J Pharmacol Sci **120**, 254 – 257 (2012)

Journal of Pharmacological Sciences

Short Communication

Comparison of High and Low Affinity Serotonin 1A Receptors by PET In Vivo in Nonhuman Primates

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Received April 22, 2012; Accepted September 7, 2012

Abstract. Serotonin (5-HT) 1A receptors exist in high and low affinity states. Agonist ligands bind preferentially to the high affinity state receptors, providing a more functionally relevant measure than antagonist binding. We now report comparison of 5-HT_{1A} binding in vivo using both [^{11}C]CUMI-101 (agonist) and [^{11}C]WAY100635 (antagonist) in nonhuman primates. PET studies show that both tracers bind to known 5-HT_{1A} receptor (5-HT_{1A}R)-rich regions of baboon brain. The binding (BP_F) of [^{11}C]CUMI-101 was lower on an average of 55% across the regions of interest (ROIs) compared to [^{11}C]WAY100635. This ratio is consistent with the in vitro binding data of agonist and antagonist 5-HT_{1A}R ligands previously reported.

Keywords: 5-HT_{1A} receptor (5-HT_{1A}R), positron emission tomography, brain imaging

The serotonin 1A receptor (5-HT_{1A}R) is implicated in the pathophysiology of a variety of neuropsychiatric and neurodegenerative disorders (1). The antagonist radiotracer [^{11}C]WAY100635 has been the most commonly used 5-HT_{1A} ligand for in vivo Positron Emission Tomography (PET) studies (2). However, being a noncompetitive antagonist at the 5-HT_{1A}R, it has equal affinity for high agonist (HA) and low agonist (LA) affinity sites of the receptors and therefore cannot detect changes in the ratio of these sites or changes specific to high agonist affinity sites. In contrast, agonists bind preferentially to the HA state of the receptor, thereby providing a more functionally meaningful measure of the 5-HT_{1A}R. This hypothesis is supported by the finding that in vitro agonist binding measured by [^{3}H]8-OH-DPAT is 35% – 63% lower than antagonist measured by [^{3}H]WAY100635 in the autoradiography studies of human, rodent brain, and cells stably expressing human 5-HT_{1A}R (3 – 5). Theoretically, the failure of the system to adjust or maintain the necessary ratio of HA and LA affinity site of receptors may be an important factor in the pathophysiology of several diseases. Obtaining PET scans with both an agonist and antagonist ligand in the same subject enables the quantification of not only the binding to active or G-protein–coupled receptors (GPCR), but also the ratio of active vs. inactive receptors, which may change without any change in the total binding. Importantly, this ratio determines the number of active receptors or the efficacy of 5-HT transmission by endogenous 5-HT. Moreover, an agonist 5-HT_{1A} radiotracer is likely to be sensitive to displacement by the endogenous agonist of the receptor and therefore may be useful as a biomarker to measure the changes in the intra-synaptic concentrations of the endogenous 5-HT, to monitor desensitization (down regulation) and sensitization (up regulation) of GPCRs, to provide a better estimate of receptor occupancy for agonist therapeutic agents, and for evaluation of the efficacy of SSRI treatment. However, no in vivo data have been published for a comparison of agonist and antagonist 5-HT_{1A} tracers as a proof of principle for measurement of high and low affinity states of this receptor. We have recently reported that [^{11}C]CUMI-101, a 5-HT_{1A} partial agonist radiotracer (K_i = 0.15 nM, E_{max} = 80%), can quantify 5-HT_{1A} binding with PET in nonhuman primates and human subjects (6 – 8). We have also demonstrated the suitability of [^{11}C]CUMI-101 to mea-

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Published online in J-STAGE on October 16, 2012 (in advance)
doi: 10.1254/jphs.12100SC

