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Rapid Communication

Age-related decline in central serotonin transporter availability with [^{123}I]β-CIT SPECT☆

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Abstract

Postmortem studies have provided limited and conflictin data regarding aging effects on the central serotonin transporter (SERT). The present study investigated the effect of age on SERT availability in the human brainstem and diencephalon with single photon emission computed tomography (SPECT) using the ligand [^{123}I]2β-carbomethoxy-3β-(4-iodophenyl)tropane ([^{123}I]β-CIT). Healthy control subjects ($n = 126$) who ranged in age from 18 to 88 were injected with 6.0 ± 0.8 (mean ± SD) mCi [^{123}I]β-CIT and imaged 23.1 ± 1.9 h later under equilibrium conditions. A ratio of specifi to nondisplaceable brain uptake (i.e., $V_3'' =$ [brainstem-diencephalon – occipital]/occipital), a measure proportional to the binding potential (B_{max}/K_D), was derived. SERT availability (V_3'') showed a significan inverse correlation with age ($r = -0.40$, $P < 0.0001$). Linear regression analysis revealed that V_3'' declined by 29.5% over the age range 18 to 88, or approximately 4.2% per decade. These results demonstrate reductions in the availability of central SERT binding sites with age in living human subjects.

Keywords: Aging; Serotonin transporter; [^{123}I]β-CIT; SPECT

1. Introduction

The central serotonin (5-HT) system has been implicated in several behaviors, including mood, feeding, aggression, cognition, and sleep [16,19,24]. Age-related changes in this system may play an important role in a number of late-life conditions, including geriatric depression, memory loss, and insomnia. Whereas several human postmortem and neuroimaging studies have revealed reductions in postsynaptic 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors with healthy aging (for a review, see [20]), reports of aging effects on the presynaptic serotonin transporter (SERT) have been scanty and contradictory [2–4,18,23,26].

As an in vivo probe for SERT, we have chosen the single photon emission computed tomography (SPECT) radioligand [^{123}I]2β-carbomethoxy-3β-(4-iodophenyl)tropane ([^{123}I]β-CIT; also referred to as [^{123}I]RTI-55). [^{123}I]β-CIT is a potent cocaine analog with high affinit in vitro for both SERT and the dopamine transporter (DAT) [7]. Previous studies in humans and nonhuman primates have shown that [^{123}I]β-CIT accumulates in two distinct brain regions: striatum and brainstem-diencephalon. Pharmacological characterization of regional [^{123}I]β-CIT uptake in baboons [13] has indicated that striatal activity is associated almost exclusively with DAT (i.e. displaceable by GBR-12909 but not citalopram), whereas binding in the brainstem-diencephalon seems to be specifi for SERT (i.e. displaceable by citalopram but not GBR-12909). Thus, regional uptake of [^{123}I]β-CIT in the brainstem-diencephalon seems to provide a pharmacologically relevant measure of SERT in humans. Previous studies with [^{123}I]β-CIT have demonstrated diminished SERT availability in patients with major depression [17] and alcoholism [11] and elevated SERT availability in

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acutely abstinent cocaine addicts [12]. The present study employed [^{123}I]β-CIT and SPECT to examine the effect of age on SERT availability in healthy human subjects.

2. Methods

2.1. Subjects

The study population consisted of 126 healthy volunteers (70 male, 56 female) who ranged in age from 18 to 88 (46 ± 19 years; 104 Caucasian, 10 African-American, 8 Asian, 4 Hispanic). Subjects underwent a clinical examination by a research psychiatrist to exclude any neurological or psychiatric disease, alcohol or substance abuse. Screening procedures included a physical and neurological examination, EKG, serum chemistries, thyroid function studies, CBC, urinalysis, and urine toxicology screen. Female subjects of childbearing potential were required to have a negative pregnancy test (serum at screening; urine immediately before tracer injection). Those subjects ≥ 55 years of age (n = 31) were also required to have no significan evidence of cognitive impairment, as indicated by a Folstein Mini-Mental State Examination (MMSE) [10] score ≥ 27. Those subjects ≥ 68 (n = 25) were required to have a normal brain MRI scan. No subject was taking medication known to affect the brain 5-HT system. All subjects gave written informed consent to the research protocol approved by the local human investigation committee. Subjects received 0.6 g of potassium iodide (SSKI solution) in the 24 h before the scan.

