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IN VIVO BINDING OF [^{18}F]GBR 13119 TO THE BRAIN DOPAMINE UPTAKE SYSTEM

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Summary

Regional rat brain uptake of [^{18}F]GBR 13119, a high specific activity, positron-emitter labeled derivative of the potent dopamine uptake antagonist GBR 12935, is reported. Striatum to cerebellum ratios of 3 are obtained at 90 minutes post injection. Specific binding in striatum can be blocked by pretreatment with dopamine uptake system antagonists (mazindol, nomifensine) but not with receptor antagonists (spiperone, flupenthixol). [^{18}F]GBR 13119 is proposed as a new positron-emitting radioligand for in vivo PET studies of the pre-synaptic dopamine uptake system.

Dysfunctions of the brain dopaminergic system have been demonstrated as the underlying biochemical change in certain neurological diseases (Parkinson's disease) (1) and have been implicated in psychiatric disorders (schizophrenia) (2). The study of the dopamine system in vivo using Positron Emission Tomography (PET) has been vigorously pursued. Most PET studies of dopamine receptor pharmacology have centered on measurements of the number (Bmax) and affinity (Kd) of post-synaptic receptors (or some combination of these terms) using carbon-11, fluorine-18 or bromine-75 labeled neuroleptics (e.g., spiperone) or benzamides (e.g., raclopride) for D_2 receptors, and benzazepines (e.g., SCH 23390) for D_1 receptors (3,4). Determination of DOPA uptake and in vivo metabolism to dopamine has been approached using [1-^{11}C]DOPA and 6-[^{18}F]fluoroDOPA. Promising results in PET studies of Parkinson's patients (5), MPTP-poisoned individuals (6), and schizophrenics (7) have been reported using these various radiopharmaceuticals.

A third component of the dopaminergic system is the pre-synaptic dopamine uptake system, which is important in the regulation of dopamine concentration in the synapse. Numerous compounds have been reported as antagonists of the uptake system, among them nomifensine, mazindol, methylphenidate, and GBR 12935: labeling of such ligands with carbon-11 or fluorine-18 would produce potential in vivo markers of dopaminergic neuronal density. Nomifensine has been recently reported in carbon-11 labeled form; in vivo studies have been described as promising but evaluation remains incomplete (8). Reported here is the regional in vivo brain uptake of [^{18}F]GBR 13119 ((1-[4-[^{18}F]fluorophenyl)phenylmethoxy] ethyl-4-(3-phenylpropyl)piperazine, Figure 1), a fluorine-18 labeled derivative of the potent and selective dopamine uptake antagonist GBR 12935. The results are also compared to recently reported in vivo data obtained with the structurally similar dopamine uptake antagonist [^{3}H]GBR 12783.

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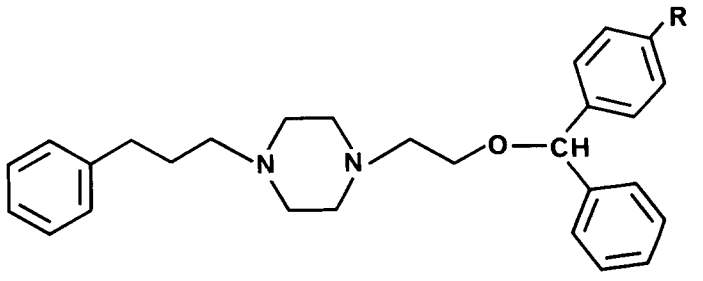

FIG 1.

Chemical structures of [^{18}F]GBR 13119 (R=^{18}F) and GBR 12935 (R=H).

Materials and Methods

Chemicals

[^{18}F]GBR 13119 was prepared in high radiochemical purity (> 99%) and high specific activity (> 1000 Ci/mmol) by a four-step synthesis reported elsewhere (9). Mazindol was obtained from Sandoz Pharmaceuticals, and nomifensine from Hoechst-Roussel.

Animal Studies

Sprague-Dawley rats (150-200 g) were anesthesized and injected with [^{18}F]GBR 13119 (10-600 μCi) in saline - 5% ethanol, via the surgically exposed femoral vein. At designated times animals were sacrificed by decapitation and the brain rapidly dissected (10). Tissue samples were weighed, then counted in an automatic gamma counter.

For blocking studies animals were injected, 30 minutes prior to radiotracer injection, with appropriate amounts of competing ligand dissolved in dilute acetic acid - saline solution. Control animals were injected with vehicle alone. Data were analyzed for statistical analysis using a two-tailed student's t-test: p values are given in the Tables.

