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Short communication

Regional brain distribution of [^{18}F]GBR 13119, a dopamine uptake inhibitor, in CD-1 and C57BL/6 mice

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We have examined the regional brain distribution of [^{18}F]GBR 13119 (^{18}F: $\beta+$, $T_{1/2} = 110$ min), a dopamine uptake inhibitor, in CD-1 and C57BL/6 mice. High levels of binding are observed in the striatum of both species, with striatum/cerebellum ratios of 3-4 at 60 min after injection of the radiotracer. Striatum radioactivity and striatum/cerebellum ratios are more than 50% reduced in C57BL/6 mice treated chronically with the neurotoxin MPTP. We conclude mice are an appropriate model for the in vivo study of the dopamine uptake system, and that [^{18}F]GBR 13119 may be a suitable in vivo marker for degeneration of striatal dopaminergic neurons.

Dopamine; Dopamine uptake inhibitors; 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); (Mouse)

1. Introduction

The aryl-1,4-dialk(en)ylpiperazines are a class of compounds which have received considerable attention as selective and high affinity inhibitors of the neuronal dopamine uptake process. Two of these compounds, GBR 12935 (1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine) and GBR 12783 (1-[2-diphenylmethoxy)ethyl]-4-(3-phenyl-2-propenyl)piperazine) have been available in tritium labeled form and extensively evaluated both in vitro and in vivo for specificity and affinity for the dopamine transport system. Recently, it was reported that [^{3}H]GBR 12935, when injected in vivo into NMRI mice, did not show a regional brain distribution (analysis of bound radioactivity after tissue removal, homogenization and filtration) consistent with dopaminergic neuron distribution (Andersen et al., 1987). These authors propose the presence of a 'piperazine acceptor site' to account for the observed uniform distribution of [^{3}H]GBR 12935 in their study. However, in vivo studies in CD-1 mice with the closely related compound [^{3}H]GBR 12783 showed specific binding of the radiotracer to expected dopaminergic neuronal fields (striatum, nucleus accumbens) as determined by ex vivo counting or autoradiography (Chagraoui et al., 1987; Leroux-Nicollet and Costentin, 1988).

We have recently prepared a fluorine-18 labeled analog, [^{18}F]GBR 13119 (1-[(4-[^{18}F]fluorophenyl)(phenyl)methoxy)ethyl]-4-(3-phenylpropyl)piperazine; Kilbourn and Haka, 1988) which differs from GBR 12935 in only a single radioactive fluorine atom (^{18}F: $\beta+$, $T_{1/2} = 110$ min). Our goal is the development of this compound as a radiopharmaceutical for the study of the dopamine uptake system in man using positron emission tomography (PET). We have extensively evaluated this radiotracer in vivo in rats (Kilbourn, 1988) and in primates (Kilbourn et al., in press)

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and have consistently observed a regional localization of the radiotracer in dopaminergic neuron-rich brain regions (caudate/putamen, nucleus accumbens, olfactory tubercle, entopeduncular nucleus, subthalamic nuclei, substantia nigra, ventral tegmental area: determined by in vivo autoradiography), and this localization can be blocked in vivo with pharmacological doses of dopamine uptake inhibitors but not dopamine receptor antagonists, not other monoamine uptake inhibitors (nisoxetine, fluoxetine). The possible existence of a high affinity but non-specific binding site, and possible species differences of regional brain distribution for this class of compounds (Andersen et al., 1987), were disturbing. We have therefore examined the regional in vivo brain distribution of [^{18}F]GBR 13119 in two species of mice (CD-1 and C57 BL/6), and in preliminary experiments have examined [^{18}F]GBR 13119 biodistribution after chronic MPTP treatment of C57BL/6 mice.

2. Materials and methods

2.1. Drugs

[^{18}F]GBR 13119 was prepared by methods previously described (Kilbourn and Haka, 1988): the product is obtained in high radiochemical purity (> 98%) and very high specific activity (> 2000 Ci/mmol). MPTP was prepared by N-methylation of 4-hydroxy-4-phenylpiperidine (Aldrich Chem. Co.) followed by acid-catalyzed dehydration.

