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# Specific binding of 3*N*-(2'-[$^{18}$F]fluoroethyl)benperidol to primate cerebral dopaminergic $D_2$ receptors demonstrated in vivo by PET

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3*N*-(2'-[$^{18}$F]Fluoroethyl)benperidol ([$^{18}$F]FEB) an $^{18}$F-labeled analogue of the $D_2$ antagonist benperidol, was evaluated as a tracer for positron emission tomography (PET). PET imaging of a living baboon showed that the fluorinated ligand rapidly localized in vivo within $D_2$ receptor-rich brain tissue, with selective retention lasting over 2 h after tracer injection. Pretreatment of the animal with unlabeled $D_2$-specific antagonist eticlopride (4 mg/kg, i.v.) 1 h before [$^{18}$F]FEB completely abolished the selective disposition of the radioligand, whereas the regional cerebral blood flow, blood volume and peripheral metabolism/protein binding of [$^{18}$F]FEB were not changed. Tracer localization when the baboon was pretreated with unlabeled ketanserin (0.55 mg/kg, i.v.) or SCH 23390 (1.1 mg/kg, i.v.) was identical to that for the control case, indicating that the [$^{18}$F]FEB did not bind to $S_2$ of $D_1$ receptors in vivo. [$^{18}$F]FEB has advantages compared to previously used PET tracers, and may be an excellent radioligand for non-invasive study of $D_2$ receptor binding.

The cerebral dopaminergic nervous system plays a key role in such neuropsychiatric disorders as schizophrenia [7, 10], Parkinson's disease [14] and Huntington's disease [15], and the potency of antipsychotic drugs correlates with their affinity for binding to central dopaminergic $D_2$ receptors [3, 17]. The prevalence of these conditions has motivated several research groups to investigate $D_2$ receptor binding in vivo using positron emission tomography (PET). A major advantage of the PET methodology is its non-invasive nature, which permits application to human subjects and allows serial studies on the same individual.

Positron-emitting $D_2$ ligands for PET [1] are generally analogues of the butyrophenone spiperone or the benzamide raclopride. Both of these ligand types have limitations as PET tracers. Spiperone and its derivatives have high affinity for binding to $D_2$ receptors, but they are relatively non-specific and bind in vivo to serotonergic $S_2$ receptors as well [2, 5, 12]. In contrast, raclopride had high $D_2$ selectivity, but the relatively low binding affinity of this ligand leads to receptor competition by endogenous dopamine for $D_2$ sites [18], thereby complicating the kinetic modeling of PET data.

The butyrophenone ligand benperidol holds promise for development as a PET tracer because it has both high affinity and specificity for binding to $D_2$ receptors [13]. We have evaluated a positron-emitting derivative of this ligand, 3*N*-(2'-[$^{18}$F]fluoroethyl)benperidol ([$^{18}$F]FEB), for suitability in measuring cerebral $D_2$ receptor binding in vivo by PET. FEB has high $D_2$ binding affinity ($K_i$ = 5.2 nM) [9], and the use of fluorine-18 ($t_{1/2}$ = 110 min) as a radiolabel is convenient for image acquisition as well as laboratory procedures.

Experiments were done with the PET VI system in the high resolution mode [19]. Transverse reconstructed resolution was about 12 mm, and axial resolution was about 14 mm. All studies were performed on a single 8.6 kg male baboon following procedures [11] approved by the Animal Studies Committee of Washington University. Separate experiments were done with and without preadministration of unlabeled receptor antagonists, which were given i.v. 1 h prior to radiotracer injection in dosages of 4 mg/kg eticlopride ($D_2$ ligand), 1.1 mg/kg SCH 23390 ($D_1$ ligand) and 0.55 mg/kg ketanserin ($S_2$ ligand). These parenteral dosages have been shown by others to be sufficient for in vivo saturation of the respective cere-

