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In Vivo Imaging of Monoaminergic Nerve Terminals in Normal and MPTP-Lesioned Primate Brain Using Positron Emission Tomography (PET) and [^{11}C]Tetrabenazine

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ABSTRACT The first successful in vivo imaging of monoamine vesicular transporters in the living primate brain is described, using [^{11}C]tetrabenazine ([^{11}C]TBZ) and Positron Emission Tomography (PET). Radioligand uptake into brain is rapid, and at short time periods (10–30 minutes) the higher uptake and retention of the radiotracer in the more densely dopaminergic innervated striatum is clearly visualized. Specific binding in striatum can be entirely blocked with co-administration of a pharmacological dose (1 mg/kg i.v.) of tetrabenazine. In a unilaterally MPTP-lesioned monkey, specific binding of radioligand was absent in the striatum on the lesioned side, with no effect on radiotracer distribution in the cortex, cerebellum or contralateral striatum. PET imaging with [^{11}C]TBZ provides a new approach to the in vivo study of monoaminergic neurons and their loss in neurodegenerative diseases.

INTRODUCTION

Considerable effort has been expended in the development of single photon emission computed tomography (SPECT) and positron emission tomography (PET) radioligands intended for in vivo quantification of monoaminergic nerve terminals and in particular for the development of potential neuronal markers for the dopaminergic system. Such efforts have resulted in the synthesis and in vivo evaluation of a number of new radiotracers, including 6-[^{18}F]fluoroDOPA and 4-[^{18}F]fluoro-m-tyrosine, and the neuronal reuptake inhibitors [^{11}C]nomifensine (Aquilonius et al., 1987), [^{11}C]cocaine (Fowler et al., 1989), [^{11}C]WIN 35,428 (also termed [^{11}C]CFT) (Wong et al., 1992), [^{123}I]RTI-55 (Shaya et al., 1992), [^{123}I]CIT (Innis et al., 1991), [^{18}F]GBR 13119 (Kilbourn et al., 1989) and [^{18}F]GBR 12909 (Koeppe et al., 1990). Although each radiotracer has unique characteristics of brain penetration, pharmacological specificity, and level of nonspecific uptake or binding, all have been successfully used to image dopaminergic neurons in primate or human brain.

As part of our studies with the aryldialk(en)ylpiperazines [^{18}F]GBR 13119 and [^{18}F]GBR 12909, we have demonstrated that the neuronal dopamine transporter (DAT) may be subject to regulation according to the chronic concentrations of endogenous dopamine (Kilbourn et al., 1992). This finding has been reported by others for the dopamine uptake system (Scheffel et al., 1991) and furthermore is common to the norepinephrine reuptake system (Lee et al., 1983). The B_{max} for DAT may also be altered by chronic treatments with drugs that bind at this site (Ikegami et al., 1990; Sharpe et al., 1991; Wiener et al., 1989). For the development of a true neuronal marker, the up- or down-regulation of the numbers of reuptake sites per neurons might serve to, respectively, obscure neuronal degeneration or provide an unrealistically large estimate of neuron loss. For these reasons, we have continued our search for radiotracers for other presynaptic, high affinity binding sites that might serve as specific markers of neuronal numbers. We have recently described the synthesis and mouse brain distribution of [^{11}C]tetrabenazine ([^{11}C]TBZ), a high affinity inhibitor of vesicular monoamine transport (DaSilva and Kilbourn, 1992). Although this transporter exhibits similar numbers and kinetics as the neuronal transporters, there is apparently no differentiation into systems specific for dopamine, serotonin, and norepinephrine (Slotkin and Bareis, 1980). Specific binding of [^{11}C]TBZ can thus be

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demonstrated in nearly all brain regions (striatum, cortex, hippocampus, hypothalamus, cerebellum), and the in vivo estimates of specific binding of [^{11}C]TBZ correlate well with both in vitro numbers of vesicular transporters (determined using [^{3}H]dihydrotetrabenazine, [^{3}H]TBZOH) or combined in vivo levels of monoamines (Scherman et al., 1986). [^{11}C]TBZ appears a suitable radioligand for the in vivo study of monoaminergic nerve loss, particularly in the striatum, which contains a high concentration of predominantly dopaminergic nerve terminals as compared to 5-HT or NE neurons. We describe here the first successful imaging of monoaminergic neurons in primate brain using a vesicular uptake inhibitor and PET and demonstrate that such binding sites are completely lost in the striatum on the affected side of a unilateral MPTP-lesioned primate brain.

MATERIALS AND METHODS

Radiotracer preparation

High specific activity [^{11}C]tetrabenazine was prepared by the [^{11}C]methylation of 9-O-desmethylTBZ, as previously described (DaSilva et al., 1993). The radiotracer was purified using semipreparative silica gel chromatography and prepared for injection by evaporation of the HPLC solvent and dissolution in phosphate buffer. Radiochemical and chemical purity was then determined by reversed phase high pressure liquid chromatography. The specific activity of final product was >1,000 Ci/mmol at time of injection, with a radiochemical purity >95%.

