *PET study of neurochemical systems*, in *Positron Emission Tomography*, P.E. Valk, D.L. Bailey, D.W. Townsend, and M.N. Maisey, Editors. 2002, Springer London. p. 309-327.
6. Buursma AR, Beerens AM, de Vries EF, van Waarde A, Rots MG, Hospers GA, Vaalburg W, Haisma HJ. The Human Norepinephrine Transporter in Combination with 11C-m-Hydroxyephedrine as a Reporter Gene/Reporter Probe for PET of Gene Therapy. J Nucl Med. 2005;46(12):2068–2075. PubMed PMID: 16330572.
7. Caldwell JH, Kroll K, Li Z, Seymour K, Link JM, Krohn KA. Quantitation of presynaptic cardiac sympathetic function with carbon-11-meta-hydroxyephedrine. J Nucl Med. 1998;39(8):1327–1334. PubMed PMID: 9708501.
8. Zahniser NR, Doolen S. Chronic and acute regulation of Na+/Cl- -dependent neurotransmitter transporters: drugs, substrates, presynaptic receptors, and signaling systems. Pharmacol Ther. 2001;92(1):21–55. PubMed PMID: 11750035.

9. Wilson AA, Johnson DP, Mozley D, Hussey D, Ginovart N, Nobrega J, Garcia A, Meyer J, Houle S. Synthesis and in vivo evaluation of novel radiotracers for the in vivo imaging of the norepinephrine transporter. Nucl Med Biol. 2003;30(2):85–92. PubMed PMID: 12623106.
10. Langer O, Halldin C. PET and SPET tracers for mapping the cardiac nervous system. Eur J Nucl Med Mol Imaging. 2002;29(3):416–434. PubMed PMID: 12002720.
11. Ding YS, Lin KS, Logan J, Benveniste H, Carter P. Comparative evaluation of positron emission tomography radiotracers for imaging the norepinephrine transporter: (S,S) and (R,R) enantiomers of reboxetine analogs ([11C]methylreboxetine, 3-Cl-[11C]methylreboxetine and [18F]fluororeboxetine), (R)-[11C]nisoxetine, [11C]oxaprotiline and [11C]lortalamine. J Neurochem. 2005;94(2):337–351. PubMed PMID: 15998285.
12. Melloni P, Carniel G, Della Torre A, Bonsignori A, Buonamici M, Pozzi O, Ricciardi O, Rocci AC. Potential antidepressant agents, alpha-Aryloxy-benzyl derivatives of ethanolamine and morpholine. Eur J Med Chem. 1984;19:235–242.
13. Lin KS, Ding YS. Synthesis, enantiomeric resolution, and selective C-11 methylation of a highly selective radioligand for imaging the norepinephrine transporter with positron emission tomography. Chirality. 2004;16(7):475–481. PubMed PMID: 15236345.
14. Ding YS, Lin KS, Garza V, Carter P, Alexoff D, Logan J, Shea C, Xu Y, King P. Evaluation of a new norepinephrine transporter PET ligand in baboons, both in brain and peripheral organs. Synapse. 2003;50(4):345–352. PubMed PMID: 14556239.