2.2. SPECT imaging

All subjects received an injection of [^{123}I]β-CIT (6.0 ± 0.8 mCi; specifi activity > 5,000 Ci/mmol) on day 1, followed 23.1 ± 1.9 h later by a 24 min scan with a Picker (Cleveland, OH, USA) PRISM 3000 (n = 89) or 3000XP (n = 37) SPECT camera equipped with a low energy, high resolution (LEHR) fanbeam collimator (128 × 128 matrix, 120° angular range, 3° angular step, 40 steps, 36 s per step, 15.5 cm radius of rotation). In this configuration the PRISM 3000 acquires images at a reconstructed full-width at half-maximum resolution of 12.3 mm as determined by an ^{123}I point source in water. Comparability of the two cameras was confirme by imaging 26 subjects on both cameras from a single [^{123}I]β-CIT injection and findin no signif-cant difference in V_3'' in brainstem-diencephalon (PRISM 3000: 2.0 ± 0.6, 3000XP: 2.1 ± 0.6, t = 0.38, P = 0.31, paired t-test). Previous studies have demonstrated that [^{123}I]β-CIT reaches equilibrium binding in the brain by 18 to 24 h [15], yielding a simple unitless ratio of regional radioactivities (V_3'' = specific/nondisplaceabl binding = [brainstem-diencephalon – occipital]/occipital) in estimating SERT number (i.e. B_{max}). Before scanning, four or fiv fiducia markers fille with 5 μCi of $Na^{99m}TcO_4$ were attached to the skin along the canthomeatal plane to identify this plane during image analysis.

Images were reconstructed from photopeak counts (159 ± 16 keV) using standard filtere backprojection methods (Butterworth, power 10, cutoff 0.24 cm^{-1}) and displayed as a 128 × 128 × 64 matrix with a voxel size of 2.07 × 2.07 × 3.56 mm (15.25 mm^3). Subsequent image analysis was performed by an operator who was unaware of subject demographics. SPECT data were reoriented to correct for deviations from the canthomeatal plane, as identifie by the fiducia markers. Eight contiguous transaxial slices with the highest uptakes in brainstem-diencephalon and striatum, respectively, were identifie from a reconstructed midsagittal image and digitally summed to yield two transaxial slices, each 28.5 mm thick (Fig. 1). Attenuation correction was performed using a Chang zero order method (attenuation coefficien μ = 0.15 cm^{-1}) within an ellipse drawn around the skull. Standard region of interest (ROI) templates for brainstem-diencephalon (432 voxels or 6.6 ml) and occipital cortex (7912 voxels or 120.6 ml) were positioned on their respective summed slices, with the occipital ROI placed at the striatal level (Fig. 1). No attempt was made to correct for scatter or partial volume effects.

V_3'' for brainstem-diencephalon was computed without conversion of SPECT cpm to absolute units of radioactivity as $[(\text{cpm/voxel})_{\text{brainstem-diencephalon}}—(\text{cpm/voxel})_{\text{occipital}}]/(\text{cpm/voxel})_{\text{occipital}}$. The effect of age on V_3'' was examined by linear regression and Pearson's product moment correlation coefficien (r).

3. Results

The values of V_3'' for brainstem-diencephalon ranged from 1.63 to 4.48 (2.70 ± 0.61) for this healthy subject sample. V_3'' showed a significan inverse correlation with age (r = −0.40, n = 126, P < 0.0001) (Fig. 2). Linear regression analysis revealed that V_3'' declined by 29.5% over the age range 18 to 88, or approximately 4.2% per decade.

The data showed no effect of gender on SERT levels. That is, the addition of gender (with or without the interaction of age and gender) did not significantl improve the prediction of V_3'' after controlling for the contribution of age. Nor was there an effect of gender when premenopausal (<50 years) and postmenopausal age ranges were examined separately. Nonlinear functions (including exponential, logarithmic, and 2nd order polynomials) did not provide a significantl better fi for the relationship between V_3'' and age.

