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Imaging the Dopamine Uptake Site with Ex Vivo [^{18}F]GBR 13119 Binding Autoradiography in Rat Brain

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Abstract: We studied the binding of [^{18}F]GBR 13119 {1-[[(4-[^{18}F]fluorophenyl) (phenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine} to rat brain with autoradiography after intravenous injection. The rank order of binding was dorsal striatum > nucleus accumbens = olfactory tubercle > substantia nigra = ventral tegmental area > other areas. Binding was blocked by prior injection of dopamine uptake blockers but not by injection of dopamine receptor antagonists or drugs that bind to the dialkylpiperazine site. Unilateral 6-hydroxydopamine lesions of dopamine neurons caused a marked decrease in striatal and nigral binding on the side of the lesion. We conclude that intravenous injection of [^{18}F]GBR 13119 provides a useful marker of presynaptic dopamine uptake sites. **Key Words:** Dopamine uptake—[^{18}F]GBR 13119—6-Hydroxydopamine lesions—Positron emission tomography scanning. **Ciliax B. J. et al.** Imaging the dopamine uptake site with ex vivo [^{18}F]GBR 13119 binding autoradiography in rat brain. *J. Neurochem.* **55**, 619–623 (1990).

The dopamine (DA) neurotransmitter systems are thought to be involved with schizophrenia, psychosis, tardive dyskinesias, and various basal ganglia disorders (Tarsy and Baldessarini, 1984; Penney and Young, 1986; Seeman, 1987). The nigrostriatal DA neurons have been shown to be destroyed selectively in Parkinson's disease (PD), causing the symptoms of bradykinesia, rigidity, and tremor (Hornykiewicz and Kish, 1987). Several groups have attempted to image quantitatively the DA system using positron emission tomography (PET) and various potential markers, including ^{18}F- or ^{11}C-labeled butyrophenone neuroleptics (Wagner et al., 1983; Wong et al., 1984; Arnett et al., 1985; Perlmutter et al., 1986) or [^{11}C]raclopride (Farde et al., 1986) to mark dopamine D2 receptors, 6-[^{18}F]fluorodihydroxyphenylalanine (6-FDOPA) as a tracer for DA syntheses (Garnett et al., 1983; Calne et al., 1985; Leenders et al., 1986), and [^{11}C]nomifensine as a marker for DA uptake sites (Tedroff et al., 1988). The receptor binding experiments thus far have not shown significant changes in striatal uptake of DA receptor ligands in patients with PD (Hagglund et al., 1987; Rutgers et al., 1987). Significant decreases in 6-FDOPA uptake in PD patients have been reported (Garnett et al., 1983; Calne et al., 1985; Leenders et al., 1986).

The 6-FDOPA results have been difficult to interpret, because several phenomena may affect the final uptake level in PD (Cumming et al., 1987; Firnau et al., 1987). 6-FDOPA is taken up by catecholamine neurons and is then decarboxylated to form 6-[^{18}F]fluorodopamine. In DA neurons, 6-[^{18}F]fluorodopamine can be packaged into synaptic vesicles, metabolized by monoamine oxidase, or released and metabolized by catechol-*O*-methyltransferase. In the periphery, 6-FDOPA is metabolized to 6-[^{18}F]fluoro-3-*O*-methyldihydroxyphenylalanine, which forms the major radioactive species in the blood, at least in primates (Melega et al., 1989).

In PD, the DA cell loss decreases the uptake capacity of the striatum. It is thought that the surviving DA neurons greatly upregulate their DA turnover to compensate for the loss of DA neurons (Hoehn et al., 1976). This increased turnover may decrease the amount of 6-FDOPA that accumulates. Therefore, quantitative evaluation of DA neuronal loss in PD may be very difficult with 6-FDOPA, because its uptake is influenced by many parameters, and PET studies are complicated by the production of radiolabeled metabolites (6-[^{18}F]fluoro-3-*O*-methyldihydroxyphenylalanine).

Although [^{11}C]nomifensine has been used to image changes in PD (Tedroff et al., 1988), nomifensine is not a site-selective ligand. Its IC_{50} for inhibiting nor-

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Abbreviations used: DA, dopamine; 6-FDOPA, 6-[^{18}F]fluorodihydroxyphenylalanine; GBR 13119, 1-[[(4-fluorophenyl) (phenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine; MFB, medial forebrain bundle; PD, Parkinson's disease; PET, positron emission tomography; SNc, substantia nigra pars compacta.

epinephrine uptake into whole brain synaptosomes is 9×10^{-8} *M* (Schacht and Heptner, 1974). Furthermore, nomifensine's sensitivity to intermediate neuronal losses is unknown. Thus, there remains a need for a marker of dopaminergic neuron degeneration for applications in in vivo imaging with PET.

