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In Vivo Imaging of Vesicular Monoamine Transporters in Human Brain Using [^{11}C]Tetrabenazine and Positron Emission Tomography

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Abstract: The pharmacokinetics of [^{11}C]tetrabenazine, a high-affinity radioligand for the monoamine vesicular transporter, were determined in living human brain using in vivo imaging by positron emission tomography (PET). The radiotracer showed high brain uptake and rapid washout from all brain regions with relatively slower clearance from regions of highest concentrations of monoamine vesicular transporters (striatum), resulting in clear differential visualization of these structures at short intervals after injection (10–20 min). As the first human PET imaging study of a vesicular neurotransmitter transporter, these experiments demonstrate that external imaging of vesicular transporters forms a new and valuable approach to the in vivo quantification of monoaminergic neurons, with potential application to the in vivo study of neurodegenerative disorders such as Parkinson's disease. **Key Words:** Positron emission tomography—[^{11}C]Tetrabenazine—Vesicle—Transporter—Monoamine—Parkinson's disease—Striatum. **Kilbourn M. R. et al.** In vivo imaging of vesicular monoamine transporters in human brain using [^{11}C]tetrabenazine and positron emission tomography. *J. Neurochem.* **60,** 2315–2318 (1993).

The availability of specific radioligands for discrete neuronal populations, suitable for in vivo imaging of nerve terminals using positron emission tomography (PET), would be of immense value in the study of neurodegenerative disorders such as Parkinson's or Alzheimer's disease. Repeated, noninvasive, quantitative measures of presynaptic nerve terminal densities would be useful in determining the rate and extent of neuronal loss during the onset and progression of these diseases and provide objective measures of therapies intended to prevent or delay further degeneration. Previous approaches to dopaminergic nerve terminal imaging have used 6-[^{18}F]fluoroDOPA (for review, see Leenders, 1991) or radioligands that bind to the neuronal membrane dopamine (DA) transporters (for review, see Salmon, 1991). The neurochemical basis for cerebral localization of radioactivity after 6-[^{18}F]fluoroDOPA injection and its relationship to nerve terminal densities continue to be debated (Gjedde et al., 1991; Leenders, 1991; McGeer et al., 1992), and recent evidence suggests that DOPA decarboxylase, the enzyme responsible for the first irreversible step in accumulation of radioactivity, is subject to regulation after acute or chronic manipulations of the dopaminergic system (Hadjiconstantinou et al., 1992; Zhu et al., 1992). Although many of the radioligands for DA transporters have been used for successful in vivo imaging of dopaminergic terminals in the basal ganglia of human brain, recent studies in animals have suggested that the numbers of neuronal membrane transporters might be regulated by changes in endogenous DA levels (Scheffel et al., 1991; Kilbourn et al., 1992), or chronic drug administration (Wiener et al., 1989; Ikegami and Prasad, 1990; Sharpe et al., 1991). For use as a quantitative measure of neuronal densities, a process or binding site that does not undergo such regulation would be preferable, and thus alternatives to the neuronal membrane transporters might be investigated.

A second specific marker for monoaminergic nerve terminals is the transporter site located on membranes of presynaptic vesicles, which is responsible for movement of monoamines from the cytosol to the lumen of the vesicle (Henry and Scherman, 1989). Three ligands—reserpine, ketanserin, and tetrabenazine (TBZ)—are high-affinity inhibitors of this transporter, although none of these ligands distinguishes among the different monoaminergic neurons (DA, noradrenaline, or serotonin). Radioligand binding assays for the vesicular transporter have been developed using [^{3}H]-reserpine and [^{3}H]dihydrotetrabenazine ([^{3}H]TBZOH), and autoradiographic studies have reported using [^{3}H]reserpine, [^{3}H]TBZOH, and 7-amino-8-[^{125}I]iodoketanserin (for review, see Henry and Scherman, 1989). Binding of such radioligands in the striatum is due predominantly to dopami-

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Abbreviations used: DA, dopamine; PET, positron emission tomography; TBZ, tetrabenazine; TBZOH, dihydrotetrabenazine.

nergic terminals, and excellent correlations between reductions in [^{3}H]TBZOH binding and dopaminergic terminal loss (measured by tyrosine hydroxylase activity) have been demonstrated following stereotactic brain lesions in animals (Masuo et al., 1990) or in autopsy samples from Parkinson's disease patients (Scherman et al., 1989). We have recently prepared TBZ in a high-specific-activity, carbon-11 (β^+, $t_{1/2}$ = 20.4 min)-labeled form (DaSilva et al., 1993), and we describe here the initial in vivo human imaging of vesicular monoamine transporters using this new radioligand.