sure robust increases in intrasynaptic endogenous serotonin in baboons induced by citalopram and fenfluramine administration (9). One study indicates that CUMI-101 behaves as a potent 5-HT$_{1A}$ antagonist in rat brain tissue in vitro and as a partial agonist in a human recombinant 5-HT$_{1A}$R preparation (10). These differences would be anticipated to dilute the effects of a serotonergic challenge on [^{11}C]CUMI-101 binding to 5-HT$_{1A}$R in rats. Many groups performed in vivo studies in humans, including finding that the increase in [^{11}C]CUMI-101 binding after intravenous citalopram is consistent with a decrease in endogenous 5-HT in post synaptic regions (11, 12). In human PET studies [^{11}C]CUMI-101 labels a substantially lower proportion of 5-HT$_{1A}$R than the classical 5-HT$_{1A}$R antagonist, [^{11}C]WAY100635, which supports that [^{11}C]CUMI-101 does act as a 5-HT$_{1A}$ partial agonist in humans, and this property explains its sensitivity to endogenous 5-HT levels at postsynaptic sites. We performed autoradiography studies of [^{3}H]CUMI-101 in postmortem human and baboon brain and found that it is very selective for 5-HT$_{1A}$R and the binding is in excellent agreement with [^{3}H]8-OH-DPAT binding on the same subjects, indicating CUMI-101 is an alternative ligand for in vitro studies. This would enable the results from in vitro studies with [^{3}H]CUMI-101 to be more directly comparable to in vivo PET studies with [^{11}C]CUMI-101 (Kumar et al., unpublished results). This further confirms that CUMI-101 is a selective 5-HT$_{1A}$R ligand. Here we report results describing the high affinity 5-HT$_{1A}$ binding using [^{11}C]CUMI-101 and [^{11}C]WAY100635 in vivo using PET in baboons (Fig. 1).

The classical approach to specifically determine HA and LA affinity of 5-HT$_{1A}$R is by measuring the differences in B_{max} and K_D of both tracers based on a Scatchard analysis of a saturation binding isotherm. This can be achieved by administering the radiotracers in a range of specific activities (SA) to determine the B_{max} and K_D for both tracers. However, this approach is problematic for agonists such as the 5-HT$_{1A}$ agonist [^{11}C]CUMI-101 because of pharmacologic effects, so we limited the cold mass of CUMI-101 for PET studies to at or below 5 μg. Higher doses of [^{11}C]CUMI-101 may cause vital changes and that would affect the binding parameter measurement

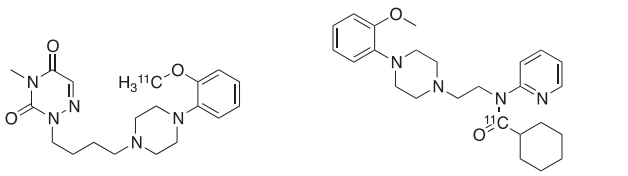

Fig. 1. Chemical structures of [^{11}C]CUMI-101 and [^{11}C]WAY100635.

(13). For the above reasons, we did not measure K_D of [^{11}C]CUMI-101 in vivo in baboons. Even though the in vivo [^{11}C]CUMI-101 K_D was not yet measured, the Ki values of WAY100635 (0.1 nM) and CUMI-101 (0.15 nM) are comparable. Also determining the ratio in a single subject is not practical as it would require repeated injections of both tracers resulting in unacceptable exposure to radiation. As an alternative approach, we set out to determine the HA and total 5-HT$_{1A}$ binding using [^{11}C]CUMI-101 and [^{11}C]WAY100635 by comparing their binding potential ($BP_F = B_{avail}/K_D$ in vivo in baboons by sequential administration of the two tracers. The assumption we make is that the differences in BP_F are not due to the difference in K_D. Radiosyntheses of [^{11}C]CUMI-101 and [^{11}C]WAY100635 were performed using procedures reported elsewhere (6 – 8, 14). All animal experiments were carried out with the approval of the Institutional Animal Care and Use Committees (IACUC) of Columbia University Medical Center (CUMC) and New York State Psychiatric Institute (NYSPI). [^{11}C]WAY100635 and [^{11}C]CUMI-101 PET scans were performed on two baboons (*Papio anubis*) using the ECAT EXACT HR+ scanner (Siemens Medical Solutions, Inc. Knoxville, TN, USA). Animal anesthesia, preparation, and PET study procedures were previously described (7 – 9, 14) [^{11}C]CUMI-101 (5 ± 0.5 mCi, SA: 2 ± 0.5 Ci/μmol) and [^{11}C]WAY100635 (4 ± 0.5 mCi, SA: 3 ± 0.4 Ci/μmol) were injected as an intravenous bolus over 30 s, and emission data were collected for 120 min in the 3-dimensional mode. Regions of interest drawn on each animal's MRI scan were transferred to registered frames of PET data. The plasma, metabolism, and free fraction were determined using the methods previously reported (6 – 8, 14). Regional distribution volumes of [^{11}C]WAY100635 and [^{11}C]CUMI-101 were determined by kinetic analysis using a two tissue compartment non-iterative (2TCNI) model using cerebellum as the reference region (6 – 8, 14). The use of the same reference regions for both tracers provided better binding comparison.

