Published in final edited form as:

Brain Res. 2013 April 24; 1507: 11–18. doi:10.1016/j.brainres.2013.02.035.

Autoradiographic evaluation of [³H]CUMI-101, a novel, selective 5-HT_{1A}R ligand in human and baboon brain

J. S. Dileep Kumar^{a,b,*}, Ramin V. Parsey^c, Suham A. Kassir^a, Vattoly J. Majo^b, Matthew S. Milak^{a,b}, Jaya Prabhakaran^b, Mark D. Underwood^{a,b}, J. John Mann^{a,b,d}, and Victoria Arango^{a,b}

^aDivision of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, New York, USA

^bDepartment of Psychiatry, Columbia University College of Physicians and Surgeons, New York, USA

^cDepartment of Psychiatry and Behavioral Sciences, Stony Brook University, Stony Brook, NY

^dDepartment of Radiology, Columbia University College of Physicians and Surgeons, New York, NY 10032

Abstract

[¹¹C]CUMI-101 is the first selective serotonin receptor (5-HT_{1A}R) partial agonist radiotracer for positron emission tomography (PET) tested *in vivo* in nonhuman primates and humans. We evaluated specific binding of [³H]CUMI-101 by quantitative autoradiography studies in postmortem baboon and human brain sections using the 5-HT_{1A}R antagonist WAY100635 as a displacer. The regional and laminar distributions of [³H]CUMI-101 binding in baboon and human brain sections matched the known distribution of [³H]8-OH-DPAT and [³H]WAY100635. Prazosin did not measurably displace [³H]CUMI-101 binding in baboon or human brain sections, thereby ruling out [³H]CUMI-101 binding to α₁-adrenergic receptors. This study demonstrates that [¹¹C]CUMI-101 is a selective 5-HT_{1A}R ligand for *in vivo* and *in vitro* studies in baboon and human brain.

Keywords

5-HT_{1A}R; brain; serotonin agonist; autoradiography

1. Introduction

Serotonin_{1A} receptors (5-HT_{1A}R) have been implicated in the pathophysiology of mood and anxiety disorders, suicidal behavior, sexual functions, eating and panic disorders, epilepsy, schizophrenia, Parkinson's disease and Alzheimer's disease as well as in the mechanism of

*Corresponding author J. S. Dileep Kumar, Ph.D., Department of Psychiatry & Division of Molecular Imaging and Neuropathology, Columbia University & New York State Psychiatric Institute, 1051 Riverside Drive, Box #42, New York, NY 10032 Telephone: (212) 543-1163 Fax: (212) 543-1054, kumardi@nyspi.columbia.edu.

action of antidepressants (Akimova et al., 2009; Drago et al., 2008; Elliott et al., 2009; Giovacchini et al., 2009; Munoz et al., 2009; Richardson-Jones et al., 2010).

There are several radioligands for the *in vivo* and *in vitro* quantification of 5-HT_{1A}R. Most tracers belong to two structural families: (i) compounds with structural similarity to the 5-HT_{1A} antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexane carboxamide (e.g. WAY-100635); or (ii) derivatives of the 5-HT_{1A} agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Kumar and Mann, 2007; Pike et al., 2001). Among these, [³H] or [¹¹C] labeled WAY-100635 has been the most commonly used 5-HT_{1A}R antagonist ligand (Assem-Hilger et al., 2010; Saijo et al., 2010; Sargent et al., 2010; Stein et al., 2008). WAY-100635 has more than 100-fold selectivity for 5-HT_{1A}R *versus* other receptors except for 5-HT_{2B}, adrenergic α_{1A} and dopamine D₄ receptors (Chemel et al., 2006). The discovery of (±)8-OH-DPAT as a 5-HT_{1A}R agonist revolutionized 5-HT_{1A}R research (Duncan et al., 1998; Simpson et al., 1996; Trillat et al., 1998). Although additional radioligands have been reported over the years, [³H]8-OH DPAT remains the most widely used agonist radioligand for *in vitro* quantification of 5-HT_{1A}R sites, despite its moderate affinity for some non-5-HT_{1A} targets including 5-HT₇R, 5-HT_{1B/D}R, α₂-adrenergic receptor (α₂-AR), D₂-like receptors and the serotonin transporter (Heusler et al., 2010; Nenonene et al., 1994; Sprouse et al., 2004). As a result, [³H]8-OH-DPAT binding in 5-HT_{1A}R knockout mice has detectable binding to 5-HT₇R and α₂-AR. [³H]8-OH-DPAT also binds to serotonin transporters in striatum and the raphe nuclei (Assié and Koek, 2000; Schoemaker and Langer, 1986). *In vivo*, 8-OH-DPAT and its derivatives have failed as PET tracers due to poor blood brain barrier (BBB) penetration or high nonspecific binding (Kumar and Mann, 2007). *Thus, a reliable comparison of in vitro vs. in vivo distribution of 5-HT_{1A}R binding sites is not possible due to the lack of a ligand works both in vivo and in vitro.*