4. Discussion

Central SERT availability demonstrated a significan age-related decline as measured in vivo by [^{123}I]β-CIT and

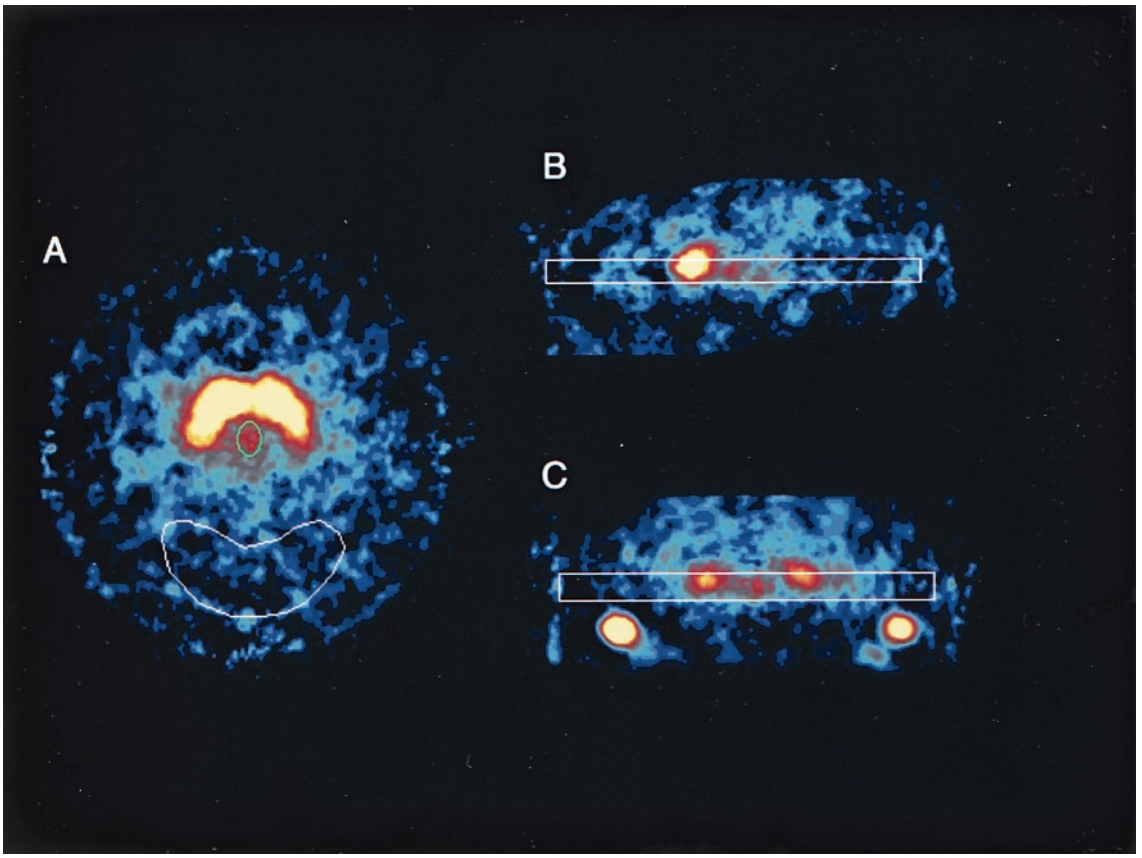

Fig. 1. Transaxial (A), midsagittal (B), and coronal (C) equilibrium images of [^{123}I]β-CIT uptake in the same healthy subject. The transaxial image (A) is representative of the digitally summed slice (28.5 mm thick) used for the analysis of brainstem-diencephalon. It displays the brainstem-diencephalon and occipital ROIs (occipital ROIs were positioned by analogy at the striatal level). The midsagittal (B) and coronal (C) images display a rectangle, which delineates the anatomic boundaries of the brainstem-diencephalon transaxial slice (A). The coronal image (C) also contains two fiducia markers.

SPECT. This findin is somewhat at odds with the majority of postmortem studies. Whereas several human postmortem and neuroimaging studies have revealed reductions in postsynaptic 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors with healthy aging (for a review, see [20]), reports of aging effects on the SERT have been more equivocal. Three postmortem studies have examined [^{3}H]imipramine binding in human brain and have reported: 1) no effect of age in temporal cortex [2], 2) increases with age in frontal and parietal cortices and in hypothalamus [23], and 3) a 50% decline in the cingulate cortex with aging, but no effect of age in frontal cortex, hippocampus, amygdala, and basal ganglia [18]. However, these studies are limited by the low specifi binding of [^{3}H]imipramine—only about 10% in frontal cortex [6]. Three postmortem studies have used the more selective ligand [^{3}H]paroxetine and have found no age-related changes in frontal and cingulate cortex, amygdala, and hypothalamus [3], frontal cortex [4] or dorsal raphe nucleus [26].

