Effects of Age on Dopamine Transporters in Healthy Humans

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99mTc-TRODAT-1 is a new radiopharmaceutical that selectively binds the dopamine transporters. This study characterized the effects of aging on its regional cerebral distribution in healthy human volunteers. Methods: The sample consisted of 27 men and 28 women with a mean age of 41.1 \pm 17.1 y (age range 18.7-73.8 y). Dynamic SPECT scans of the brain were obtained with a standardized acquisition and processing protocol on a triple-head camera. Mean counts per pixel were measured in multiple regions of interest within each basal ganglia. Regression analyses were used to relate the specific uptake values at 3-4 h after administration to age. Both linear and nonlinear models of aging were tested. Results: The relative concentration of radioactivity in most subregions of the basal ganglia decreased significantly with age (all P values < 0.0001). Nonlinear models of aging fit the data significantly better than a straight line. The rate of decline was significantly faster in young adults than in older volunteers (P < 0.001). The break-point age at which the rate of change slowed down and became more stable was 36 y old for the whole striatum and ranged from 32 to 44 y old depending on subregion. Conclusion: The effects of aging on central nervous system dopamine transporters do not appear to be linear. Most effects seem to occur during young adulthood before people reach their 40s. The distribution then appears to remain relatively stable until late in life. The findings suggest that the adult life cycle is better characterized as a series of phases than as a continuum.

Key Words: 99mTc; normal; tomography emission computed

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Let dopamine transporter participates in the regulation of dopaminergic tone (1). Manipulation of the transporter causes dysfunction and abnormal behavior in animals (2) and people (3).

Neuroimaging studies have consistently shown that transporter concentrations decrease with advancing age in healthy human volunteers (4-8). The magnitude of the drop with normal aging appears significantly smaller than the decline produced by several dopaminergic degenerative diseases (9-11). Therefore, if a new radiopharmaceutical can detect the effects of aging on healthy people, then it may be an

effective probe for investigating several neurodegenerative disorders.

The strategy of validating the effectiveness of new radiopharmaceuticals in this way has contributed to the formation of new questions about the dynamic nature of the aging process in healthy people. Hypotheses suggest that middle age represents a prolonged period of relatively stable cerebral physiology. Alternative hypotheses suggest that brain function deteriorates at a constant rate throughout adulthood. The issue may have vocational, as well as clinical, implications, because the dopaminergic system mediates some aspects of manual dexterity as well as cognition and emotion. The purpose of this study was to determine whether a new ^{99m}Tc-labeled tropane could characterize the effects of normal aging on the dopamine transporter.

MATERIALS AND METHODS

Participants

All procedures were approved by the local Committee on Research Involving Human Beings. The sample consisted of 55 consenting healthy volunteers whose demographic characteristics reflected those of the local community (27 men, 28 women; age range 18.7–73.8 y; mean age 41.1 \pm 17.1 y). Their mean educational level was 16.1 \pm 2.4 y. The histogram in Figure 1 shows the distribution of participants by age.

None of the participants had a lifetime history of a psychiatric disorder, including drug or alcohol abuse. Their past medical histories and physical examinations were not remarkable for a disease or event that could have affected brain structure or function. There were no clinically significant abnormalities on their electrocardiograms (ECGs).

Radiopharmaceutical

The radiopharmaceutical, $[^{99m}Tc][2-[[2-[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2 0.1]oct-2-yl]methyl](2-mercaptoethyl)amino]ethyl]amino] ethane-thiolato(3-)-N2,N2',S2,S2']oxo-[1R-(exoexo)]- (^{99m}Tc-TRODAT-1), has been described previously ($ *12–18*). Each dose was prepared immediately before administration.