EXPERIMENTAL PROCEDURES

Human subjects

Two 26-year-old healthy volunteers participated in this study. There were free of significant medical, neurologic, and psychiatric illness, and they were nonsmokers receiving no centrally acting medications. The studies were approved by the University of Michigan Institutional Review Board, and written informed consent was obtained for both studies.

PET imaging and experimental design

A cutaneous vein was cannulated for injection of the radioisotope, and the subjects were positioned supine in the gantry of a CTI/Siemens model 931/08-15 tomograph. This scanner simultaneously acquires 15 contiguous slices over an ~10-cm axial field of view, with a reconstructed in-plane spatial resolution of 7–8 mm. [^{11}C]TBZ (27.5 or 27.6 mCi; specific activities, >1,000 Ci/mmol) in a volume of 3–4 ml was injected as a bolus. A dynamic series of PET scans was obtained over a 60-min period, beginning with frames of short duration (30 s) and progressing to 10-min frames at the end of the imaging period.

Realignment of images

Reconstructed images were realigned using radioactive scalp fudiciary beads to correct for subject motion during the scan session. Beads were labeled with 5–10 μCi of ^{11}C and securely taped to positions on the forehead and behind and above each ear. All image frames obtained were then reoriented to a single frame acquired at 5–7.5 min. This method allows for correction of three translational and three rotational degrees of freedom to within <0.5 mm and 1°, respectively.

Data analysis

Regions of interest for striatum, thalamus, cortex, and cerebellum were determined on the early images (summation of images from 3 to 5 min), which most reflect initial radiotracer distribution. These regions of interest were then mapped to the entire temporal image sequence to determine the time-dependent concentration of radiotracer in different brain regions.

RESULTS AND DISCUSSION

Of the three high-affinity ligands for the vesicular monoamine transporter, ketanserin, reserpine, and TBZ, we have chosen to study TBZ because of its low toxicity, rapid and short-lived pharmacological actions, and ease of radiolabeling. It is a well-characterized drug that has been used clinically for more than 2 decades (Pletscher et al., 1962).

[^{11}C]TBZ, labeled with the short half-life positron-emitter radionuclide carbon-11, was injected into two normal human subjects, and the brain distribution of the radiochemical was imaged for 60 min. Representative images at 1–3 min and from 20 to 60 min are shown in Fig. 1. Brain uptake (estimated from count rates in early frames of the study) of radioligand was high, similar to that observed with [^{11}C]flumazenil (Koeppe et al., 1991) and [^{11}C]-tropanylbenzilate (Frey et al., 1990), radioligands that readily cross the blood–brain barrier. As shown in Fig. 2, egress of radioactivity from brain regions was very rapid, with the best differentiation of basal ganglia from cortex visible at 20–30 min after injection. Longer retention of the radioligand in the basal ganglia is most likely due to binding to the higher numbers of monoaminergic nerve terminals in these regions (Henry and Scherman, 1989). Although we have not performed blocking studies in humans to assign this binding definitively to the vesicular transport site, studies in mice (DaSilva and Kilbourn, 1992) and monkeys (DaSilva et al., 1992) have shown that striatal retention of this radiotracer can be blocked with reserpine, TBZ, and ketanserin but not inhibitors of neuronal DA transport (GBR 12909) or DA D_2 receptors (haloperidol). PET studies of [^{11}C]TBZ in a unilateral MPTP-lesioned monkey have clearly demonstrated that at tracer doses there is only a presynaptic localization for this radioligand (DaSilva et al., 1993).

This work represents the first in vivo PET imaging of a human vesicular neurotransmitter transporter. Although [^{11}C]TBZ appears very suitable for qualitative imaging of