We have developed [*O*-Methyl-¹¹C]2-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-4-methyl-1,2,4-triazine-3,5(2*H*,4*H*)dione ([¹¹C]CUMI-101; Figure 1) and reported its properties as a specific 5-HT_{1A}R partial agonist PET tracer in human and nonhuman primates (Kumar et al., 2012; Kumar et al., 2011; Kumar et al., 2007; Milak et al., 2008; Milak et al., 2010; Milak et al., 2011). Compared to 8-OH-DPAT, CUMI-101 has higher affinity (*K_i*= 0.15 nM), better selectivity for 5-HT_{1A}R, and it is a partial agonist (*E_{max}* = 88%; *EC*₅₀ = 0.1 nM, Kumar et al., 2007). It therefore has the potential to be both a PET tracer and when tritiated, a ligand for *in vitro* studies. This would allow for direct comparison of *in vitro* and *in vivo* findings with the same ligand.

A detailed study of the characteristics of CUMI-101 as a 5-HT_{1A}R ligand *in vitro* has not been published. We therefore carried out such a study using [³H]CUMI-101. We believe, that in addition to having a superior pharmacological profile, [³H]CUMI-101 offers several other advantages over [³H]8-OH-DPAT including: 1. ease of synthesis; 2. minimal autoradiolysis; 3. possibility of measurement of endogenous competition with intrasynaptic serotonin; 4. comparison of *in vivo* human/animal data obtained from PET studies and *in vitro* postmortem data in human/animal brain using [¹¹C]CUMI-101 and [³H]CUMI-101 respectively; 5. quantification of the high affinity (HA) state of the receptor; and 6. a useful tool for agonist drug development and pharmacology studies based on 5-HT_{1A}R. Herein we

report quantitative receptor autoradiography data of regional, specific binding of [^3H]CUMI-101 to postmortem human and baboon brain sections, as well as the absence of $\alpha 1$ -adrenergic receptor ($\alpha 1$ -AR) binding.

2. RESULTS

Binding of [^3H]CUMI-101 to baboon brain

The regional distribution of [^3H]CUMI-101 binding is shown in Figure 2. [^3H]CUMI-101 binding to 5-HT_{1A} sites varied across brain regions. The highest binding density was found in the pyramidal layer of CA1 of the hippocampus (86.0 ± 1.8 fmol/mg tissue), followed by the molecular layer of the dentate gyrus (47.3 ± 0.8 fmol/mg tissue), layer II of prefrontal cortex (45.2 ± 5.1 fmol/mg tissue) and most other neocortical areas, including the insular cortex (41.1 ± 1.9 fmol/mg tissue). Other regions had less binding: the total thickness of gray matter (when all 6 cortical layers are measured together) in frontal cortex (28.1 ± 1.8 fmol/mg tissue), claustrum (23.7 ± 3.9 fmol/mg tissue), amygdala (20.4 ± 3.1 fmol/mg tissue), striatum (7.7 ± 3.0 fmol/mg tissue), thalamus (3.1 ± 3.1 fmol/mg tissue) and cerebellum (1.4 ± 1.2 fmol/mg tissue).

We also carried out autoradiography studies of [^3H]8-OH-DPAT in baboon brain from the same animal and the results are quite comparable to [^3H]CUMI-101 binding in the tested regions (Figures 2 and 4). Figure 5 shows the high correlation of binding of the two ligands across brain regions ($R^2=0.94$). In terms of relative levels of binding, CA1 pyramidal layer, DG-molecular layer and insular layer II have comparable binding for both radioligands, but [^3H]CUMI-101 has higher binding than [^3H]8-OH-DPAT in the other tested brain regions.

