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Repeated reserpine administration reduces in vivo [^{18}F]GBR 13119 binding to the dopamine uptake site

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The effects of repeated reserpine on the in vivo regional brain distribution of [^{18}F]GBR 13119 (1-[(4-[^{18}F]fluorophenyl)(phenyl)methoxy)ethyl]-4-(3-phenylpropyl)piperazine), a dopamine uptake inhibitor, have been examined. Repeated parenteral administration of reserpine (2 mg/kg i.p., once daily for three days) causes a decrease of the in vivo specific binding of [^{18}F]GBR 13119 in mouse striatum, consistent with a down-regulation of available uptake sites in response to dopamine depletion. These results indicate that modification of endogenous dopamine concentrations, either due to pathological disturbance or pharmacological interventions, may affect in vivo studies of the dopamine uptake system using radioligands of the 1,4-dialk(en)ylpiperazine class, and complicate the intepretation of in vivo human studies of these radioligands using positron emission tomography.

Dopamine uptake; Reserpine; [^{18}F]GBR 13119 binding; (In vivo); (Mouse)

1. Introduction

Dopamine (DA) reuptake by presynaptic neurons is an important component of the termination of neurotransmission, and serves a dual function as a means for recovery and conservation of synthesized DA. The specific, membrane-bound dopamine uptake carrier has been proposed by ourselves and others as a candidate for new radioligand development for in vivo imaging of dopaminergic neurons. These efforts have resulted in successful imaging of the DA uptake site in living human brain using [^{11}C]nomifensine (Aquilonius et al., 1987), [^{18}F]GBR 12909 (Koeppe et al., 1990), and [^{11}C]cocaine (Fowler et al., 1989).

The interpretation of changes in the in vivo distribution of such radiotracers is not straightforward. To date, decreases in radiotracer uptake due to stereotactic lesioning (Ciliax et al., 1990) or neurotoxic insult (Leenders et al., 1988; Kilbourn et al., 1991a) in animals, or disease in humans (Salmon et al., 1990), has been attributed to the loss of dopaminergic neurons. Such conclusions would be supported by experimental data which demonstrates that in vivo radioligand binding is not affected by levels of endogenous neurotransmitter. Previous investigations have provided contradictory results on the effects of DA depletion or enhancement on the binding of radioligands for the DA uptake site (Janowsky et al., 1985; Vaugeois et al., 1990; Maurice et al., 1991). These different results could be due to methodological issues, or the use of structurally different radioligands. We have examined here the effects of repeated treatments with reserpine, a vesicular monoamine uptake blocker which depletes tissue DA, on the in vivo binding of [^{18}F]GBR 13119 (1-((4-[^{18}F]fluorophenyl)(phenyl)methoxy)ethyl]-4(-3-phenylpropyl)piperazine), a DA uptake inhibitor of the 1,4-dialk(en)ylpiperazine class.

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2. Materials and methods

2.1. Animals and treatments

All studies were done in female CD-1 mice, 20–25 g (Charles River). Six treated animals and an equal number of untreated control animals were used. Reserpine treatment regimen was 2 mg/kg. i.p., once daily for three days. Animals were used for in vivo radiotracer experiments 24 h after the last reserpine injection.

2.2. Radioligands

No-carrier-added, high specific activity (> 1000 Ci/mmol) [^{18}F]GBR 13119 was prepared as previously described (Kilbourn and Haka, 1989).

2.3. In vivo radiotracer studies

Mice were anaesthetized (diethyl ether) and injected with 2–10 μCi of [^{18}F]GBR 13119 (mass dose 17 to 86 ng/kg), then allowed to recover. After 1 h, the animals were killed by decapitation and the brain rapidly excised and dissected into striatum, whole cortex, and whole cerebellum. A blood sample was also obtained. The tissue samples were weighed and then counted for fluorine-18 using an automated γ-counter. These data were used to calculate the % injected dose/g (%ID/g) for each brain region and for blood.

2.4. Statistical analysis

Statistical analysis was conducted using an unpaired Student's t-test.