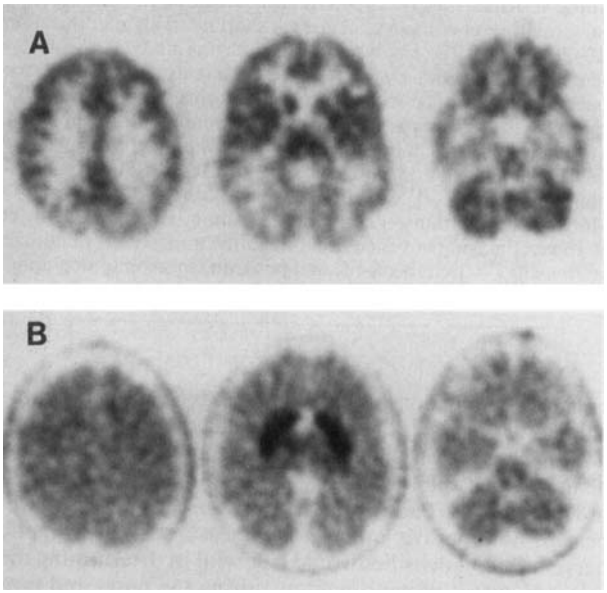

FIG. 1. Representative images of human brain distribution of [^{11}C]TBZ at 1–3 min (**A**) and 20–60 min (**B**) following intravenous injection. Shown are three of the 15 simultaneously acquired transaxial slices, which represent planes 8.4 (left), 5.7 (center), and 3.0 (right) cm above and parallel to the canthomeatal line. These planes pass through the levels of the cingulate cortex; caudate nucleus, putamen and inferior thalamus; and cerebellum, pons, and inferior frontal and temporal lobes, respectively. Distribution at early times (A) resembles that obtained with blood flow agents. Higher residual radioactivity levels are clearly seen in the basal ganglia by 20 min postinjection (B, center), consistent with greater retention of [^{11}C]TBZ in this region of highest monoaminergic innervation. Images in B have been scaled up by a factor of 3 to allow comparison with the early images in A; absolute radioactivity levels are significantly lower in the later images (see Fig. 2).

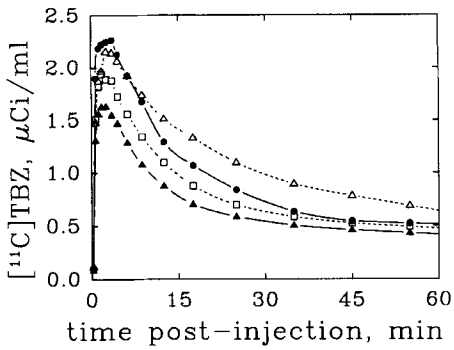

FIG. 2. Tissue time–activity curves following intravenous injection of [^{11}C]TBZ. Regional brain levels in striatum (△), thalamus (●), cerebellum (□), and cortex (▲) were determined using region of interest analysis of PET images. Time points shown are the midpoints of the acquired images.

vesicular transporters and may have application in PET studies of dopaminergic terminal losses in various neurodegenerative diseases, it is not likely to be the radioligand of choice for quantitative in vivo PET imaging because of the possible formation of a radiolabeled metabolite [^{11}C]-TBZOH, formed by reduction of [^{11}C]TBZ in vivo. This metabolite shows nearly identical in vitro binding affinity for the monoamine vesicular transporter (Henry and Scherman, 1989), and thus it is possible that the images obtained here represent the combined localizations of [^{11}C]TBZ and the metabolite [^{11}C]TBZOH. Existence of a radiolabeled metabolite, particularly one with similar pharmacological properties but perhaps different physiochemical properties, would complicate pharmacokinetic modeling of the in vivo data. Our work does demonstrate, however, that in vivo imaging studies of the monoaminergic vesicular uptake system are feasible, and qualitative studies of loss of this site in neurodegenerative disorders might immediately be undertaken. With development of an improved radioligand that does not form radiolabeled metabolites, this presynaptic site might serve as a quantitative measure of dopaminergic terminals. In addition, the possible physiological regulation of vesicular transporters by either endogenous DA or chronic dopaminergic drugs is unknown at this time and will need to be examined.

These in vivo PET results also confirm the interactions of [^{11}C]TBZ with multiple monoamine systems, as uptake and retention of radioligand are evident in the thalamus (noradrenaline) and cerebellum (noradrenaline and serotonin), although pharmacokinetic modeling of radiotracer distribution will be necessary to characterize fully [^{11}C]TBZ distribution in these tissues. In rodents the in vivo retention of [^{11}C]TBZ in various brain regions correlates very well (r^2 = 0.96; data not shown) with in vitro binding of [^{3}H]-TBZOH (Henry and Scherman, 1989), and this specific binding in all tissues can be blocked with pharmacological doses of unlabeled TBZ or reserpine. Vesicular transporters for monoamines have only recently been cloned and sequenced (Liu et al., 1992); although they can be considered as members of the 12 membrane-domain transporter superfamily (Uhl, 1992), they are not homologous with the neuronal monoamine transporters, which do show monoamine selectivities (Giros et al., 1992). An intriguing possibility is the potential existence of multiple subtypes of vesicular transporters, exhibiting unique monoamine specificities.

In conclusion, we have demonstrated for the first time in vivo imaging of the vesicular monoamine transport system in human brain. In combination with development of radioligands for the specific vesicular transporter for acetylcholine (Jung et al., 1990; Kilbourn et al., 1990; Widen et al., 1992), including successful single photon emission computed tomographic imaging in humans (D. E. Kuhl et al., unpublished data), it may soon be possible routinely to image and quantify these important, specific neuronal populations in the living human brain.

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