Binding of [^3H]CUMI-101 to postmortem human brain

Figure 6 shows autoradiograms of [^3H]CUMI-101 binding to human brain sections. As in the baboon brain, binding is highest in pyramidal layer of CA1 (96.5 ± 1.6 fmol/mg tissue), followed by molecular layer of DG (43.3 ± 5.7 fmol/mg tissue), layer II of insula (44.5 ± 3.0 fmol/mg tissue) layer II of prefrontal cortex (39.7 ± 1.3 fmol/mg tissue) and claustrum (25.5 ± 2.5 fmol/mg tissue). Amygdala has intermediate levels of binding (8.1 ± 1.3 fmol/mg tissue). The cerebellar vermis (2.0 ± 0.4 fmol/mg tissue) and cerebellar cortex have lower binding (0.65 ± 0.9 fmol/mg tissue), whereas, thalamus (1.4 ± 0.4 fmol/mg tissue) and striatum (0.5 ± 0.3 fmol/mg tissue) have the least tracer binding (Figure 7). The specific binding of [^3H]CUMI-101 was determined by 1 μM WAY100635, and non-specific binding in all areas is low (<10%). Autoradiography results in human brain sections of the same subjects using [^3H]8-OH-DPAT are comparable with [^3H]CUMI-101 binding. Figure 8 shows correlation of [^3H]8-OH-DPAT and [^3H]CUMI-101 binding in tested brain regions ($R^2=0.92$). Both radioligands show comparable binding in CA1 pyramidal layer, insular layer II and claustrum. [^3H]CUMI-101 shows higher binding in DG-molecular layer, whereas, [^3H]8-OH-DPAT shows higher binding in PFC-layer II and amygdala.

α -1AR binding of [^3H]CUMI-101 in postmortem baboon and human brain

CUMI-101 binding affinity for $\alpha 1$ -AR ($K_i = 6.75$ nM) is 45 times less than the affinity for the 5-HT_{1A}R ($K_i = 0.15$ nM) (Kumar et al, 2006). Nevertheless, we determined the

proportion of binding across baboon brain regions to $\alpha 1$ -AR by autoradiography using 1 μ M of prazosin to displace [3 H]CUMI-101 binding (Figure 3). There was no detectable $\alpha 1$ -AR binding in brain regions with reportedly abundant $\alpha 1$ -AR such as thalamus, cortical regions, striatum, amygdala, and cerebellum, suggesting that [3 H]CUMI-101 does not bind to $\alpha 1$ -AR. The molecular layer of the dentate gyrus showed the highest displacement with prazosin corresponding to a binding of 2.7 ± 4 fmol/mg tissue (Figure 3). In agreement with baboon data, prazosin (1 μ M) on human brain sections did not result in any statistically significant reduction of [3 H]CUMI-101 binding, indicating no measurable specific binding to $\alpha 1$ -AR (Figure 7). However, in contrast with baboon, the molecular layer of the human dentate gyrus shows 6.5 fmol/mg tissue binding in prazosin displacement studies.

3. DISCUSSION

CUMI-101 is a high affinity partial agonist for 5-HT_{1A}R and its C-11 analogue is the only PET ligand currently available for the *in vivo* quantification of high affinity 5-HT_{1A}R in human and nonhuman primates (Kumar et al., 2009, Hines et al., 2011; Milak et al., 2010; Milak et al., 2011, Selvaraj et al. 2012, Kumar et al. 2012). The present study examined the binding characteristics of [3 H]CUMI-101 in postmortem brain sections of human and baboon using quantitative autoradiography and found the pattern of relative regional brain radioligand binding in the tested regions is comparable to previously reported binding of [3 H]8-OH-DPAT and [3 H]WAY100635 (Burnet et al., 1997). Moreover, the *in vitro* data obtained for [3 H]CUMI-101 in the tested brain regions are in agreement with *in vivo* data obtained for [11 C]CUMI-101 in human (Milak et al, 2010) with PET. The highest *in vivo* binding for [11 C]CUMI-101 in human was found in entorhinal cortex, followed by hippocampus, and cortical regions which is similar to *in vivo* findings in baboon. We find that the striatum, thalamus and cerebellum show the least *in vitro* binding, and this is also the case for *in vivo* binding in human (Milak et al, 2010).

We examined whether [3 H]CUMI-101 binds to $\alpha 1$ -AR in baboon and human brain by evaluating binding of [3 H]CUMI-101 in human and baboon brain *in vitro* and found 1 μ M of prazosin did not cause statistically significant blockade of binding in the tested brain regions except in the DG-molecular layer of the hippocampus where a small level of binding was detected. Prazosin did not displace [3 H]CUMI-101 binding in $\alpha 1$ -AR rich areas such as claustrum, thalamus, cerebral cortex, striatum or cerebellum. Hence the DG-molecular layer binding based on prazosin displacement is not likely due to $\alpha 1$ -AR binding. Since the $\alpha 1$ -AR antagonist prazosin produces cardiovascular changes in monkeys even at a 0.01 mg/kg dose (Schindler et al., 1992), we did not perform blocking studies *in vivo* in baboons to test the $\alpha 1$ -AR selectivity of [11 C]CUMI-101 as cardiovascular changes themselves can alter the binding outcome with PET (Ohira et al., 2011), and larger doses of prazosin could threaten the health of baboons under anesthesia. One *in vivo* study in rodents and monkeys with [11 C]CUMI-101 reported binding in thalamus and cerebellum, which was partially displaced with prazosin; that binding was attributed to $\alpha 1$ -AR and 5HT_{1A}R binding in these brain regions in these particular species (Liow et al, 2010). It has been reported that brain $\alpha 1$ -AR regional distribution in mammals is species dependent (Szot et al 2005, Palacios et al. 1987). As described above, our studies indicate no significant $\alpha 1$ -AR binding in the tested brain