We have proposed a new DA uptake inhibitor labeled with fluorine-18, [^{18}F]GBR 13119 {1-[[(4-[^{18}F]fluorophenyl) (phenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine} (Kilbourn, 1988; Kilbourn and Haka, 1988; Kilbourn et al., 1989*a,b*), as a potential marker for in vivo imaging of DA neurons with PET. We report here the application of ex vivo autoradiography to study the regional distribution of [^{18}F]GBR 13119, the pharmacological specificity of [^{18}F]GBR 13119 for the DA uptake site, and the effects on [^{18}F]GBR 13119 distribution of selective, unilateral dopaminergic neuron destruction with 6-hydroxydopamine.

MATERIALS AND METHODS

Materials

GBR 13119 was a gift of Dr. P. Van der Zee (Gist-Brocades, Delft, The Netherlands); fluoxetine and nisoxetine, of Dr. David Robertson (Eli Lilly, Indianapolis, IN, U.S.A.); *cis*-flupenthixol, of Dr. John Hyttel (H. Lunbeck A/S, Copenhagen, Denmark); nomifesin, of Hoechst-Roussel Pharmaceuticals (Somerville, NJ, U.S.A.); and desipramine, of Merrill-Dow Research Institute. Xylazine was purchased from Mobay Corp. (Shawnee, KN, U.S.A.), and ketamine was from the Parke-Davis Division of Warner Lambert Co. (Morris Plains, NJ, U.S.A.). All other compounds were purchased from Sigma (St. Louis, MO, U.S.A.).

Ex vivo autoradiography

Male Sprague–Dawley rats (weighing 225–450 g) were anesthetized with ethyl ether, catheterized via the left femoral vein with polyethylene tubing, and placed in plastic restraining tubes to recover for at least 2 h (Ciliax et al., 1986). Each rat was injected with [^{18}F]GBR 13119 (0.5–5 mCi) and decapitated 60 min later. The brains were rapidly removed by blunt dissection, frozen on dry ice, thin-sectioned (20 μm) in a cryostat/microtome, and thaw-mounted on gelatin-coated microscope slides. X-ray film (SB-5; Eastman Kodak) was exposed to the sections along with ^{18}F-calibrated ^{14}C-labeled standards for 12 h (Olds et al., 1985). The films were transferred to separate cassettes and developed the next day. The optical densities of the resultant film images were determined using a computer-based image-processing system (Imaging Research, St. Catherines, Ontario, Canada). Radioactivity levels were determined by a computer-generated polynomial regression that compared optical densities of the areas of interest with those of the radioactive standards.

Pharmacologic studies

To evaluate the pharmacology of in vivo [^{18}F]GBR 13119 binding, rats were predosed 30 min before injection of radiotracer with either saline vehicle or one of several possible competitors. The following drugs and doses were injected intravenously: GBR 13119, 10 mg/kg (n = 1); fluoxetine, 20 mg/kg (n = 3); nisoxetine, 5 mg/kg (n = 3); nomifensine, 8 mg/kg (n = 3); and *cis*-flupenthixol, 20 mg/kg (n = 2).

Lesioning

[^{18}F]GBR 13119 was injected into three rats that had been given 6-hydroxydopamine lesions of the medial forebrain bundle (MFB) and substantia nigra pars compacta (SNc) (Pan et al., 1985). To make the lesions, the animals were pretreated with desipramine (15 mg/kg), pargyline (50 mg/kg), and atropine (1.0 mg/kg). Ten minutes later, the rats were anesthetized with xylazine (3.8 mg/kg) and ketamine (77 mg/kg) and placed in a David Kopf small-animal stereotaxic apparatus. The scalp was incised, and the skull was exposed. A small hole was made in the cranium with a dentist drill at each of the two lesion sites. To lesion the SNc, the coordinates used were 5.0 mm posterior to bregma, 2.0 mm lateral of the midline, and 7.4 mm below the cranial surface. The coordinates for the MFB were 3.0, 1.8, and 8.4 mm, respectively. At each site, 10 μg of 6-hydroxydopamine was injected over an 8-min period. The lesioned rats were given 3 weeks to recover and tested for ipsilateral rotations after 2.5 mg/kg of apomorphine i.p. (Ungerstedt, 1971), followed by another 2 weeks of recovery. Only rats that tested positive (>5 rotations/min) were used.