Monkey PET studies

PET imaging studies were done using two female pigtail monkeys (M. nemistrina, 6.6 and 4.6 kg). One monkey had, 3½ years earlier, undergone a right unilateral carotid injection of MPTP (3 mg total) using a modified method of Bankewicz et al. (1986). The animal developed persistent hemiparkinsonism on the contralateral side. Although used in pharmacological studies of dopamine agonists, the monkey had been drug-free for 1 month prior to the imaging study.

For each imaging session, the animals were anaesthetized with ketamine (15 mg/kg i.m. and repeated as needed) and administered xylazine hydrochloride (2 mg/kg i.m.). Studies were done using the TCC 4600 PET scanner (three-ring, five-slice tomograph) operating in the high resolution mode (12 mm FWHM). Cerebral blood flow studies using i.v. injections of 3–5 mCi of ^{15}O-labeled water were done prior to the [^{11}C]TBZ studies, to aid in positioning of the animals. The animals were then injected with 4.0–6.0 mCi of [^{11}C]TBZ and sequentially imaged (30-second frames early, progressing to 10-minute frames at late times) for a total of 40 minutes. For the blocking study in the control animal, a no-carrier-added injection of [^{11}C]TBZ and imaging study was followed 1 hour later by injection of low specific activity [^{11}C]TBZ containing 1 mg/kg TBZ (Fluka Chem. Co.).

RESULTS AND DISCUSSION

After i.v. injection, [^{11}C]tetrabenazine exhibits rapid brain uptake and clearance with a maximum brain concentration at 5 minutes. The slower release of the radioligand from the striatum, the site of the highest concentrations of vesicular transporters, results in clear visualization of this brain structure beginning at ~10 minutes after injection. The striatal to cerebellar ratio of radioactivity reaches a maximum value of about 2 at 20 minutes after injection; striatum to cortex values in monkeys ranged from 1.3 to 1.6: a representative PET image is shown in Figure 1. Unlike radioligands specific for the dopaminergic system, [^{11}C]TBZ binds to vesicles of noradrenergic and serotonergic nerve terminals; as these are clearly present in the cerebellum and cortex, these tissues do not represent reference regions for only non-specific binding, and reporting of striatum-to-cerebellum or striatum-to-cortex ratios are at best just approximations of the contrast between areas of high and low binding site densities. Evidence that the radioligand retention in the striatum was due to binding to specific vesicular transporters was provided by a blocking study, where a co-injection of 1 mg/kg unlabeled tetrabenazine completely abolished any region-selective accumulation of radioligand (data not shown). In previous studies in mice we have demonstrated that the in vivo binding of tetrabenazine is unaffected by neuronal dopamine uptake inhibitors or dopamine D_2 receptor antagonists, but is sensitive to competition with tetrabenazine, reserpine, and ketanserin, all known inhibitors of the vesicular transport of monoamines (DaSilva and Kilbourn, 1992).

In contrast to the symmetrical uptake of radioligand in brain of the control monkey, in the unilateral MPTP-lesioned animal there was localization of the radiotracer in the striatum on only one side of the brain, corresponding to the unlesioned hemisphere (Fig. 1). Radiotracer uptake was unaffected in the cortex or unlesioned striatum (STR/CTX = 1.55 at 30 min), but was reduced to or below unity on the lesioned side (Fig. 2). This is consistent with the many reports of imaging of presynaptic dopaminergic functions in unilateral MPTP-lesioned animals using [^{18}F]fluoroDOPA or a variety of radiolabeled dopamine reuptake inhibitors (Leenders et al., 1988; Shaya et al., 1992).

Vesicular monoamine transporters are located exclusively in presynaptic monoaminergic terminals, and along with the neuronal transporters and the biosynthetic enzymes, tyrosine hydroxylase and tryptophan hydroxylase might serve as specific markers of presynaptic neuronal loss. Synaptic vesicular amine transporters (SVAT) are clearly distinct proteins that are quite different than the neuronal amine transporters; both the SVAT and DAT have been recently cloned and