regions in either baboon or human, and perhaps species differences are the explanation for the discrepancies with the literature.

The high specific/nonspecific binding ratio of [^3H]CUMI-101 in all brain regions tested in human and baboon compared favorably with [^3H]8-OH-DPAT, indicating CUMI-101 is an alternative ligand for *in vitro* studies. This would also allow for results from *in vitro* studies with [^3H]CUMI-101 to be more directly comparable to *in vivo* PET studies with [^{11}C]CUMI-101. The minimal binding to α_1 -AR in baboon and human brain indicates [^3H]CUMI-101 is a sensitive tool for *in vitro* and [^{11}C]CUMI-101 for *in vivo* studies in baboon and human to quantify high affinity 5-HT $_1$ AR in the central nervous system.

4. Materials and Methods

The use of human samples for this study were approved by the Institutional Review Board of the New York State Psychiatric Institute (NYSPI) and were determined to be non-human subjects research. Postmortem sections from two nonpsychiatric control human brains (20 μm) that included prefrontal cortex, striatum, amygdala, hippocampus, cerebellum and thalamus were used. Baboon brain (20 μm) sections from prefrontal, midcallosal and cerebellum anatomical levels containing the regions of interest were used for *in vitro* studies. [^3H]CUMI-101 (NIMH Code: M-906) was obtained from the NIMH drug supply program. [^3H]8-OH-DPAT was purchased from Sigma Life Science. Prazosin hydrochloride and WAY100635 maleate were purchased from Tocris and RBI, respectively.

Tissue sections were incubated in Tris-Sucrose buffer (pH 7.6) containing 170mM Tris-HCl, 4mM CaCl $_2$, 138 mM sucrose 2 nM ^3H -CUMI-101 (Specific Activity 77.7 Ci/mmol) for 60 min at 37°C. Adjacent sections were incubated with 2 nM ^3H -CUMI and 1 μm prazosin or 1 μm WAY 100635 to determine specific binding to α_1 -AR and 5-HT $_1$ ARs, respectively. Sections were washed for 10 minutes (2 X 5) minutes in Tris-Sucrose buffer at 4°C followed by a dip in cold distilled H $_2$ O to remove buffer salts. Sections were then dried under a stream of desiccated cold air, dried and exposed to 4% paraformaldehyde vapor over night. Slides were exposed to tritium-sensitive screen (Fuji) for three days, alongside ^3H -containing polymer standards (ART-123 and -123A from American Radiolabeled Chemicals, Inc.) and developed using a Packard Cyclone phosphor imager. All slides were laid out in X-ray film cassettes and exposed to Biomax MS film (Kodak) for 6 weeks, developed using Kodak D-19 developer and fixative. The autoradiograms were sampled using a computer-based image analysis system (MCID, Imaging Research Inc., St. Catharines, Ontario, Canada) as previously described (Arango et al., 1993; Arango et al., 1995; Boldrini et al., 2008). All the sections were corrected for light transmission inhomogeneities and the known nCi/mg values from the standards slides were half-life corrected, and converted to femtomoles of radioligand per unit mass of tissue.

References

- Akimova E, Lanzenberger R, Kasper S. The serotonin-1A receptor in anxiety disorders. *Biol Psychiatry*. 2009; 66:627–35. [PubMed: 19423077]