RESULTS

The autoradiograms revealed that the radioactivity concentrated in classical dopaminergic nerve terminal fields in rats injected with [^{18}F]GBR 13119 (Table 1). The rank order of distribution was dorsal striatum > nucleus accumbens = olfactory tubercle = SNc/ventral tegmental area > cortex = cerebellum. This range of binding encompassed values from 0.4 to 1.5 nCi/g of brain. The ratio of striatal-to-cerebellar uptake was 3:1.

Results of the pharmacological studies are given in Fig. 1. Excess unlabeled competitors were injected intravenously 30 min before administration of [^{18}F]GBR 13119. A 10 mg/kg dose of unlabeled GBR 13119 blocked striatal uptake. The antidepressant nomifensine (8 mg/kg), a DA uptake inhibitor (Raiteri et al., 1979), also blocked specific binding in striatum, and the norepinephrine and 5-hydroxytryptamine uptake inhibitors, nisoxetine (5 mg/kg) and fluoxetine (20 mg/kg), respectively, did not block in vivo binding. *cis*-Flupenthixol, which acts as a D1 antagonist but has also been reported to bind to the "dialkylpiperazine

TABLE 1. *Regional distribution of ex vivo [^{18}F]GBR 13119 binding in rat brain*

	nCi/g of brain	
	Left	Right
Dorsal striatum	1.40 ± 0.30	1.46 ± 0.31
Nucleus accumbens	0.94 ± 0.20	0.92 ± 0.19
Olfactory tubercle	0.87 ± 0.19	0.81 ± 0.16
Substantia nigra/ventral tegmental area	0.84 ± 0.15	0.80 ± 0.13
Cortex	0.44 ± 0.06	
Cerebellum	0.37 ± 0.07	

Data are mean ± SEM values.

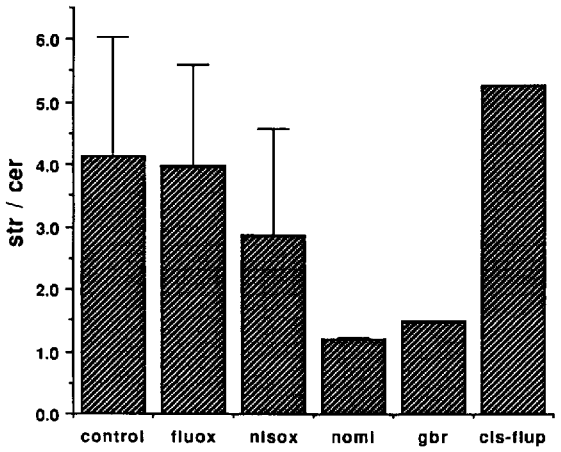

FIG. 1. Pharmacology of ex vivo [^{18}F]GBR 13119 binding in rat brain, shown as the striatum/cerebellum ratio of uptake in rats pretreated with saline vehicle, 20 mg/kg of fluoxetine (fluox), 5 mg/kg of nisoxetine (nisox), 8 mg/kg of nomifensine (nomi), 10 mg/kg of GBR 13119 (gbr), or 20 mg/kg of *cis*-flupenthixol (cis-flup).

site" (Andersen, 1987; Chagraoui et al., 1987), did not decrease the striatal/cerebellum ratio.

Unilateral 6-hydroxydopamine lesions of the dopaminergic nigrostriatal pathway caused significant reductions ($p < 0.005$ by paired t test) in radioactive uptake in dorsal striatum. The levels decreased to those of cerebellum (Fig. 2 and Table 2). Binding in nucleus accumbens and olfactory tubercle was also significantly decreased ($p < 0.02$ by paired t test), and binding in SNc was consistently reduced.

DISCUSSION

A presynaptic marker for dopaminergic neurons would be of considerable value in PET studies of patients with neurological disorders generally ascribed to a loss of DA (for example, PD, progressive supranuclear palsy, and striatonigral degeneration). Such a marker might allow presymptomatic diagnosis, in vivo study of the progression of such diseases, and better evaluation of methods for treatment.