2.2. Regional brain distribution of [^{18}F]GBR 13119

Mice (male CD-1, 20-25 g; female C57BL/6, 25-30 g, all from Charles River) were injected via the tail vein with 7-10 μCi of [^{18}F]GBR 13119. At 60 min the animals were killed by decapitation, and the brain rapidly removed and dissected into regions of interest (striatum, cortex and cerebellum). Blood samples were obtained, and the remainder of the brain tissue also collected. The tissue samples were weighed and then counted for fluorine-18 in an automatic gamma counter. Data were calculated as %ID/g for all tissues including blood.

2.3. MPTP lesioning of C57BL/6 mice

Lesioning of the C57BL/6 mice was done by a modification of a literature procedure (Ogawa et al., 1985). Male mice (age 10 weeks) were treated twice daily for four days with 30 mg/kg s.c. of a solution of MPTP in saline. Unfortunately this regimen produced a high mortality rate, with only two survivors from a group of 12 animals. This study of [^{18}F]GBR 13119 uptake was performed 4.5 weeks after the cessation of MPTP treatments. Animals showed no outward signs of neurological disorder.

TABLE 1

In vivo regional brain distribution of [^{18}F]GBR 13119 at 60 min after i.v. injection into mice and rats. Data are given as means ± S.D.

	CD-1 mice [a]	C57BL/6 mice [a]	C57BL/6 MPTP-treated		SD rats [b,c]
			1	2	
%ID/g					
Striatum	2.8 ±0.11	2.9 ±0.9	0.63	0.93	0.294±0.06
Cortex	0.98±0.08	1.13±0.21	0.45	0.75	0.187±0.03
Cerebellum	0.66±0.06	0.86±0.1	0.39	0.52	0.132±0.03
Blood	0.89±0.16	0.70±0.14	0.49	0.56	0.035±0.004
Ratios					
str/cer	4.24±0.48	3.35±0.97	1.61	1.79	2.58 ±0.95
str/cor	2.98±0.32	2.51±0.52	1.4	1.24	–
str/blood	3.3 ±0.58	4.4 ±2.0	1.29	1.67	–

[a] N = 4; [b] N = 11; [c] data from Kilbourn, 1988.

3. Results

3.1. Regional brain distribution of [^{18}F]GBR 13119 in control mice

The distribution of [^{18}F]GBR 13119 in the brains of the two species of mice is shown in table 1; for comparison similar results obtained in rats (Kilbourn, 1988) are shown. The regional distribution of [^{18}F]GBR13119 is quite similar for rats and mice, with higher levels in the dopaminergic neuron-rich striatal tissue. Striatum to cerebellum ratios for both species of mice are greater than 3 at 60 min, higher than that found in rats. Absolute levels of radiotracer in mouse brain is higher than found with rats, most likely due to the smaller body mass and greater delivery of tracer to the brain.

3.2. [^{18}F]GBR 13119 distribution in MPTP-treated mice

The uptake and retention of [^{18}F]GBR 13119 in the two MPTP-treated C57BL/6 mice, expressed both as %ID/g in striatum and as striatum-to-cerebellum ratios, were clearly lower. Because of inter-subject variability in animal data, the striatum/cerebellum ratio is more reliable; as can be seen in table 1 there is approximately a 50% reduction in this ratio, as compared to controls, in the MPTP-treated C57BL/6 mice.

4. Discussion

The diarylalk(en)ylpiperazines, exemplified by GBR 12935, GBR 12783 and GBR 13119, are high affinity and very selective inhibitors of the dopamine uptake process and are very valuable tools for both in vitro and in vivo studies. In response to a published report of species differences in the binding of this class of drugs to the dopamine uptake site (Andersen et al., 1987), we examined here the regional brain distribution of [^{18}F]GBR 13119 in CD-1 and C57BL/6 mice, and compared this data to that previously obtained in Sprague-Dawley rats. Contrary to the results obtained with NMRI mice (striatum/cerebellum ratio of 1; Andersen et al., 1987), we find that regional differences in [^{18}F]GBR 13119 uptake and retention are observed in both CD-1 and C57BL/6 mice. Striatum/cerebellum ratios are greater than 3 at 60 min after injection.