Image Acquisition

The protocol began with the insertion of a catheter in an antecubital vein. The participants were then placed at rest on the imaging table for a minimum of 20 min while vital signs were recorded every 3 min and ECG rhythm strips were obtained continuously. A dose of 740 \pm 74 MBq (20 mCi) was injected

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FIGURE 1. Distribution of participants by age.

through the indwelling catheter while planar images of the brain were acquired for 1 s per frame for 1 min. Dynamic SPECT images were then acquired at a framing rate of 5 min per scan. All scans were obtained with the same triple-head gamma camera equipped with ultra-high-resolution fanbeam collimators (Picker 3000; Picker International, Cleveland, OH). The characteristics of this particular instrument have already been described (19). The acquisition parameters included a continuous mode with 40 projection angles over a 120° arc to obtain data in a 128 \times 128 matrix with a pixel width of 2.11 mm and a slice thickness of 3.56 mm. The center of rotation was always 14.0 cm regardless of body habitus. The first 20 participants had a total of 60 intermittent scans acquired over 7 h. Subtle repositioning errors were corrected with software. The remainder of the participants had 4 scans obtained from 5 to 25 min after administration and then 24 consecutive scans obtained at 3-5 h after administration without their heads being moved.

Image Processing

All the images were reconstructed according to the same protocol. After backprojection, a simple, low-pass filter was applied with an order of 4 and a cutoff of 0.351 cm⁻¹. A uniform ellipse was used to estimate attenuation. The images were then reinterpolated into $2 \times 2 \times 4$ mm voxels on another platform.

Manual Image Analysis

A set of standardized templates representing the basal ganglia and the whole supratentorial brain was fit on a summed image and then was transposed without manipulation on each frame. The template has already been described (7, 11). Briefly, within the x-y plane, each region of interest (ROI) in the template was smaller than the actual structure it represented, to minimize resolutioninduced problems with ill-defined edges. To reduce the effects of volume averaging in the axial direction, we placed the ROIs on the 3 contiguous slices that contained the most intense activity. Of these, the slice with the lowest counting rate was then retrospectively discarded. This tended to limit the ROIs to the central 8 mm of structures they represented. Boundaries for the whole brain (WB) were drawn beginning 12 mm above the uppermost slice containing basal ganglia activity.

Statistical Analyses

The scans that were acquired from 180 to 240 min after administration were used to calculate the standardized uptake values (SUVs), which, at equilibrium, represent the ratio of k3:k4in a conventional three-compartment model. This time interval was chosen on the basis of preclinical studies in baboons (20) and data in healthy humans. We calculated the mean activity per pixel in each ROI across two slices by adding up the total number of counts in each ROI and dividing by the total number of corresponding pixels. Homotopic regions of the right and left hemisphere were averaged together and were compared to the mean activity per pixel in the whole supratentorial brain.

Correlation coefficients between age and the regional values were calculated for each ROI. The process began by plotting age against the mean WB ratio for each region. Each graph contained one data point for each of the 55 participants in the sample. Curve fitting was performed as previously described (8) with a nonlinear, "broken-stick" regression model defined as:

> $y = b_0 + m_0 \times age$ for age < X and $y = b_1 + m_1 \times age$ for age > X,

where, for each ROI, m_0 represents the rate of change with age before an unknown age of X, m_1 represents the rate of change after the age of X and X is the break-point age at which the two separate straight lines describing the changes with age intersect. The data were iteratively fit to simultaneously minimize the root-meansquare error of the slopes for the two regression lines as well as their intercepts. The region-to-WB ratios at the break points were calculated to complete the descriptions of the aging curves and to define the average values in middle-aged adults when the terminal slopes of the aging curves decreased.

Corroboration with Statistical Parametric Mapping

The analyses were performed independently with statistical parametric mapping (SPM-96) (21). We coregistered the images to each other using a count difference algorithm (22) and affine (linear) transformations, with a randomly selected image as the baseline template. All the coregistered images were then added together, each scaled by their mean background counts, into a single average image. The coregistration was then repeated; this time each image was registered to the average template. This had the effect of reducing the bias that was introduced by using one single image as a template. The registration procedure was repeated two additional times, which reduced any residual bias still further. Each registered image was scaled by its mean background counts, which normalized the images for further analysis and effectively gave parametric images of SUVs (23,24). Pixel-by-pixel statistical tests were applied to the images, and the resulting statistic images for nonindependent multiple comparisons were corrected using the



FIGURE 2. SPECT scans of brain in 26-y-old healthy volunteer.

theory of random Gaussian fields (25). Pixel-level thresholds on the statistic maps were set to P < 0.001, and surviving clusters of pixels were thresholded at P < 0.05.

