^{123}I-ADAM Binding to Serotonin Transporters in Patients with Major Depression and Healthy Controls: A Preliminary Study

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The serotonergic system may play an important role in the pathophysiology of major depressive disorder (MDD). Few imaging studies have examined serotonin transporter (SERT) binding in patients with MDD. We hypothesized that SERT binding activity may be altered in patients with MDD. This study compared SERT binding in patients with MDD with that in healthy controls. **Methods:** We studied SERT activity in 7 patients (22–50 y old) with moderate to severe MDD and 6 healthy controls (24–56 y old) using ^{123}I-labeled 2-((2-((dimethylamino)methyl) phenyl)thio)-5-iodophenylamine (ADAM) and SPECT brain imaging. Subjects underwent SPECT 4 h after intravenous administration of 185 MBq (5 mCi) of ^{123}I-ADAM. Images were reconstructed in the axial plane, and region-of-interest demarcations were placed on the midbrain, medial temporal region, and basal ganglia region. **Results:** ^{123}I-ADAM binding to SERT in the midbrain was significantly lower ($P = 0.01$) in MDD patients (1.81 ± 0.07) than in controls (1.95 ± 0.13). Age-adjusted ^{123}I-ADAM binding in the midbrain correlated significantly with scores on the Hamilton Depression Rating Scale ($r = 0.82$; $P = 0.02$). A significant negative correlation was observed between ^{123}I-ADAM SERT binding in the midbrain and age in the healthy control group ($r = 0.98$; $P = 0.0002$). SERT binding in the basal ganglia or medial temporal regions of interest did not significantly differ between groups. **Conclusion:** The findings from this preliminary study suggest the possibility of decreased SERT binding in the midbrain region of patients with MDD, with the degree of decrease correlating with the severity of depressive symptoms. There also appears to be an age-related decline in midbrain ^{123}I-ADAM SERT binding in healthy subjects.

Key Words: depression; serotonin transporter; SPECT; ADAM

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The serotonergic system may play a key role in the pathophysiology of major depressive disorder (MDD). In particular, the serotonin transporter (SERT) may serve an important function as a key site of antidepressant drug action. Several postmortem studies have found a reduction in the density of SERT sites in the brains of depressed patients (*1,2*). In vivo neuroimaging studies of SERT binding in patients with MDD have been few.

The development of radiopharmaceuticals selective for imaging SERT has been of considerable interest. Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, sertraline, citalopram, and paroxetine have been labeled with ^{11}C (*3–6*) or ^{18}F (*7–9*) for PET, but many of these agents were found not to be suitable for imaging SERT because of their high degree of nonspecific binding to other receptors (*10–12*). Previously, [^{11}C](+)McN5652 has been used for quantitative PET of SERT in the human brain (*13,14*). Using [^{11}C](+)McN5652, members of our research group recently demonstrated that drug-free patients with MDD had significantly more SERT binding sites in the left frontal cortex ($P = 0.013$) and right cingulate cortex ($P = 0.043$) than did healthy controls (*15*). However, [^{11}C](+)McN5652 is difficult to produce, and its uptake can be difficult to quantify. ^{11}C-labeled 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (DASB) is another PET compound that binds to SERT and has recently been used in human studies. An ^{11}C-DASB PET study found no significant difference in SERT binding between drug-free MDD patients and healthy controls (*16*). However, this same study did show an 80% decrease in SERT binding in MDD patients after treatment with paroxetine for 4 wk. Further studies will need to evaluate the ability of ^{11}C-DASB to quantitatively measure SERT binding (*17*).

^{123}I-ADAM (2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine) is a selective radioligand for imaging SERT using SPECT and provides several important

advantages over other PET and SPECT tracers (*18*). In contrast to PET agents for SERT, ^{123}I-ADAM can be manufactured in kits and has a long shelf life that simplifies the radiolabeling process. ^{123}I-ADAM was initially developed at the University of Pennsylvania and is now being used to study SERT in humans. This paper presents preliminary data on the use of ^{123}I-ADAM in patients with MDD compared with healthy controls.

MATERIALS AND METHODS

Subjects. All subjects signed consent forms approved by the human Institutional Review Board. The study was conducted under Investigational New Drug application 65,542 from the Food and Drug Administration. We studied 7 patients with unipolar MDD using the same ^{123}I-ADAM imaging protocol as for the control subjects. The patients with MDD included 3 men and 4 women ranging in age from 22 to 50 y (mean, 38 y). They were clinically assessed by a psychiatrist, met the DSM-IV criteria for MDD (*19*), and had had the disease for a mean of 10 y (range, 6 mo to 20 y). All patients received a score of ≥16 on the 21-item Hamilton Depression Rating Scale (*20*) and had been free from prior psychotropic medication for ≥5 elimination half-lives or ≥2 wk for monoamine oxidase inhibitors. More specifically, 2 patients were drug naïve, 2 patients had discontinued their non-SSRI antidepressant more than 3 wk previously, and the other 3 patients had discontinued their SSRI antidepressant at least 6 mo previously. Patients were excluded if they had a primary Axis I diagnosis other than MDD, current alcohol or drug abuse, a significant medical (e.g., uncontrolled diabetes or hypertension), neurologic (e.g., seizure disorder), or comorbid psychiatric (e.g., dementia) condition that could affect cerebral function or were actively suicidal or required hospitalization.