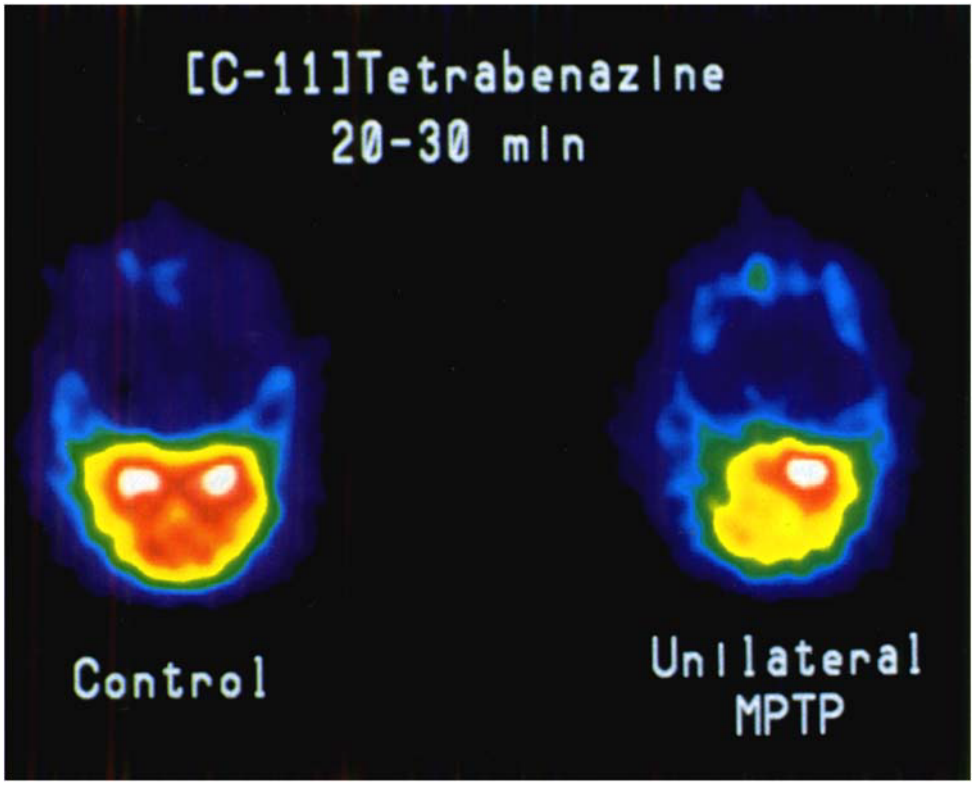

Fig. 1. PET images of [^{11}C]TBZ accumulation in striatum of normal (left) and unilateral MPTP-lesioned (right) monkeys. Images represent accumulated counts for the period 20–30 minutes after i.v. injection of radiotracer.

sequenced (Giros et al., 1992; Liu et al., 1992), and although they both belong to the 12-transmembrane region transporter superfamily (Uhl, 1992), there is no sequence homology between them. Although it is recognized that the neuronal reuptake systems and the biosynthetic enzymes (Zigmond, 1985) are subject to regulation upon changes in physiological conditions, very little is known of the sensitivity of the vesicular transporter to regulation by endogenous dopamine levels. We are encouraged by preliminary experiments in mice where regulation of [^{11}C]TBZ binding could not be observed (DaSilva and Kilbourn, unpublished results) despite large alterations of brain dopamine levels induced by chronic pargyline treatment (Buu, 1989).

In this study, we have demonstrated that the vesicular transporters can be imaged in the living primate brain and that we can study the loss of such sites upon destruction of the dopaminergic terminal field in the striatum. Our results are consistent with the in vitro studies of 6-hydroxydopamine lesioned rats (Masuo et al., 1990) and of postmortem human brain tissue of Parkinson's disease patients (Scherman et al., 1989), which have shown a clear and dramatic loss of vesicular

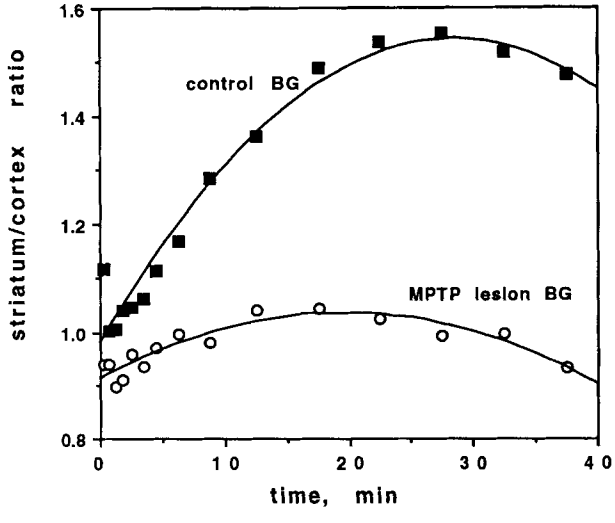

Fig. 2. Striatum/cortex ratios of radioactivity in PET scans of a unilateral MPTP-lesioned monkey. Regions of interest were placed over the right and left striatum, and cortex on both sides, and the time-dependent concentrations of radioactivity in those areas determined. Ratios represent the division of the striatal radioactivity by the average cortical radioactivity.

binding sites (measured using [^{3}H]dihydrotetrabenazine) consistent with the loss of nerve animals. The specific targeting of radiopharmaceuticals for the vesicular transporters is a new approach to in vivo imaging of the monoaminergic nervous system, but the concept is similar to the successful development in our laboratory (Jung et al., 1990; Kilbourn et al., 1990) and others (Widen et al., 1992) of specific ligands for the vesicular acetylcholine transporter. [^{11}C]Tetrabenazine and related labeled benzoisoquinolines (DaSilva et al., 1992) show good potential for PET imaging of monoaminergic nerve terminals; development of analogous agents for single photon emission computed tomography (SPECT) is being pursued by others (Kung et al., 1992). In vivo imaging with such agents provides a new approach to the study of monoaminergic nerve terminals.

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