Results

The time course of [^{18}F]GBR 13119 uptake and retention in various brain regions (striatum, cortex and cerebellum) is shown in Table I. Uptake of radiotracer is rapid, with 0.608% of the injected dose in the brain at 2 minutes. Whole brain radioactivity decreases thereafter. Radioactivity is well retained in striatum, and the striatum to cerebellum ratio (target to nontarget ratio) increases to 3 at 90 minutes post injection.

Pharmacological blocking studies of [^{18}F]GBR 13119 are shown in Tables II and III. Pretreatment with 8 mg/kg mazindol or nomifensine, compounds which inhibit monoamine uptake (including dopamine), completely blocks selectivity in retention of [^{18}F]GBR 13119 in striatum, with striatum-to-cerebellum ratios reduced to one. Pretreatment with the dopamine receptor antagonists spiperone (D_2 receptors) or cis-flupenthixol (D_1 receptors) has no effect on either striatal uptake and retention or striatum-to-cerebellum ratios.

TABLE I*

Time (min)	1	5	30	60	90	120
Cerebellum	0.378 ± .034	0.348 ± .013	0.148 ± .024	0.132 ± .031	0.096 ± .008	0.091 ± .005
Cortex	0.415 ± .043	0.385 ± .018	0.218 ± .028	0.187 ± .035	0.127 ± .002	0.113 ± .016
Striatum	0.408 ± .022	0.405 ± .036	0.307 ± .049	0.294 ± .064	0.287 ± .044	0.244 ± .032
Blood	0.429 ± .019	0.125 ± .015	0.0597 ± .013	0.035 ± .036	0.039 ± .004	0.035 ± .009
Striatum / Cerebellum	1.08 ± .09	1.16 ± .096	2.06 ± .106	2.58 ± .95	2.97 ± .28	2.68 ± .31
Striatum / Cortex	0.98 ± .06	1.04 ± .06	1.40 ± .124	1.55 ± .28	2.25 ± .38	2.18 ± .34
Cortex / Cerebellum	1.10 ± .13	1.11 ± .04	1.47 ± .138	1.51 ± .22	1.33 ± .10	1.24 ± .13
Striatum / Blood	0.95 ± .026	3.28 ± .59	5.31 ± 1.3	9.41 ± 3.3	7.66 ± 2.5	7.50 ± 2.1
% ID/brain	0.671 ± .044	0.642 ± .03	0.356 ± .055	0.283 ± .077	0.221 ± .011	0.206 ± .023

*3-11 animals per data point.

Time Course of [^{18}F]GBR 13119 Uptake In Vivo in Rat Brain

TABLE II*

	control	8 mg/kg mazindol	control	8 mg/kg nomifensine	300 ug/kg nomifensine	300 ug/kg mazindol
time	30 min	30 min	60 min	60 min	60 min	60 min
striatum	0.307 ± .049	0.169 ± .009**	0.294 ± .064	0.157 ± .023**	0.204 ± .004**	0.203 ± .023**
cortex	0.218 ± .028	0.227 ± .010	0.187 ± .035	0.188 ± .019	0.146 ± .013	0.152 ± .005
cerebellum	0.148 ± .024	0.171 ± .033	0.132 ± .031	0.147 ± .017	0.117 ± .003	0.121 ± .005
blood	0.059 ± .013	0.053 ± .012	0.035 ± .0036	0.040 ± .005	0.042 ± .004	0.032 ± .002
striatum / cerebellum	2.06 ± .106	0.99 ± .076**	2.58 ± .95	1.08 ± .062**	1.75 ± .02***	1.68 ± .20***

*Pretreatment 30 minutes before radiotracer injection, with 4-11 animals per data point.
**$p < 0.05$
***$0.14 < p < 0.18$

Effects of Dopamine Uptake Antagonists on [^{18}F]GBR 13119
Uptake In Vivo in Rat Brain

TABLE III*

	control	300 ug/kg flupenthixol	300 ug/kg spiperone
time	60 min	60 min	
		% ID/g	
striatum	0.294 ± .064	0.361 ± .009	0.277 ± .010
cortex	0.187 ± .035	0.190 ± .010	0.172 ± .050
cerebellum	0.132 ± .031	0.138 ± .006	0.141 ± .031
blood	0.035 ± .0036	0.059 ± .006	0.038 ± .012
striatum / cerebellum	2.58 ± .95	2.58 ± .007**	1.91 ± .330**