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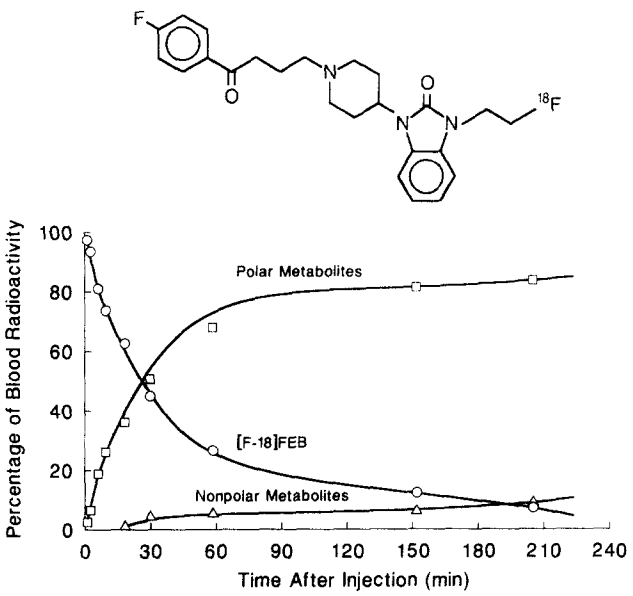


Fig. 1. Structure of [$^{18}$F]FEB and its metabolism in blood as a function of time. ○, [$^{18}$F]FEB; □, polar metabolites; △, non-polar metabolites

bral receptor sites in primates [12, 16, 21]. [$^{18}$F]FEB was prepared in 25–30% radiochemical yield and specific activity > 37 GBq/mmol [9], and dissolved in 1 M lactic acid in 0.9% saline for injection. Unlabeled antagonist drugs were dissolved in the same solvent for use in the receptor-blocking experiments.

Details of the imaging protocol (including anesthesia of the animal) for the control and receptor-blocked studies have been described previously [8]. A head holder permitted precise repositioning of the baboon [11], and for each experiment an attenuation scan was obtained using a ring source of $^{68}$Ge/$^{68}$Ga. Regional cerebral blood flow (rCBF) and cerebral blood volume (rCBV) were determined using [$^{15}$O]$H_2O$ and [$^{15}$O]CO, respectively [20]. Sequential scans were obtained for 3 h, and scan durations were adjusted to maintain coincidence counts above 100,000/slice [8].

For each imaging experiment, the free fraction and peripheral metabolism of [$^{18}$F]FEB were determined. The free fraction of the tracer in arterial blood was measured in triplicate for each study using an in vitro microfiltration technique [11]. Radio-TLC ($SiO_2$; $CH_3OH$/$CH_2Cl_2$ = 1/9) was used to ascertain metabolites from 1 ml arterial blood samples that were removed in duplicate from the animal at intervals ranging from 1 s to 3 h after injection of [$^{18}$F]FEB. The radiochromatographic plates were scanned with a Berthold Tracemaster 20 Automatic TLC Linear Analyzer interfaced with an Epson Equity III+ computer. The peripheral metabolism of [$^{18}$F]FEB is illustrated in Fig. 1. Note that [$^{18}$F]FEB was metabolized predominantly to polar metabolites ($R_f$ < 0.2). Because only non-polar compounds partition into brain, the vast majority of the radiometabolites of [$^{18}$F]FEB are anticipated to be excluded from brain, and thus do not complicate tracer kinetic modeling of PET data.