- Arango V, Ernsberger P, Sved AF, Mann JJ. Quantitative autoradiography of alpha 1- and alpha 2-adrenergic receptors in the cerebral cortex of controls and suicide victims. *Brain Res.* 1993; 630:271–82. [PubMed: 8118693]
- Arango V, Underwood MD, Gubbi AV, Mann JJ. Localized alterations in pre- and postsynaptic serotonin binding sites in the ventrolateral prefrontal cortex of suicide victims. *Brain Res.* 1995; 688:121–33. [PubMed: 8542298]
- Assem-Hilger E, Lanzenberger R, Savli M, Wadsak W, Mitterhauser M, Mien LK, Stogmann E, Baumgartner C, Kletter K, Asenbaum S. Central serotonin 1A receptor binding in temporal lobe epilepsy: a [carbonyl-(11)C]WAY-100635 PET study. *Epilepsy Behav.* 2010; 19:467–73. [PubMed: 20850389]
- Assié M-B, Koek W. [3H]-8-OH-DPAT binding in the rat brain raphe area: involvement of 5-HT1A and non-5-HT1A receptors. *Br J Pharmacol.* 2000; 130:1348–1352. [PubMed: 10903975]
- Boldrini M, Underwood MD, Mann JJ, Arango V. Serotonin-1A autoreceptor binding in the dorsal raphe nucleus of depressed suicides. *J Psychiatr Res.* 2008; 42:433–42. [PubMed: 17574270]
- Burnet PW, Eastwood SL, Harrison PJ. [3H]WAY-100635 for 5-HT1A receptor autoradiography in human brain: a comparison with [3H]8-OH-DPAT and demonstration of increased binding in the frontal cortex in schizophrenia. *Neurochem Int.* 1997; 30:565–74. [PubMed: 9152998]
- Chemel BR, Roth BL, Armbruster B, Watts VJ, Nichols DE. WAY-100635 is a potent dopamine D4 receptor agonist. *Psychopharmacology (Berl).* 2006; 188:244–51. [PubMed: 16915381]
- Drago A, Ronchi DD, Serretti A. 5-HT1A gene variants and psychiatric disorders: a review of current literature and selection of SNPs for future studies. *Int J Neuropsychopharmacol.* 2008; 11:701–21. [PubMed: 18047755]
- Duncan GE, Knapp DJ, Breese GR, Crews FT, Little KY. Species differences in regional patterns of 3H-8-OH-DPAT and 3H-zolpidem binding in the rat and human brain. *Pharmacol Biochem Behav.* 1998; 60:439–48. [PubMed: 9632227]
- Elliott MS, Ballard CG, Kalaria RN, Perry R, Hortobagyi T, Francis PT. Increased binding to 5-HT1A and 5-HT2A receptors is associated with large vessel infarction and relative preservation of cognition. *Brain.* 2009; 132:1858–65. [PubMed: 19433439]
- Giovacchini G, Conant S, Herscovitch P, Theodore WH. Using cerebral white matter for estimation of nondisplaceable binding of 5-HT1A receptors in temporal lobe epilepsy. *J Nucl Med.* 2009; 50:1794–800. [PubMed: 19837769]
- Gozlan H, Thibault S, Laporte AM, Lima L, Hamon M. The selective 5-HT1A antagonist radioligand [3H]WAY 100635 labels both G-protein-coupled and free 5-HT1A receptors in rat brain membranes. *Eur J Pharmacol.* 1995; 288:173–86. [PubMed: 7720779]
- Hall H, Lundkvist C, Halldin C, Farde L, Pike VW, McCarron JA, Fletcher A, Cliffe IA, Barf T, Wikstrom H, Sedvall G. Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]WAY-100635. *Brain Res.* 1997; 745:96–108. [PubMed: 9037397]
- Hamon M, Gozlan H, el Mestikawy S, Emerit MB, Bolanos F, Schechter L. The central 5-HT1A receptors: pharmacological, biochemical, functional, and regulatory properties. *Ann N Y Acad Sci.* 1990; 600:114–29. discussion 129–31. [PubMed: 2252305]
- Hendry N, Christie I, Rabiner EA, Laruelle M, Watson J. In vitro assessment of the agonist properties of the novel 5-HT1A receptor ligand, CUMI-101 (MMP), in rat brain tissue. *Nucl Med Biol.* 2011; 38:273–7. [PubMed: 21315283]
- Heusler P, Raully-Lestienne I, Tourette A, Tardif S, Ailhaud MC, Croville G, Cussac D. Actions of the prototypical 5-HT1A receptor agonist 8-OH-DPAT at human alpha2-adrenoceptors: (+)8-OH-DPAT, but not (-)8-OH-DPAT is an alpha2B subtype preferential agonist. *Eur J Pharmacol.* 2010; 640:8–14. [PubMed: 20450907]
- Hines CS, Liow JS, Zanolli-Fregonara P, Hirvonen J, Morse C, Pike VW, Innis RB. Human biodistribution and dosimetry of (1)(1)C-CUMI-101, an agonist radioligand for serotonin-1a receptors in brain. *PLoS One.* 2011; 6:e25309. [PubMed: 21980419]
- Liow J-S, Lu S, Zoghbi S, Shrestha S, Gladding R, Morse C, Hirvonen J, Parsey R, Pike V, Innis R. Selectivity in rodents and monkeys of 11C-CUMI-101, an agonist radioligand for serotonin 5-HT1A receptors. *J Nucl Med.* 2010; 51:164.