Both tracers showed high binding in 5-HT$_{1A}$R rich areas such as the hippocampus, insula, cingulate, and prefrontal cortex, while the amygdala and the thalamus exhibited lower binding. Minimal binding was seen in the striatum and the cerebellum had the least uptake. BP_F was calculated as $(V_T - V_{ND})/f_p = B_{max}/K_D$, where V_{ND} and V_T are defined as the distribution volumes of the non-displaceable and total (ND + specific) compartments, respectively. V_T is defined as the total regional equilibrium distribution volume, whereas, f_P is the free fraction of radioligand in the plasma. Figure 2 compares BP_F of [^{11}C]CUMI-101 and [^{11}C]WAY100635 across the regions of interest. The BP_F of the antagonist tracer

[^{11}C]WAY100635 represents high and low affinity binding (total binding) and the agonist tracer [^{11}C]CUMI-101 represents high affinity 5-HT$_{1A}$R binding. The binding of [^{11}C]WAY100635 is higher for all brain regions except for the occipital cortex, which is statistically insignificant in comparison to agonist radiotracer. The BP_F of the caudate, midbrain, thalamus, and putamen are lower in comparison to cortical regions and hippocampus for both radiotracers. [^{11}C]CUMI-101 BP_F values, on average, were 55% lower compared with [^{11}C]WAY100635 in the tested brain regions. The highest difference in BP_F was found for the parahippocampal gyrus (60%) ranging between 49% – 60% for cortical regions, whereas hippocampus and dorsal raphe nucleus show 48%. The amygdala and putamen show approximately 35% HA sites. There is a strong correlation of BP_F of the two tracers (Fig. 3) but the slope of this correlation is almost twice that of identity, suggesting that with increased total binding the proportion of HA 5-HT$_{1A}$R declines across brain regions.

This finding is in agreement with the report of approximately 50% higher [^{3}H]WAY100635 binding sites in comparison to the agonist [^{3}H]8–OH–DPAT binding in vitro by different methods independently in human brain, rat brain, and CHO cells expressing 5-HT$_{1A}$R (3 – 5). We also calculated $BP_{ND} = (V_T - V_{ND})/V_{ND} = f_{ND}B_{max}/K_D$ (f_{ND} is the free fraction of radioligand in the non-displaceable compartment) of both tracers and found HA binding is in close agreement with the results obtained using BP_F. These studies imply that the difference in BP_F values corresponds to the ratio of high to low affinity binding sites, suggesting that the radioligands measure different population of 5-HT$_{1A}$R in vivo and supports the utility of [^{11}C]CUMI-101 to measure high affinity 5-HT$_{1A}$ binding in vivo. It could be argued that the binding differences of these two radiotracers are caused by differences in K_D or differences in sensitivity

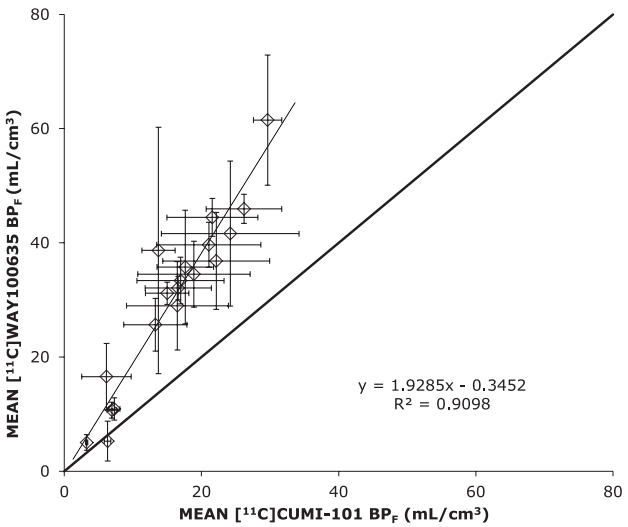

Fig. 3. Correlation of the average BP_F's of [^{11}C]CUMI-101 and [^{11}C]WAY-100635 in anesthetized baboons (n = 2).