RESULTS

The radiopharmaceutical localized in the basal ganglia of all the volunteers without causing any side effects or changes in cardiac electrophysiology (Fig. 2). The SUVs in the caudate were on average 11.2% higher than the values in the putamen (P < 0.0001). Only 3 participants had values in the anterior putamen that were higher than in the caudate. All the values in the posterior putamen were lower.

Dynamic image analysis showed that the activity in the basal ganglia peaked within 90–120 min, after which the rate of elimination was very slow (Fig. 3). Activity in the remainder of the brain peaked within the same time period, after which the rate of elimination was also slow. Counting rates in the supratentorial brain region were slightly higher, but less noisy, than the rates in the cerebellum. The time-activity curves for the supratentorial brain and the cerebellum were otherwise identical. As the time-activity curves in Figure 3 show, the final outcome measure between 3 and 4 h after administration was taken after the kinetics became relatively stable.

Simple, linear models showed that there were significant correlations with age. The regression coefficients ranged from -0.76 for the whole striatum (P < 0.0001) to a low of -0.48 (P < 0.001) for the left anterior putamen. Curve fitting showed that two lines forming a broken-stick model fit the data better than one straight line (Fig. 4). Most of the effects of normal aging could be accounted for by changes before the break-point age, which ranged from 32.6 to 42.2 y in this sample. The break-point age was 36 ± 4 y for the whole striatum; the break-point age was younger for all the putamen ROIs than in the caudate ROIs. After the breakpoint age, age effects became less pronounced (Table 1). The rate of decline in the whole striatum at age 20 was 10.9% per decade, but it dropped to only 2.9% per decade by age 40 (Table 2). The terminal slope for the central caudate actually increased by +2.2% per decade on each side ($r^2 = 0.13$, which was not significant).

The results were virtually identical with SPM. Like the manual ROI analyses, the findings showed that the specific uptake of this new ^{99m}Tc-labeled dopamine transporter imaging agent decreased with advancing age, and two lines







FIGURE 4. Diagram showing age effects. Two lines fit data better than one. SUV = standardized uptake value.

fit the data significantly better than one (P < 0.05). The findings held in almost every pixel of the basal ganglia. No other pixels in the brain were significant.

DISCUSSION

The results of this study showed that the specific uptake of this new 99m Tc-labeled dopamine transporter imaging agent decreases with advancing age. When simple linear models were used to estimate the rate of decline in the whole striatum, the group mean values of 6.2% per decade for the whole sample and about 11% per decade for the younger adults were consistent with several previous estimates using other transporter imaging agents (3–8). The values were also

 TABLE 1

 Parameters Describing Aging Curves

Region	Slope* if age < BP†	BP age (y)	Slope* if age > BP
Whole striatum	-0.018 ± 0.004	36 ± 4	-0.0039 ± 0.001
Caudate	-0.019 ± 0.004	44 ± 4	$+0.0056 \pm 0.004$
Putamen	-0.017 ± 0.007	35 ± 5	-0.0041 ± 0.002

*Units of change in standardized uptake value per year \pm SE. †Break-point age at which $y_1 = y_2$. BP = break point.

 TABLE 2

 Regional Effects of Aging (% Change per Decade)

Region	18 < age < 74	lf age <40 y	If age >40 y
Right caudate	-4.7	-10.1	+2.2
Left caudate	-5.7	-12.0	+2.2
Right anterior putamen	-6.9	-13.1	-5.0
Left anterior putamen	-4.1	- 11.3	-2.1
Right posterior putamen	-7.9	- 11.9	-4.4
Left posterior putamen	-7.8	-9.8	-7.4
Whole striatum	-6.1	-12.1	-2.4
Mean	-6.2	- 11.3	-4.1

consistent with published rates for fluorodopa (26-28), as well as several postsynaptic dopamine receptor imaging agents (29-32).