3. Results

Repeated reserpine (2 mg/kg i.p., once daily for three days) significantly decreased the concentration of [^{18}F]GBR 13119 in the mouse striatum, as shown in table 1. Specific binding, expressed as either the difference between striatum and cerebellum or as the striatum/cerebellum ratio, were also significantly reduced by > 39%. Tissue concentrations of the radiotracer was significantly increased in cortex, cerebellum and blood, as compared to controls, but there was no specific binding of [^{18}F]GBR 13119 in the cortex of either controls or reserpine-treated animals. For this study, radioligand accumulation in cerebellum was assigned as due only to non-specific binding, as there are no DA uptake carriers in cerebellum and the uptake of [^{18}F]GBR 13119 in this brain region is unaffected by pharmacological pretreatment with the DA uptake inhibitors mazindol, nomifensine of GBR 12909 (Kilbourn, 1988).

TABLE 1

Effects of repeated reserpine (2 mg/kg i.p., once daily for three days, last injection 24 h prior to injection of radiotracer) on the regional mouse brain accumulation of [^{18}F]GBR 13119. Radiotracer was injected i.v. and animals killed at 1 h after injection. Values are means ± S.D. (n = 6). Numbers in parentheses are the differences between control and reserpine-treated animals.

Tissue	Control (% injected dose/g)	Reserpine (% injected dose/g)
Striatum	2.33 ± 0.63	1.77 ± 0.19 (−25%) [a]
Cortex	0.64 ± 0.13	0.91 ± 0.14 (+42%) [a]
Cerebellum	0.58 ± 0.13	0.75 ± 0.16 (+29%) [a]
Blood	1.03 ± 0.11	1.55 ± 0.27 (+50%) [a]
Striatum − cerebellum	1.66 ± 0.6	1.05 ± 0.39 (−58%) [a]
Striatum/cerebellum	3.66 ± 0.71	2.25 ± 0.39 (−39%) [a]

[a] $P < 0.05$ vs. control.

4. Discussion

Radiolabeled DA uptake inhibitors have been proposed as in vivo markers of neuronal integrity, with an anticipated use in monitoring the progression of degenerative processes such as Parkinson's disease (Aquilonius et al., 1987; Koeppe et al., 1990; Salmon et al., 1990). To function as a marker of neurons, radioligand binding should not be influenced by transient alterations in endogenous DA levels. Acute reserpine (5 mg/kg, 4 h prior) or L-DOPA (200 mg/kg i.p. + 50 mg/kg benzeraside, 2 h prior) have been reported not to affect the specific striatal in vivo accumulation of [^{3}H]GBR 12783 in mouse brain (Vaugeois et al., 1990), but the same treatments have been reported to increase (reserpine treatment) and decrease (L-DOPA treatment) the specific in vivo binding of [^{3}H]BTCP ([^{3}H]N-[1-(2-benzo(b)thiophenyl)-cyclohexyl]piperidine) (Maurice et al., 1991). These studies cannot be directly compared, however, as they use different radioligands and different methods of determining in vivo specific binding.

In our hands, repeated reserpine treatment (table 1) decreased the in vivo specific striatal binding, defined as striatum minus cerebellum, of [^{18}F]GBR 13119 by nearly 60%. This reduction in specific striatal binding of [^{18}F]GBR 13119 provides support for down-regulation of available DA uptake sites upon DA depletion, which would be consistent with an adaptive process to reduce the uptake capacity and maintain synaptic levels of DA (Zigmond et al., 1990), and is similar to the reported 59% reduction in norepinephrine reuptake sites in rat brain cortex after repeated reserpine pretreatment (Lee et al., 1983). The observed reduction in [^{18}F]GBR 13119 binding is also similar in magnitude but opposite in direction to the change in striatal binding of [^{3}H]raclopride in reserpine-treated rats.

In the reserpinized animals there was also observed an increase of radiotracer uptake in non-target tissues such as cerebellum (+29%), which most likely reflects an increased delivery of radioligand to all brain tissues due either to the elevated blood levels or increased blood-brain barrier permeability for the radioligand. Increased radioligand concentrations in cerebellum have been previously noted in the uptake of [^{3}H]GBR 12783 (+48%; Vaugeois et al., 1990) and [^{3}H]BTCP (+90%; Maurice et al., 1991), and also in unrelated studies of in vivo uptake of [^{3}H]raclopride (Ross and Jackson, 1989). These studies employing reserpine pretreatment demonstrate the importance of considering radiotracer delivery when interpreting apparent increases in radiotracer accumulation after pharmacological interventions. Increases in the absolute amount of specifically bound radioligand, as observed for [^{3}H]BTCP, can be attributed to increased radioligand availability for binding to the DA uptake site, rather

than an increase in the number of available sites. On the other hand a decrease in radiotracer binding, as observed for [^{18}F]GBR 13119, with a concomitant increase in radiotracer delivery cannot be so explained, and the observed decrease in specific binding may in fact be an underestimation of the true 'loss' of available binding sites. In the extreme case, increased delivery may obscure smaller 'losses' of apparent binding sites, resulting in no apparent effect of drug treatment.