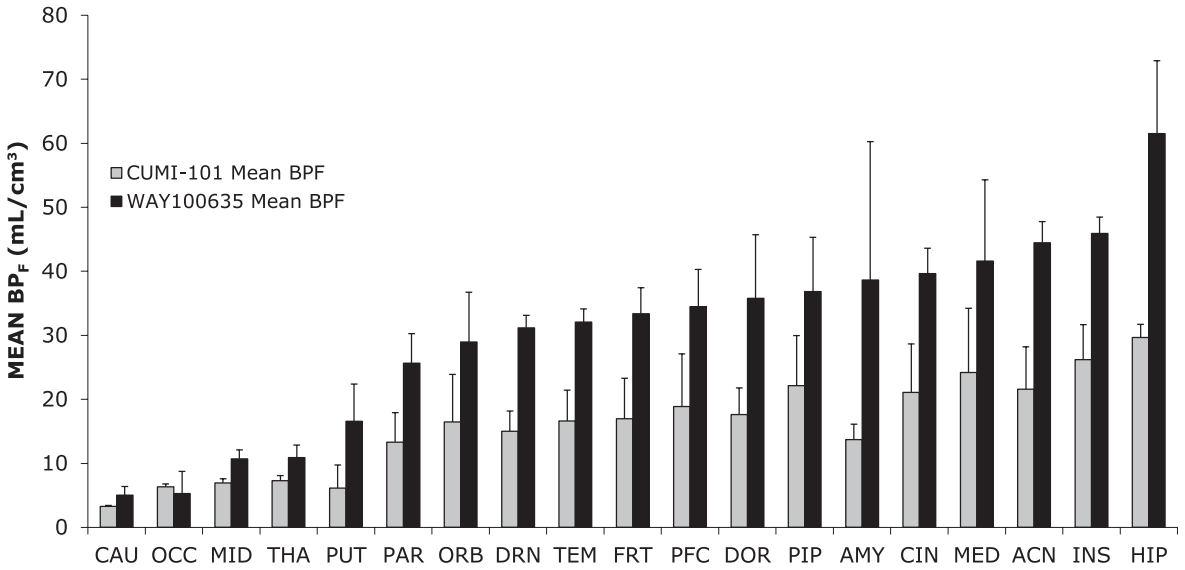

Fig. 2. Comparison of the average BP_F's of [^{11}C]CUMI-101 and [^{11}C]WAY100635 in anesthetized baboons (n = 2). ACN: anterior cingulate, AMY: amygdala, CAU: caudate, CIN: cingulate, DOR: dorsal and lateral prefrontal cortex, DRN: dorsal raphe nucleus, HIP: hippocampus, THA: ahalamus, MID: midbrain, PUT: putamen, OCC: occipital cortex, PAR: parietal cortex, ORB: orbital cortex, FRT: frontal cortex, TEM: temporal cortex, PFC: prefrontal cortex, PIP: parahippocampal gyrus, MED: medial prefrontal cortex, INS: insula.

to displacement by endogenous 5-HT. The previous studies indicate no evidence of [^{11}C]WAY100635 competition with endogenous 5-HT. Our studies indicate that [^{11}C]CUMI-101 did not compete with 5-HT at baseline conditions with tracer dose administration (9). Therefore, the interference of endogenous 5-HT displacement of the radioligands at tracer doses can be ruled out. However, being a partial agonist, CUMI-101 is expected to displace endogenous 5-HT at higher doses.

In summary, for the first time, we have compared the ratio of HA to LA binding sites for 5-HT$_{1A}$R in vivo in the baboon with PET. BP_F of [^{11}C]CUMI-101 was approximately 55% lower across the ROIs than that of [^{11}C]WAY100635. These findings are consistent with the known in vitro pharmacology of 5-HT$_{1A}$ receptors (agonist vs. antagonist). Studies comparing B_{max} of these tracers will provide further verification of this finding. These data support the notion that [^{11}C]CUMI-101 may be used to measure the effects of disease and/or treatment altering the ratio of high to low affinity 5-HT$_{1A}$ binding sites.

Acknowledgments

This publication was made possible by grant number R21 MH077161 and K08 MH76258-01A1 from the National Institute of Mental Health (NIMH) at the National Institutes of Health (NIH).

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