*Pretreatment 30 minutes before radiotracer injection.
4-10 animals per data point.
**p > 0.12

Effects of Dopamine Receptor Antagonists on [^{18}F]GBR 13119 Uptake In Vivo in Rat Brain

Discussion

The disubstituted piperazines of structure similar to GBR 12935 (Figure 1) are a class of compounds which exhibit high affinity (K_D=2-3 nM for [^{3}H]GBR 12935) for the dopamine uptake system and good selectivity for the dopamine system relative to other monoamine uptake systems (11). These characteristics make this class of compounds more appealing as candidates for carbon-11 or fluorine18 labeling for PET studies than nomifensine or mazindol, drugs which are actually better antagonists of norepinephrine than dopamine uptake and have lower affinities *in vitro* for the dopamine uptake system (nomifensine K_D=230 nM, mazindol K_D=18 nM (12)). In addition, substitution of the aromatic rings of GBR 12935 with one or more fluorines was known to produce derivatives with similar affinities and selectivities to that of GBR 12935 (11).

The in vivo behavior of [^{18}F]GBR 13119 in rat brain is consistent with in vitro studies with [^{3}H]GBR 12935 (13,14) and a single in vivo study using [^{3}H]GBR 12783 (1,2-(diphenylmethoxy)ethyl-4-(3-phenyl-2-propenyl)-piperazine) (15). Retention of radioactivity is highest in the striatum (Table I), with radioactivity in cerebral cortex and cerebellum steadily decreasing with time. The striatum-to-cerebellum ratio reaches a maximum of 3 at 90 minutes, similar to the ratio obtained in vivo with [^{3}H]GBR 12783 at 60 minutes. Pharmacological blocking studies (Tables II and III) are consistent with radiotracer binding to the dopamine uptake system in vivo, and not to dopamine receptors. Uptake into the striatum is completely blocked by a pharmacological dose (8 mg/kg) of mazindol or nomifensine: cortex and cerebellum are unaffected, suggesting uptake in those brain regions is largely non-specific. Striatum-to-cerebellum ratios remain unchanged by pretreatment with the dopamine receptor antagonists spiperone (D_2) and flupenthixol (D_1). The dose of spiperone utilized is sufficient to effectively block [^{18}F]spiperone uptake in rat striatum (16). Equivalent doses (300 ug/kg) of nomifensine or mazindol only partially block the striatal uptake and retention of [^{18}F]GBR 13119: in this study the decrease in striatum/cerebellum ratio was not as significant. A similar decrease in striatum radioactivity levels (but not str/cer ratio) was observed in the in vivo

dose-response study of [^{3}H]GBR 12783 (15).

In one published in vitro study of [^{3}H]GBR 12935, Andersen proposes two separate binding sites for this radioligand (14). The first is the classical dopamine uptake system, which could be blocked by drugs such as mazindol and nomifensine. The second site proposed was a high affinity, uniformly distributed binding of disubstituted piperazines, which could be blocked in vitro with flupenthixol and piflutixol. In this study, it was not possible to distinguish if the binding in non-target tissue (cerebellum) was due to binding to this purported piperazine binding site, or was simply non-specific binding as observed with many other lipophilic radiotracers (3,4,17).

[^{18}F]GBR 13119 would thus appear to be a good candidate for further evaluation as a marker for the presynaptic dopamine uptake system. Brain uptake, regional selectivity, and pharmacological selectivity are all consistent with successful application in in vivo PET imaging. Non-specific binding in brain tissue is high, reflective of the lipophilic nature of this class of compounds. Determination of kinetic parameters (association and dissociation rates) and B_{max} values will most likely require mathematical modeling of tissue-activity curves obtained in PET studies (17). The studies reported here are, of course, only the first steps in the development and validation of a radiopharmaceutical for PET studies in humans. Questions of brain uptake and selectivity in primates (possible species differences), confounding aspects of radiotracer metabolism, difficulties in quantitative estimation of relevant biochemical parameters, and radiation dosimetry problems all remain unanswered. Successful development of a marker for the dopamine uptake system would be of great potential in the study of degenerative neurological diseases, such as Parkinson's disease: decreased binding of [^{3}H]GBR 12935 *in vitro* to caudate nucleus of Parkinson's patients (post-mortem) has been recently reported (18). Although others have cautioned, appropriately, with extending *in vitro* results to *in vivo* situations (19,20), such results are clearly an impetus for further evaluation of [^{18}F]GBR 13119.

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