- Khawaja X. Quantitative autoradiographic characterisation of the binding of [3H]WAY-100635, a selective 5-HT_{1A} receptor antagonist. *Brain Res.* 1995; 673:217–25. [PubMed: 7606435]
- Kumar JSD, Milak MS, Majo VJ, Prabhakaran J, Pratap M, Savenkova L, Mann JJ, Parsey RV. Comparison of high and low affinity serotonin 1A receptors by PET in vivo in nonhuman primates. *J Pharmacol Sci.* 2012; 120:254–57. [PubMed: 23076129]
- Kumar JSD, Bai B, Ng HH, Mirsalis JC, Erlandsson K, Milak MS, Majo VJ, Prabhakaran J, Mann JJ, Parsey RV. Biodistribution, toxicology, and radiation dosimetry of 5-HT_{1A}-receptor agonist positron emission tomography ligand [11C]CUMI-101. *Int J Toxicol.* 2011; 30:611–8. [PubMed: 21994241]
- Kumar JS, Majo VJ, Hsiung SC, Millak MS, Liu KP, Tamir H, Prabhakaran J, Simpson NR, Van Heertum RL, Mann JJ, Parsey RV. Synthesis and in vivo validation of [O-methyl-11C]2-{4-[4-(7-methoxynaphthalen-1-yl)piperazin-1-yl]butyl}-4-methyl-2H-[1,2,4]triazine-3,5-dione: a novel 5-HT_{1A} receptor agonist positron emission tomography ligand. *J Med Chem.* 2006; 49:125–34. [PubMed: 16392798]
- Kumar JS, Mann JJ. PET tracers for 5-HT(1A) receptors and uses thereof. *Drug discovery today.* 2007; 12:748–56. [PubMed: 17826688]
- Kumar JS, Prabhakaran J, Majo VJ, Milak MS, Hsiung SC, Tamir H, Simpson NR, Van Heertum RL, Mann JJ, Parsey RV. Synthesis and in vivo evaluation of a novel 5-HT_{1A} receptor agonist radioligand [O-methyl-11C]2-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-4-methyl-1,2,4-triazine-3,5(2H,4H)dione in nonhuman primates. *Eur J Nucl Med Mol Imaging.* 2007; 34:1050–60. [PubMed: 17221184]
- Michel MC, Hanft G, Grob G. Radioligand binding studies of α 1-adrenoceptor subtypes in rat heart. *Br J Pharmacol.* 1994; 111:533–38. [PubMed: 7911718]
- Milak MS, Severance AJ, Ogden RT, Prabhakaran J, Kumar JS, Majo VJ, Mann JJ, Parsey RV. Modeling considerations for 11C-CUMI-101, an agonist radiotracer for imaging serotonin 1A receptor in vivo with PET. *J Nucl Med.* 2008; 49:587–96. [PubMed: 18344443]
- Milak MS, DeLorenzo C, Zanderigo F, Prabhakaran J, Kumar JS, Majo VJ, Mann JJ, Parsey RV. In vivo quantification of human serotonin 1A receptor using 11C-CUMI-101, an agonist PET radiotracer. *J Nucl Med.* 2010; 51:1892–900. [PubMed: 21098796]
- Milak MS, Severance AJ, Prabhakaran J, Kumar JS, Majo VJ, Ogden RT, Mann JJ, Parsey RV. In vivo serotonin-sensitive binding of [11C]CUMI-101: a serotonin 1A receptor agonist positron emission tomography radiotracer. *J Cereb Blood Flow Metab.* 2011; 31:243–9. [PubMed: 20571518]
- Millan MJ. Improving the treatment of schizophrenia: focus on serotonin (5-HT)(1A) receptors. *J Pharmacol Exp Ther.* 2000; 295:853–61. [PubMed: 11082417]
- Munoz A, Carlsson T, Tronci E, Kirik D, Bjorklund A, Carta M. Serotonin neuron-dependent and -independent reduction of dyskinesia by 5-HT_{1A} and 5-HT_{1B} receptor agonists in the rat Parkinson model. *Exp Neurol.* 2009; 219:298–307. [PubMed: 19500572]
- Nagai T, Murai R, Matsui K, Kamei H, Noda Y, Furukawa H, Nabeshima T. Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D1 and serotonin 5-HT_{1A} receptors. *Psychopharmacology (Berl).* 2009; 202:315–28. [PubMed: 18679658]
- Narendran R, Hwang DR, Slifstein M, Talbot PS, Erritzoe D, Huang Y, Cooper TB, Martinez D, Kegeles LS, Abi-Dargham A, Laruelle M. In vivo vulnerability to competition by endogenous dopamine: comparison of the D2 receptor agonist radiotracer (–)-N-[11C]propyl-norapomorphine ([11C]NPA) with the D2 receptor antagonist radiotracer [11C]-raclopride. *Synapse.* 2004; 52:188–208. [PubMed: 15065219]
- Nenonene EK, Radja F, Carli M, Grondin L, Reader TA. Heterogeneity of cortical and hippocampal 5-HT_{1A} receptors: a reappraisal of homogenate binding with 8-[3H]hydroxydipropylaminotetralin. *J Neurochem.* 1994; 62:1822–34. [PubMed: 8158133]
- Ohira H, Matsunaga M, Kimura K, Murakami H, Osumi T, Isowa T, Fukuyama S, Shinoda J, Yamada J. Chronic stress modulates neural and cardiovascular responses during reversal learning. *Neuroscience.* 2011; 193:193–204. [PubMed: 21763760]
- Parsey RV, Slifstein M, Hwang DR, Abi-Dargham A, Simpson N, Mawlawi O, Guo NN, Van Heertum R, Mann JJ, Laruelle M. Validation and reproducibility of measurement of 5-HT_{1A}