Regions of interest (ROIs) including striatal, cortical and cerebellar regions were identified from the blood flow images with use of proportional measurements of corresponding sections from a stereotaxic atlas of the baboon [4, 11]. ROIs were kept in identical positions for studies with and without preadministration of unlabeled

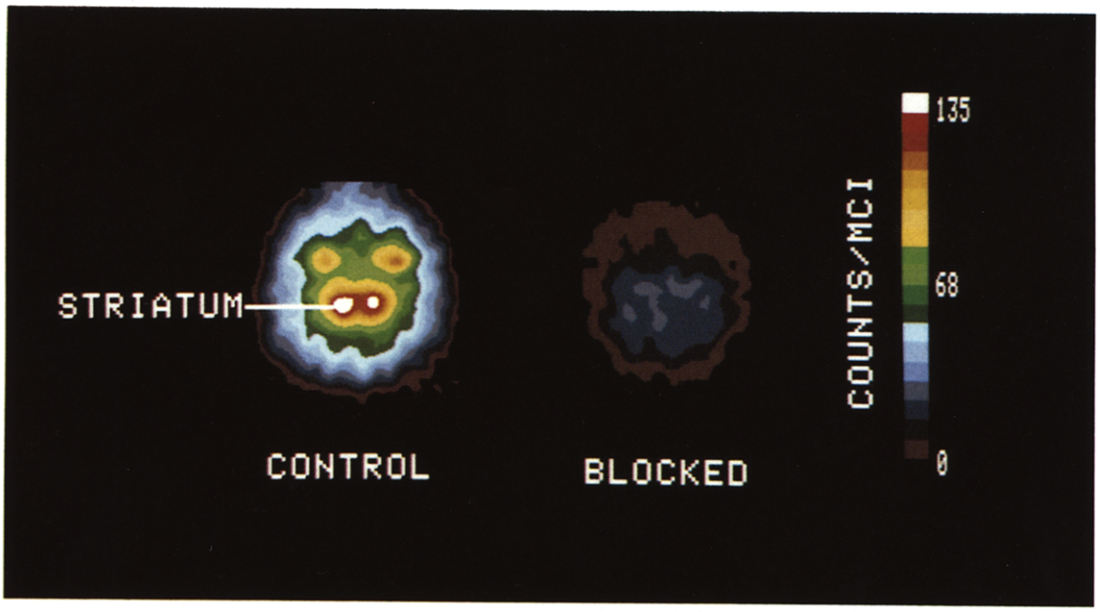


Fig. 2. PET images of the same tomographic slice of a baboon 40 min after i.v. injection of [$^{18}$F]FEB. The image on the left is reconstructed from the control study; that on the right is after administration of unlabeled eticlopride 1 h preceding injection of [$^{18}$F]FEB. The same color scale applies to both images.

receptor antagonists. Typical images obtained with [$^{18}$F]FEB in the control and eticlopride pretreatment studies are presented in Fig. 2. The PET slices are at the base of the frontal lobes and through the striata, and were reconstructed from data obtained for 5 min about 38 min after injection of [$^{18}$F]FEB. In the control image, tracer disposition within the basal ganglia is evident, and to a lesser extent within the eyes of the animal. These data demonstrate selective accumulation in vivo of [$^{18}$F]FEB during the control experiment that parallels the distribution of $D_2$ receptors in primate brain as determined in vitro with [$^3$H]raclopride [6]. The close concordance underscores the utility of [$^{18}$F]FEB and PET for non-invasive investigation of $D_2$ receptor binding in vivo.

$D_2$ receptor densities in primate brain are high (109–124 fmol/mg) in basal ganglia and low (4.4 fmol/mg) in the cerebellum [6]. Fig. 3 illustrates the tracer kinetics of [$^{18}$F]FEB in these two regions as a function of time post-injection. Radioactivity cleared from the striatum as well as the cerebellum, although the uptake and retention of [$^{18}$F]FEB was greater for the $D_2$ receptor-rich striatum. Selective retention within the basal ganglia was seen for over 2 h after tracer injection. The apparent clearance of radioactivity from $D_2$-rich tissue is probably due to a combination of partial volume averaging attributable to the low resolution of PET VI, together with a slightly low $K_1$ for this radioligand.