The hypothesis that the numbers of dopamine uptake carriers may be regulated by the chronic concentration of DA would be consistent with the concept of synaptic homeostasis (Zigmond et al, 1990) in normal animals. In this study, we have assigned a reduction in [^{18}F]GBR 13119 binding to a loss of DA uptake sites, although a change in the K_D for the radioligand might also reduce the in vivo binding; however, there is no precedence for changes of in vivo K_D values for this class of radioligands. Separation of K_D and B_{max} was not attempted in this study, as for imaging purposes these variables are often lumped together into a combined term (such as binding potential (B_{max}/K_D), volume of distribution, or related terms). The possible regulation of 'available' DA uptake sites by levels of endogenous DA would have important ramifications for the interpretation of in vivo imaging studies of this system, as a lower radiotracer accumulation may in fact reflect less available DA uptake sites per surviving neurons rather than a loss of neurons. An interesting question remains as to the mechanism for this apparent loss of DA uptake sites, as chronic reserpine did not reduce the number of [^{3}H]threo-($\pm$)-methylphenidate binding sites in rat brain determined using in vitro assays of rat striatal membranes (Janowsky et al., 1985). The possibility of intact but non-functional DA transporters would have further impact on both the interpretation of in vivo imaging studies, as well as new therapeutic regimens for treatment of Parkinson's disease.

A second important consideration for in vivo studies will be the possible competition of endogenous DA for radioligand binding. In normal animals the in vivo binding of [^{3}H]GBR 12783 does not seem to be affected by raising DA levels through L-DOPA treatment (Vaugeois et al., 1990). In preliminary studies, we have found that repeated pargylline treatment (10 mg/kg i.p., once daily for 14 days) at a dose which should inhibit $> 80\%$ of monoamine oxidase B (Zsilla et al., 1983) does not alter specific [^{18}F]GBR 13119 binding (Kilbourn et al., 1991b). Together these studies suggest that this class of high affinity radioligands is not affected by competition from endogenous DA, in contrast to the apparent reduction of in vivo binding of [^{3}H]BTCP after L-DOPA treatment (Maurice et al., 1991). Only a direct comparison of [^{18}F]GBR 13119 (or [^{3}H]GBR 12783) and [^{3}H]BTCP in reserpinized animals would answer the question of whether different kinetics of association and dissociation, or different methods for tissue analysis, are responsible for the divergent results. That endogenous DA might have different effects on these structurally unrelated drugs might not be a surprise, as similar results have been observed in studies of the effects of reserpine or amphetamine on DA D_2 radioligands in vivo. Spiperone and structurally related butyrophenone neuroleptics have been reported to show no change (DeJesus et al., 1986; Seeman et al., 1989; Young et al., 1991) or decreased (Bischoff et al., 1991; Chugani et al., 1988) binding in reserpinized animals, yet also exhibit decreased in vivo binding after pretreatment with the DA releaser amphetamine (Dewey et al., 1991). Conversly, benzamide neuroleptics such as raclopride showed an increased specific binding in the striatum (higher striatum/cerebellum ratios) after pretreatment with reserpine (Seeman et al., 1989; Young et al., 1991), and decreased binding after amphetamine pretreatment (Ross and Jackson, 1989).

In summary, we have demonstrated that the in vivo specific binding of the radioligand [^{18}F]GBR 13119 to the DA uptake carrier can be altered by depletion of endogenous DA. These results suggest that in vivo studies of the DA uptake system in neurodegenerative diseases, including human brain imaging of the distribution of carbon-11 or fluorine-18 labeled radioligands using positron emission tomography, may not simply reflect loss of dopaminergic terminals but may also provide additional information on the functional status of the uptake system in the diseased state.

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