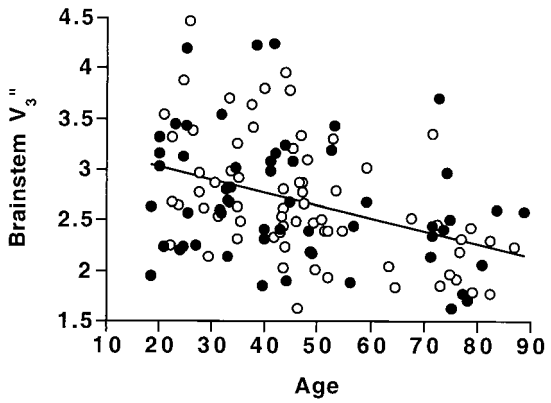

Fig. 2. Serotonin transporter (SERT) availability (V_3'') in brainstem-diencephalon, as measured by [^{123}I]β-CIT and SPECT, versus age in 126 healthy volunteers. $V_3'' = -0.013\times$ age $+ 3.292$; $r = -0.40$; $P < 0.0001$ (Pearson's test). Each dot denotes an individual subject; closed circles represent females and open circles males.

One other study [21] has examined aging effects on SERT availability with [^{123}I]β-CIT in healthy subjects and reported a similar age-related decline (3.2% per decade in thalamus-hypothalamus; 4.5% per decade in brainstem) to that of the present investigation. Two previous studies of SERT availability with [^{123}I]β-CIT in patient populations of limited age range have incidentally analyzed aging effects. No significan effect of age on brainstem SERT availability was seen in alcoholic and healthy subjects [11]. Whereas a

weakly significan ($P = 0.03$) effect of age on brainstem but not diencephalic SERT availability was observed in acutely abstinent cocaine addicts and healthy controls [12].

The divergence between the present in vivo results with [^{123}I]β-CIT and the majority of postmortem studies likely accrues from several important methodological differences, including the selection of subjects (postmortem studies have generally employed small samples and limited age ranges), radioligands (see below), and brain regions. Notably, one postmortem study that examined a brain region (dorsal raphe nucleus) subsumed in our brainstem-diencephalon ROI [26] studied only seven subjects over a limited age range (46–67).

Limitations of the present study include: 1) [^{123}I]β-CIT's dual affinit for SERT and DAT, 2) the use of occipital cortex as a SERT-free reference region, and 3) the absence of MRI-based partial volume correction. Given [^{123}I]β-CIT's affinit for both SERT and DAT, age-related decline in [^{123}I]β-CIT uptake in brainstem-diencephalon cannot definitivel be ascribed to SERT alterations alone. DAT binding is also known to decline with age in human striatum as determined by in vitro homogenate binding [1,9,28] as well as in vivo studies with [^{123}I]β-CIT [27]. Although displacement binding studies in primates have demonstrated that [^{123}I]β-CIT uptake in brainstem-diencephalon is primarily associated with SERT [13] (i.e., displaceable by citalopram but not GBR-12909), a small component of this uptake is likely attributable to DAT. Ex-vivo autoradiography studies in three monkeys from our group (unpublished data) suggest that [^{123}I]β-CIT uptake in brainstem-diencephalon is distributed (starting from the region with highest uptake) in: 1) superior colliculus, 2) dorsal and median raphe nuclei, 3) substantia nigra, and 4) several hypothalamic and thalamic nuclei. Although this distribution closely mirrors that of SERT binding in homogenate binding and autoradiographic studies in human and nonhuman primates [5,8,14], the substantia nigra also contains significan levels of DAT. This relatively small DAT component of brainstem uptake may account for the less than complete displacement of [^{123}I]β-CIT binding in human brainstem-diencephalon by citalopram [22]. Therefore, replication of the present finding using a more selective PET or SPECT tracer will be valuable.

A second limitation involves the use of occipital cortex as a SERT-free reference region. Although our previous SPECT primate study [13] failed to demonstrate a displaceable component of cortical [^{123}I]β-CIT uptake, human postmortem studies have shown low but measurable levels of SERT in occipital cortex [5,8,14]. Thus, the present study might underestimate an age-related decline in SERT availability if occipital levels were proportionately reduced. Finally, the present results may be susceptible to partial volume effects [25], related to age-related atrophy in diencephalic structures and brainstem.

Despite these limitations of interpretation, the present results clearly demonstrate that in clinical studies of SERT using [^{123}I]β-CIT, careful control for age effects is essential.

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