We also studied 6 healthy controls, who included 2 men and 4 women ranging in age from 24 to 56 y (mean, 37 y). Control subjects had no prior history of MDD or any other DSM-IV Axis I diagnosis. All received a 21-item Hamilton score of ≤6, and none had a significant underlying medical, neurologic, or psychiatric condition that could affect cerebral function. Subjects were excluded if they had a history of allergic reaction to iodine or shellfish. Women in both groups could not be breast feeding, and any woman of childbearing age had to have tested negatively for pregnancy within 48 h before injection of ^{123}I-ADAM.

Scanning Procedure. Subjects were initially given 18 drops of concentrated Lugol's solution to block the thyroid. An intravenous catheter was inserted and capped. Intravenous administration of 185 MBq (5 mCi) of ^{123}I-ADAM then took place. Because ^{123}I-ADAM was administered under an Investigational New Drug application, all subjects had their vital signs and electrocardiogram monitored from 10 min before injection to 10 min after injection. After injection of the ^{123}I-ADAM, the intravenous catheter was removed. Subjects returned to the nuclear medicine imaging room 4 h after ^{123}I-ADAM administration for a 60-min brain scan. The reason for this delay is that kinetic modeling data on ^{123}I-ADAM have demonstrated that binding potential can be estimated using the reference regions without arterial sampling at this time (*21*). After the scan, the imaging was complete and patients could begin treatment for MDD from the referring physician.

Image Processing and Analysis. SPECT scans of the brain were acquired on a triple-head γ-camera equipped with ultra-high-resolution fanbeam collimators (3000XP; Picker), which provide a spatial resolution of 6.7 mm in full width at half maximum at 10 cm. Images were reconstructed using a low-pass filter and Chang's first-order attenuation correction (coefficient of 0.11 cm^{-1}). The acquisition parameters include a continuous mode with 40 projection angles over a 120° arc to obtain data in a 128 × 128 matrix with a pixel width of 2.11 mm and a slice thickness of 3.56 mm with a center of rotation of approximately 14 cm.

All SPECT images were resliced according to the anteroposterior commissure, and regions of interest (ROIs) based on a previously described template (*22*) were placed on the scans. The initial use of this template was based on corresponding MRI scans of several control subjects. However, MRI scans were not obtained because of the preliminary nature of this investigation and the use of larger ROIs that did not require anatomic imaging for delineation. Future studies of smaller regions may necessitate MRI coregistration. These ROIs included the basal ganglia, medial temporal region, brain stem, and cerebellum. To reduce the effects of volume averaging in the axial direction, we did not place ROIs on the slices that contained the uppermost and lowermost portions of the structures they represented, thus limiting the small ROIs to the central aspect of structures they represented. The primary outcome measure was the SERT uptake ratio, in which the ROI was compared with a reference region (cerebellum) at 4–5 h after administration, when the distribution of ^{123}I-ADAM had approached a transient, near-equilibriumlike state that reflects the k3/k4 ratio and is related to the binding potential. Use of this outcome measure allowed for a quantitative assessment of SERT binding as described previously (*23*).

Statistical Analysis. All statistics were analyzed using the Minitab statistical program. Measures of ^{123}I-ADAM uptake ratios in the midbrain, medial temporal lobes, and basal ganglia were compared between the MDD and control groups using the Student *t* test. Correlation coefficients between ^{123}I-ADAM uptake ratios and depression scores were determined using a linear regression model. Values were adjusted for age on the basis of the average yearly decline observed in the healthy control group.