As shown in Fig. 2, the regionally specific distribution of radioactivity that was observed in the control study was lost in the study in which [$^{18}$F]FEB was given after administration of the unlabeled $D_2$ antagonist eticlopride. Regional CBF and CBV were not substantially altered by unlabeled receptor ligands. In addition, the

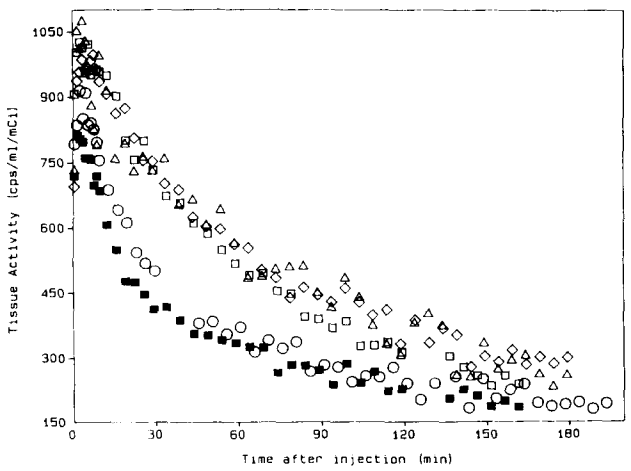


Fig. 3. Tracer kinetics of [$^{18}$F]FEB in cerebral tissues of baboon. □, basal ganglia (control); ■, cerebellum (control); ○, basal ganglia (eticlopride pretreatment); △, basal ganglia (ketanserin pretreatment); ◇, basal ganglia (SCH 23390 pretreatment).

fraction of [$^{18}$F]FEB in arterial blood that was non-protein bound (3.5 ± 1.0%) did not change after antagonist pretreatment nor did the metabolism differ from the control (Fig. 1). Thus, absence of selective accumulation of [$^{18}$F]FEB after pretreatment with eticlopride can be attributed to blockade of specific binding to $D_2$ receptors rather than to non-receptor based mechanisms. The ratio $R$ of the radioactivity in the basal ganglia relative to cerebellum can be used as a crude approximation of $D_2$ receptor binding in vivo, based on various assumptions [8]. In the control experiment, [$^{18}$F]FEB preferentially localized in vivo within $D_2$-receptor rich tissues. For a 5-min scanning interval centered at 42 min, the $R$ values decreased in the rank order left and right basal ganglia (1.69 ± 0.21, $n$ = 6) > frontal cortex (1.13 ± 0.16, $n$ = 3) > occipital cortex (1.02 ± 0.09, $n$ = 3), in agreement with the relative $B_{max}$ values for $D_2$ receptors as determined in vitro [6]. $R$ was not significantly different for the studies involving pretreatment with unlabeled $S_2$ or $D_1$ receptor antagonists, but was dramatically reduced from the control values upon pretreatment with unlabeled $D_2$ ligand eticlopride. For the latter case, the $R$ values were left and right basal ganglia (1.14) ≈ frontal cortex (1.11) ≈ occipital cortex (0.97). These results underscore the utility of [$^{18}$F]FEB for non-invasive identification of $D_2$ receptors of primate brain, as well as the specificity of this tracer in localizing to these binding sites in vivo.

To summarize, [$^{18}$F]FEB is a useful radiotracer for investigation of central $D_2$ receptor binding in vivo. It can be prepared in high radiochemical yield and specific activity for application with PET, and the half-life of the radiolabel is convenient for imaging and laboratory procedures. The radioligand rapidly localized in vivo to $D_2$ receptor sites, and is metabolized in the periphery mainly to polar metabolites that do not partition into brain. A major advantage of [$^{18}$F]FEB as a PET tracer is its high selectivity for binding to $D_2$ receptors in vivo, which permits PET studies to be accomplished without the complicating effects of binding to non-$D_2$ receptor sites of the brain. Our findings thus suggest that this radioligand has great potential for use with PET to distinguish specific changes in $D_2$ receptor binding in pathological conditions and to non-invasively evaluate pharmacotherapeutic effects of centrally acting drugs.

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