We have developed [^{18}F]GBR 13119 as a potential ligand for the in vivo study of DA terminals using PET. In vitro binding and uptake studies have indicated that drugs of this class [dialk(en)ylpiperazines] bind to and inhibit the high-affinity DA uptake site (Bonnet and Costentin, 1986; Bonnet et al., 1986; Dawson et al., 1986; Janowsky et al., 1986, 1987; Andersen, 1987; Benmansour et al., 1987; Chagraoui et al., 1987). The in vivo regional brain distribution of radioactivity in

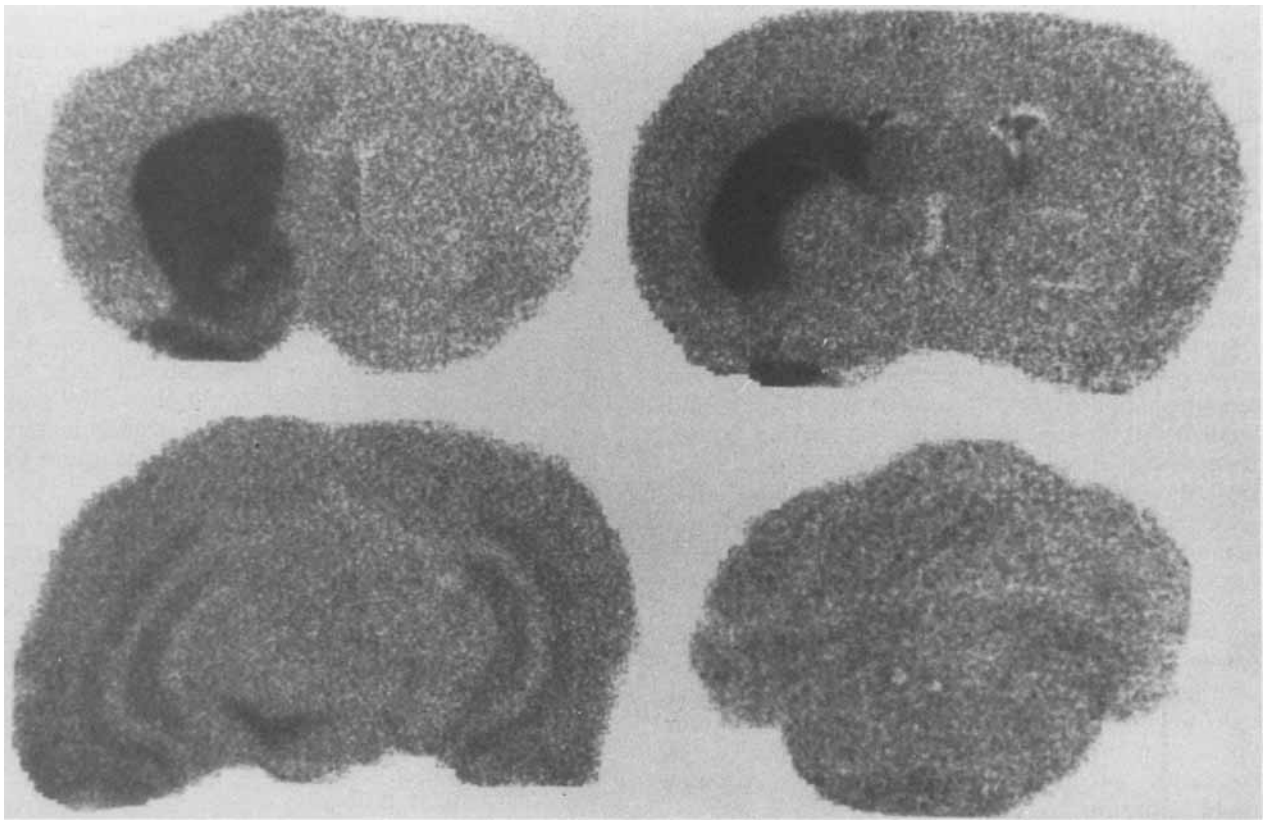

FIG. 2. Autoradiogram of ex vivo [^{18}F]GBR 13119 binding in a MFB-lesioned rat. Coronal sections pass through (**top left**) the striatum, (**top right**) the globus pallidus, (**bottom left**) the substantia nigra, and (**bottom right**) the cerebellum. Binding in striatum and substantia nigra is reduced on the side of the lesion.

TABLE 2. *Effects of unilateral 6-hydroxydopamine lesions of the MFB on regional distribution of ex vivo [^{18}F]GBR 13119 binding in rat brain*

	nCi/g of brain	
	Unlesioned	Lesioned
Dorsal striatum	2.81 ± 1.31	0.91 ± 0.20[a]
Nucleus accumbens	1.91 ± 0.76	0.99 ± 0.18[a]
Olfactory tubercle	1.80 ± 0.75	0.89 ± 0.15[b]
Substantia nigra/ventral tegmental area	2.46 ± 0.93	1.25 ± 0.30
Cortex	0.91 ± 0.18	
Cerebellum	0.85 ± 0.18	

Data are mean ± SEM values. Values are twice those of Table 1 because twice as much radioactivity was used.
[a] $p < 0.005$ by paired t test.
[b] $p < 0.02$ by paired t test.

rats after [^{18}F]GBR 13119 injection (Table 1) is consistent with the known distribution of dopaminergic innervation, with higher levels in striatum, nucleus accumbens, olfactory tubercle, and SNc/ventral tegmental area and low levels in cortex and cerebellum.