receptor parameters with [carbonyl-¹¹C]WAY-100635 in humans: comparison of arterial and reference tissue input functions. *J Cereb Blood Flow Metab.* 2000; 20:1111–33. [PubMed: 10908045]

- Palacios JM, Hoyer D, Cortés R. alpha 1-Adrenoceptors in the mammalian brain: similar pharmacology but different distribution in rodents and primates. *Brain Res.* 1987; 419:65–75. [PubMed: 2823969]
- Pike VW, Halldin C, Wikstrom HV. Radioligands for the study of brain 5-HT1A receptors in vivo. *Prog Med Chem.* 2001; 38:189–247. [PubMed: 11774795]
- Pinborg LH, Feng L, Haahr M, Gillings N, Ettrup A, Hansen HD, Yndgaard S, Svarer C, Knudsen GM. No change in [(11) C]CUMI-101 binding to 5-HT(1A) receptors after intravenous citalopram in human. *Synapse.* 2012;10.1002/syn.21579
- Richardson-Jones JW, Craige CP, Guiard BP, Stephen A, Metzger KL, Kung HF, Gardier AM, Dranovsky A, David DJ, Beck SG, Hen R, Leonardo ED. 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron.* 2010; 65:40–52. [PubMed: 20152112]
- Saijo T, Takano A, Suhara T, Arakawa R, Okumura M, Ichimiya T, Ito H, Okubo Y. Effect of electroconvulsive therapy on 5-HT1A receptor binding in patients with depression: a PET study with [11C]WAY 100635. *Int J Neuropsychopharmacol.* 2010; 13:785–91. [PubMed: 20067660]
- Sargent PA, Rabiner EA, Bhagwagar Z, Clark L, Cowen P, Goodwin GM, Grasby PM. 5-HT(1A) receptor binding in euthymic bipolar patients using positron emission tomography with [carbonyl-(11)C]WAY-100635. *J Affect Disord.* 2010; 123:77–80. [PubMed: 19726088]
- Schindler CW, Tella SR, Goldberg SR. Adrenoceptor mechanisms in the cardiovascular effects of cocaine in conscious squirrel monkeys. *Life Sciences.* 1992; 51:653–60. [PubMed: 1354322]
- Schoemaker H, Langer SZ. [3H]8-OH-DPAT labels the serotonin transporter in the rat striatum. *Eur J Pharmacol.* 1986; 124:371–3. [PubMed: 2942413]
- Selvaraj S, Turkheimer F, Rosso L, Mouchlianitis E, Faulkner P, Roiser JP, McGuire P, Cowen PJ, Howes O. Measuring endogenous changes in serotonergic neurotransmission in humans: a [11C]CUMI-101 PET challenge study. *Molecular Psychiatry.* 2012;1–7. [PubMed: 21483438]
- Simpson MD, Lubman DI, Slater P, Deakin JF. Autoradiography with [3H]8-OH-DPAT reveals increases in 5-HT(1A) receptors in ventral prefrontal cortex in schizophrenia. *Biol Psychiatry.* 1996; 39:919–28. [PubMed: 9162203]
- Sprouse J, Reynolds L, Li X, Braselton J, Schmidt A. 8-OH-DPAT as a 5-HT7 agonist: phase shifts of the circadian biological clock through increases in cAMP production. *Neuropharmacology.* 2004; 46:52–62. [PubMed: 14654097]
- Stein P, Savli M, Wadsak W, Mitterhauser M, Fink M, Spindelegger C, Mien LK, Moser U, Dudczak R, Kletter K, Kasper S, Lanzenberger R. The serotonin-1A receptor distribution in healthy men and women measured by PET and [carbonyl-11C]WAY-100635. *Eur J Nucl Med Mol Imaging.* 2008; 35:2159–68. [PubMed: 18542956]
- Szot P, White SS, Greenup JL, Leverenz JB, Peskind ER, Raskind MA. alpha1-Adrenoreceptor in human hippocampus: Binding and receptor subtype mRNA expression. *Mol Brain Res.* 2005; 139:367–71. [PubMed: 16039007]
- Trillat AC, Malagie I, Mathe-Allainmat M, Anmela MC, Jacquot C, Langlois M, Gardier AM. Effects of WAY 100635 and (-)-5-Me-8-OH-DPAT, a novel 5-HT1A receptor antagonist, on 8-OH-DPAT responses. *Eur J Pharmacol.* 1998; 347:41–9. [PubMed: 9650846]