The estimated rates of decline were significantly higher in young adults than in older volunteers. Similarly shaped aging curves have been explicitly described for several other components of the dopaminergic system (29-32). Most of the variance between the calculated aging curves and the experimental data could be accounted for by underestimates of the values in young adults. This suggests that even more elaborate mathematical models may fit the data better.

The experimentally calculated break-point age for the whole striatum of 36 y olds is consistent with several other social and biologic conceptualizations of when middle age begins. The results suggest that dopamine transporter levels remain relatively stable from the mid 30s until the late 60s in most healthy people, a period that may define middle age for the mesocortical dopaminergic nervous system. Relatively prolonged biologic stability during this period may explain why some investigators have not found any age effects in their samples (33). This stability is consistent with the observation that the 99mTc-TRODAT-1 uptake values in some 70 y olds were comparable with the mean values in the group of 30 y olds, whereas the lowest values were found in some of the oldest volunteers. Similar findings have been reported by other investigators (27,28). This set of observations suggests that the biological capacity to perform some dopaminergically mediated brain functions may not decline until very late in the life cycle.

The shapes of the aging curves were qualitatively similar for all subregions of the basal ganglia in this study, but the rates of decline were higher in the putamen than in the caudate. After the break-point age, further decreases in the caudate could not be detected at all in this sample. In fact, the terminal aging curves had a small, upward slope. Similar results were observed in a different group of healthy volunteers who were imaged with the iodinated forerunner of ^{99m}Tc-TRODAT-1, [¹²³I]iodinated phenyl tropane (7). The findings suggest that the effects of age on dopaminergic physiology may not be regionally uniform.

The consistency between these observations and several behavioral studies of aging has been noted (8,34). The capacity to perform some motor tasks declines linearly with

age, but performance scores on other tasks do not deteriorate in any measurable way until very late in life (35-39).

The conclusions seem to deserve further testing even when all the other technical and biological contributions to the uptake values are considered. It is possible that nonspecific age effects on the delivery or elimination of the tracer influenced the results independently of any changes in the dopamine transporters themselves. However, estimates of age effects on regional cerebral blood flow appear to be comparable in the basal ganglia and the supratentorial brain that served as the reference region (34), making perfusion an unlikely source of much variance. Age-related expansion of cerebrospinal fluid volumes were not corrected for in this study, but, as previously noted (8) and as subsequently corroborated by independent investigators (40), the problem should have produced the opposite effect on the data by artifactually accelerating the apparent rates of decline in older volunteers. Even if several other potential contributions to the uptake values are eventually shown to be important, the results still suggest that the effects of age on the biological factors that determine dopamine transporter measurements are not optimally modeled as a linear continuum.

The issue of how healthy aging affects dopaminergic physiology and function may deserve further study for several reasons. Clinically, proper assessment of older patients with transporter imaging agents requires knowledge of the extent to which uptake values are decreased in healthy people of that age. The results of this study suggest that middle-aged patients should normally resemble older controls more than younger adults; this finding, if verified, will require that caution be used in some clinical situations. Scientifically, the results deserve further consideration because they seem to show that some aspects of cerebral physiology change differently during young adulthood than during middle age, implying that these periods represent distinct phases of the life cycle and not a continuum.

The findings also tend to validate the development of 99m Tc-TRODAT-1 as a dopamine transporter imaging agent in clinical practice, because changes produced by pathology appear to be substantially greater than the effects of aging (9-11).

CONCLUSION

Aging significantly affects the specific uptake of ^{99m}Tc-TRODAT-1 in healthy humans. However, the changes do not appear to be linear. The findings suggest that the adult life cycle is better characterized as a series of phases than as a continuum.

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