The specificity of [^{18}F]GBR 13119 binding to the DA uptake site was examined by preadministration of pharmacological doses of various competing drugs. Binding of [^{18}F]GBR 13119 (expressed as striatum/cerebellum ratios; Fig. 1) was blocked by drugs specific for the DA uptake site (GBR 13119 and nomifensine) but was not changed after pretreatment with high-affinity and selective serotonin (fluoxetine) or norepinephrine (nisoxetine) uptake inhibitors or after pretreatment with *cis*-flupenthixol, a D1 antagonist. The ex vivo specificity of this class of compounds for DA uptake sites thus agrees with the selectivity shown in vitro (Van der Zee et al., 1980; Janowsky et al., 1986).

The degree of variability is high in these pharmacological results. We believe this variability is inherent in work with short-lived isotopes that must be synthesized separately for each experiment. For example, the autoradiographic experiments are similar in results and variability to those of our previous dissection experiments with [^{18}F]GBR13119, in which regional brain distribution of [^{18}F]GBR 13119 was unchanged after pretreatment with dopamine D1 (*cis*-flupenthixol) and dopamine D2 (spiperone) receptor antagonists (Kilbourn, 1988) or pretreatment with nisoxetine or fluoxetine but was significantly decreased by pretreatment with GBR13119 and nomifensine (Kilbourn et al., 1989*a*).

Unilateral 6-hydroxydopamine lesions are a good model for in vivo dopaminergic neuron degeneration (Ungerstedt, 1971). If [^{18}F]GBR 13119 is a marker for presynaptic uptake sites, then its binding in striatum should be diminished on the side of such a lesion.

6-Hydroxydopamine lesions of the nigrostriatal pathway were confirmed by assays of rotational locomotion following apomorphine injections. Rats testing positive should have >95% of the DA neurons eliminated (Ungerstedt, 1971). [^{18}F]GBR 13119 binding in lesioned animals was greatly decreased ipsilateral to the side of the lesion (Fig. 2). Thus, [^{18}F]GBR 13119 binding sites appear to be presynaptic and could be useful markers of viable DA nerve terminals.

Several reports have cautioned against using GBR drugs for in vivo markers, because they bind to the "dialkylpiperazine site" in vitro (Andersen et al., 1987; Chagraoui et al., 1987; Filloux et al., 1989). This secondary site obscured in vitro binding of [^{3}H]GBR 12935 (the nonfluorinated analog of GBR 13119) to the DA uptake site, effectively masking any detectable changes of the DA uptake sites caused by 6-hydroxydopamine lesions of the MFB. Our results indicate that this secondary site may be artifactual, caused by the in vitro conditions. After in vivo injections (present study; Kilbourn, 1988; Kilbourn et al., 1989*b*), we see good striatal-to-cerebellar binding ratios; binding in striatum is significantly decreased after nigrostriatal lesions; drugs specific for the DA uptake site block striatal binding; and 20 mg/kg of *cis*-flupenthixol, which does not bind to the DA uptake site but does bind to the "dialkylpiperazine site," does not block [^{18}F]GBR 13119 binding or alter its regional brain distribution. These findings strongly suggest that [^{18}F]GBR 13119 binds to the DA uptake site in vivo with good specificity and that under in vivo conditions the secondary "dialkylpiperazine site" accounts for a negligible percentage of specific [^{18}F]GBR 13119 binding. Another possibility is that the fluorine substituent on GBR 13119 dramatically decreases its affinity for the "dialkylpiperazine site." However, preliminary in vitro binding autoradiography experiments using [^{18}F]GBR 13119 have shown problems similar to those encountered with [^{3}H]GBR 12935 in that the binding is largely insensitive to mazindol and is not quickly washed away.

In vivo [^{18}F]GBR 13119 binding may be a useful presynaptic marker for DA neurons. It is specific for the DA uptake site, and [^{18}F]GBR 13119 binding is sensitive to the destruction of nigrostriatal cells. Because we also are able to image [^{18}F]GBR 13119's distribution in primate brain using PET (Kilbourn et al., 1989*a*), we are highly encouraged about its future application in clinical PET studies.

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