RESULTS

In healthy controls, a significant decrease ($r = -0.98$; $P < 0.001$) in ^{123}I-ADAM binding was observed with age (Fig. 1). This decrease in ^{123}I-ADAM binding corresponded to a decrease of approximately 3.0% per decade in SERT binding. Midbrain ^{123}I-ADAM binding was significantly less in MDD patients than in healthy controls (1.81 ± 0.07 and 1.95 ± 0.13, respectively, $P = 0.01$) (Fig. 2). This difference remained statistically significant ($P = 0.03$) even after adjustment for age, since the mean age was slightly less for the controls than for the patients (36.7 y and 38.3 y, respectively, $P = 0.77$). After age adjustment, 2 depression subjects had values close to the mean of controls, whereas the others remained less than the mean of controls. The 2 drug-naïve patients had the lowest uptake values. There were no such differences in SERT binding in the basal

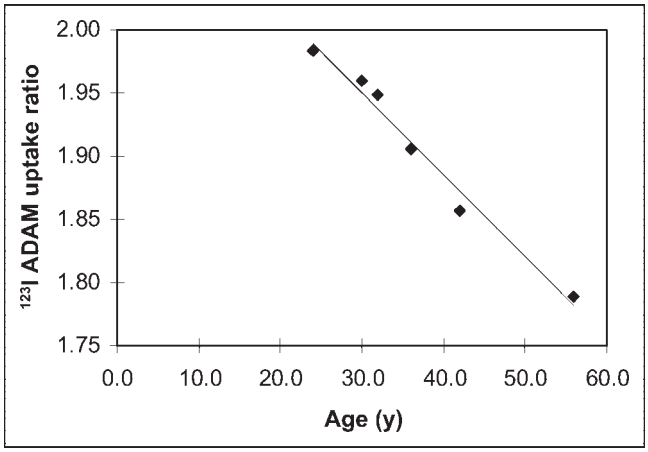

FIGURE 1. Graph shows correlation between ^{123}I-ADAM binding in midbrain with increasing age ($r = 0.98$; $P = 0.0002$). Relative decline of 3.0% per decade was found for ^{123}I-ADAM binding.

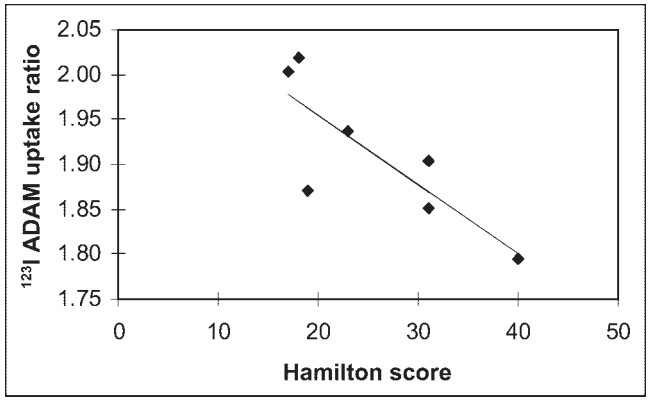

FIGURE 3. Graph correlates ^{123}I-ADAM binding in midbrain and degree of depressive symptoms as measured by Hamilton score. Binding correlated significantly ($r = 0.82$; $P = 0.02$) with lower uptake in patients with worst depressive symptoms.

ganglia or medial temporal lobe. Symptom severity as determined by the Hamilton Depression Rating Scale correlated significantly with age-adjusted ^{123}I-ADAM uptake ratios in the midbrain ($r = 0.82$; $P = 0.02$) (Fig. 3).

DISCUSSION

The results from this preliminary study revealed age-related changes in our limited sample of healthy volunteers, with ^{123}I-ADAM uptake ratios in the midbrain correlating negatively with age for an average decline of 3.0% per decade. This result is comparable to one reported earlier (*24*) for a study using ^{123}I-labeled 2β-carbomethoxy-3β-(4-iodophenyl)tropane (β-CIT) and is slightly lower than previous findings from our research group with respect to the dopamine transporter using ^{99m}Tc-TRODAT SPECT (*25*).

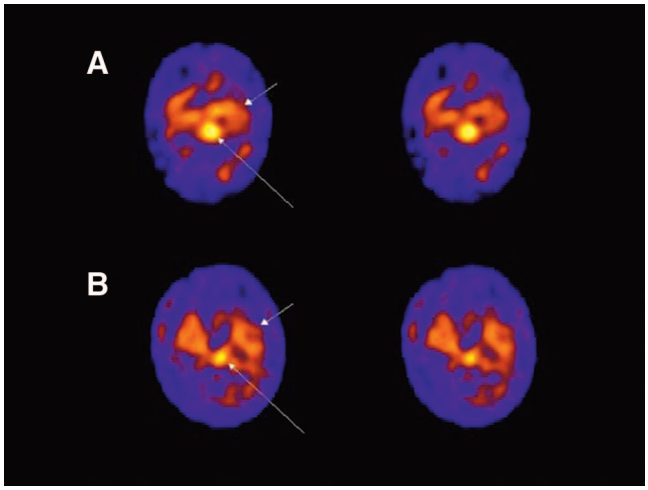

FIGURE 2. Transaxial ^{123}I-ADAM SPECT images are shown of a control subject (A) and a depression subject (B). Long arrows point to midbrain uptake, which is markedly less in depression subject than in control subject. Nonspecific binding in cerebellum is observed below midbrain uptake (posteriorly). Short arrows point to ^{123}I-ADAM binding in medial temporal region.