Figure 1.
Chemical structure of [³H]CUMI-101

Figure 2.

Autoradiograms of [^3H]CUMI-101 binding to baboon brain sections. Total binding was defined with [^3H]CUMI-101 (images A, C, E, and G) and nonspecific binding of [^3H]CUMI-101 was determined in presence of WAY100635 (images B, D, F, and H).

Amyg: Amygdala; CA1-PL: CA1 pyramidal layer; Caud: Caudate Nucleus; Cb: Cerebellum; Claust: Claustrum; DG-ML: Dentate Gyrus Molecular Layer; Ins: Insula, PFC-LII: Prefrontal cortex layer II, Put: Putamen; Thal: Thalamus

Figure 3. Autoradiograms of [³H]CUMI-101 binding to 5-HT_{1A}R and α₁-AR in baboon brain. Amyg: Amygdala, CA1-PL: CA1-pyramidal Layer, DG-ML: Dentate Gyrus- Molecular Layer, PFC-LII: Prefrontal cortex layer II, Ins LII: Insular cortex layer II, FC: Frontal cortex

Figure 4.

Autoradiograms of [³H]8-OH-DPAT binding to baboon brain sections. PFC-Gr: Prefrontal cortex grey; PFC-LII: Prefrontal cortex-layer II; Ins-LII: Insula Layer II, Caud: Caudate Nucleus, Put: Putamen, Claust: Claustrum, Cx-LII Cortex layer II, Cx-Gr: Cortex grey, Thal: Thalamus, DG-ML: Dentate Gyrus-Molecular Layer, CA1-PL: CA1-pyramidal Layer.

Figure 5. Correlation of [^3H]CUMI-101 and [^3H]8-OH-DPAT binding in baboon brain. CA1-PL: CA1- Pyramidal Layer, DG-ML: Dentate Gyrus-Molecular Layer, FC-LII: Frontal cortex Layer II, Ins-LII: Insula Layer II, PFC-LII: Prefrontal cortex-layer II

Figure 6.

Autoradiograms of [^3H]CUMI-101 total (A, B, C, G, H, I) and nonspecific binding *in presence of* WAY100635 (D, E, F, J, K, L) in human brain sections. Amyg: Amygdala; CA1-PL: CA1 pyramidal layer; Caud: Caudate nucleus; Cb-Cx: Cerebellar Cortex; Cb-Verm: Cerebellar Vermis; Claust: Claustrum; DG-ML: Dentate Gyrus, Molecular Layer; Ins LII: Insula Layer II, PFC-LII: Prefrontal cortex layer II, Put: Putamen; Thal: Thalamus

Figure 7. [3H]CUMI-101 binding to 5-HT_{1A}R and α₁-AR in human brain. Cer-Cx: Cerebellar cortex, Cer-Verm: Cerebellar vermis, CA1-PL: CA1 pyramidal layer, DG-ML: Dentate Gyrus Molecular Layer, Ins LII: Insula Layer II, PFC-LII: Prefrontal cortex layer II

Figure 8.

Correlation of [^3H]CUMI-101 and [^3H]8-OH-DPAT binding in human brain. CA1-PL: CA1 pyramidal layer, DG-ML: Dentate Gyrus Molecular Layer, Ins LII: Insula Layer II, PFC-LII: Prefrontal cortex layer II