Our results are consistent with the previous reports of regional uptake of [^{3}H]GBR 12783 (26 Ci/mmol) in CD-1 mice (Chagraoui et al., 1987; Leroux-Nicollet and Costentin, 1988). Striatum/cortex ratios in CD-1 mice determined with [^{18}F]GBR 13119 (2.5-3) are higher than reported with [^{3}H]GBR 12783 (approximately 1.3-1.5 by autoradiography (Leroux-Nicollet and Costentin, 1988); 2.1 by ex vivo counting (Chagraoui et al., 1987)). In the development and applications of in vivo radiotracers, it is often assumed that levels of non-specific binding are uniform throughout the brain. Thus, binding of dopaminergic tracers in the cerebellum (a tissue containing few dopaminergic neurons) is taken to represent non-specific binding, and subtraction of radioactivity levels in cerebellum from levels in target tissue can be used to roughly estimate specific binding. By such calculations 70-80% of the radioactivity in the striatum of mice is specifically bound to the DA uptake system. The discrepancy between our data and that obtained by autoradiography with [^{3}H]GBR 12783 is not easily explained; we have in fact previously obtained *better* striatum-to-cerebellum ratios in rats using quantitative in vivo autoradiography than with ex vivo counting (Ciliax et al., submitted for publication). As we have used a markedly different type of radiotracer in our studies, the explanation may lie in the higher specific activities of [^{18}F]GBR 13119, different chemical stabilities of the radioactive labels, or different metabolites (with different in vivo biodistributions). Significantly, the striatum/cerebellum ratio obtained with [^{18}F]GBR 13119 in CD-1 mice continues to improve at longer time periods (str/cer = 4.96 at 5 h), in contrast to the decline in this ratio reported for [^{3}H]GBR 12783 using a very similar protocol (Chagraoui et al., 1987).

C57BL/6 mice are extremely sensitive to neurochemical poisoning with MPTP (Heikkila et al., 1984). We therefore attempted to determine if [^{18}F]GBR 13119 uptake and binding will be altered in such mice, using a chronic MPTP treat-

ment regimen. In our hands the dose of 30 mg/kg s.c. (twice daily, 4 days) proved fatal to a large proportion of the animals, and we obtained only two survivors from a group of 12. Previous attempts made in our laboratories, using dose regimens of 30 mg/kg (i.p. or s.c.) for 5 days (Ogawa et al., 1985), had proved uniformly fatal. We have not been able to determine the reasons for our difficulties in reproducing this animal model, but toxicity of MPTP in mice has been noted by others (Ricaurte et al., 1987). Nevertheless, the two mice which did survive the treatment protocol were then utilized in a study of [^{18}F]GBR 13119 brain distribution and found to exhibit significantly lower striatum/cerebellum ratios (table 1). Previously, such MPTP treatment effects on the dopamine reuptake system of mice had been determined by in vitro analysis of excised and homogenized tissue, where reductions of 80% of [^{3}H]mazindol binding were observed (Sundstrom et al., 1988). Our results suggest that [^{18}F]GBR 13119 will be a suitable radiotracer for the in vivo measurement of dopaminergic neuron degeneration; studies to correlate levels of [^{18}F]GBR 13119 binding and dopamine concentrations are underway.

In conclusion, we have examined the in vivo brain distribution of [^{18}F]GBR 13119 in CD-1 and C57BL/6 mice and found that it is consistent with dopaminergic neuron distribution. It is not clear if the previous report of a lack of regional [^{3}H]GBR 12935 binding in NMRI mice is due to methodological problems or if it represents a true species difference. Our results, however, are consistent with those striatum/cerebellum ratios we have found in rats, and in primates using PET (Kilbourn et al., in press), and suggests that mice are suitable subjects for in vivo studies with radiolabeled dopamine uptake inhibitors, including the use of MPTP treatment as an animal model of loss of dopaminergic neurons.

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