The serotonergic system likely plays an important role in the pathophysiology of depression. This likelihood is based both on the efficacy data from SSRIs in the treatment of patients with depression and on postmortem studies and studies of animal models (*2,26*). Neuroimaging studies of the serotonergic system have been an important focus of recent research. Several SPECT and PET studies have demonstrated changes in the serotonergic system in patients with MDD. A SPECT study using ^{123}I-β-CIT revealed lower binding in the brain stem in drug-free depressed patients than in controls (*27*). Another ^{123}I-β-CIT SPECT study of depression, in patients with Wilson's disease, showed a significant correlation between Hamilton scores and SERT binding (*28*). A small ^{123}I-β-CIT SPECT study of patients with seasonal affective disorder reported less SERT availability in patients than in healthy controls (*29*). However, ^{123}I-β-CIT binds to both serotonin and dopamine transporters. Findings are therefore more difficult to interpret, since the uptake in various regions on ^{123}I-β-CIT SPECT scans represents a combination of both dopamine and serotonin binding. In addition, ^{123}I-β-CIT typically requires a 2-d imaging protocol. For these reasons, there has been a strong impetus to find a better candidate for imaging SERT with SPECT. The current study describes initial work with a new ^{123}I isotope, ADAM, that selectively binds SERT. It is hoped that this tracer, if proven useful, may be more widely available for use in both research and clinical work. SPECT is generally more economical and widely available than PET, despite the relatively costly ^{123}I isotope.

The results from this preliminary investigation showed significantly lower midbrain ^{123}I-ADAM binding in MDD patients than in healthy controls. The possible pathophysiologic mechanism includes 1 of 2 possibilities. One scenario would be an overall decrease in serotonin levels and downregulation of the SERT concentration to attempt to

maintain normal levels of serotonin in the synapse. The alternative would be a disease-specific decrease in SERT concentration (either a direct decrease or a decrease related to other neurotransmitter systems) that may result in dysregulation of serotonin concentrations within the synapse. Future studies will be required to delineate these possible mechanisms. There were no such differences in binding in the basal ganglia or medial temporal regions, possibly because of the high variability among these values. This variability has also been demonstrated in test/retest studies of ^{123}I-ADAM uptake (*30*). These findings are somewhat consistent with those previously reported for a study using ^{123}I-β-CIT; that study found SERT binding to decrease in patients with depression. However, the studies with ^{123}I-β-CIT reported some decrease in SERT binding in the basal ganglia—a finding that we did not observe. This finding may in part be explained by the higher specificity of ^{123}I-ADAM for SERT, since ^{123}I-β-CIT also binds to the dopamine transporter. Thus, measures of striatal uptake of ^{123}I-β-CIT may represent both serotonin and dopamine transporter binding. A PET study with ^{11}C-DASB did not show significant differences between depression patients and control subjects in cortical regions (*16*). In the present study, we did not observe significant differences in ^{123}I-ADAM in the temporal regions or striatum. The midbrain decrease in the present study does not agree with the data obtained with ^{11}C-DASB, although in that study the analysis of the midbrain was not as clearly determined. Therefore, future studies will be necessary to corroborate the current findings and to more fully evaluate other structures such as the basal ganglia. With regard to a more direct comparison of ^{123}I-ADAM and ^{11}C-DASB, both show high binding affinity for SERT (dissociation constant = 0.15 nmol/L for ADAM (*31*); inhibition constant = 1.1 nmol/L for DASB (*32*)). Both ligands also display highly selective binding toward SERT (1,000-fold greater than the binding toward other monoamine transporters). When the 2 tracers are compared with regard to the signal (specific vs. nonspecific binding), which can affect the imaging contrast, DASB has a higher ratio (9.0 to 1) than does ADAM (5.5 to 1) in studies on rats. However, for the purposes of the areas described in this paper, ^{123}I-ADAM was able to measure age-related changes in uptake and differences between controls and patients with depression.

Our preliminary study also found a significant correlation between age-adjusted ^{123}I-ADAM binding in the midbrain and Hamilton scores. This correlation further supports the relationship between decreased SERT binding and depression. However, because all studies of SERT binding in depression, including ours, have had small samples, more detailed measures in a larger number of subjects will be needed to elucidate the relationship between SERT binding and depression, particularly the extent of depressive symptoms. These findings support the potential role of SERT in the pathophysiology of depression and depressive symptoms. These findings also suggest that ^{123}I-ADAM SPECT may be useful in furthering understanding of the role of the serotonin system in depression and normal aging.

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