Quantitative Evaluation of D2 Dopamine Receptor Density in Canine Striatum with C-11 Labeld YM-09151-2 and Positron Emission Tomography

III. 2. Quantitative Evaluation of D2 Dopamine Receptor Density in Canine Striatum with C-11 Labeled YM-09151-2 and Positron Emission Tomography

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Novel positron-emitting ligand, [^{11}C]YM-09151-2, for imaging of D2 dopamine receptor has been developed.[1,2] Distribution of the ligand in a brain and specific binding to striatal D2 dopamine receptor were previously examined in rat brain.[3] Here, analytical method for measuring receptor densities with this ligand in living canine brain was studied using positron emission tomography.

Materials and Methods

Ligand preparation

[^{11}C]YM-09151-2 was synthesized with the methods developed by our group.[2] [^{11}C]CH_3I was produced from [^{11}C]Co_2 using $LiAlH_4$ and introduced into the nor-derivative of YM-09151-2 and NaH in dry DMF. After the reaction mixture was applied to a reversed phase column (SEP-PAK C18 cartridge), [^{11}C]YM-09151-2 was eluted with 5 mL of EtOH. Radiochemical purity and specific activity were measured with HPLC.

In the present study, specific activity of [^{11}C]YM-09151-2 ranged from 32 to 298 Ci/mmol at the time of administration.

Animal preparation

Two beagle dog weighed 16 Kg was anesthetized under 1 to 1.5% halothane, 2 L/min of N_2O and 1 L/min of O_2. A canule was inserted to femoral artery to take blood samples. Arterial PCO_2 and PO_2 were measured during the study. Each beagle was studied at least twice with 2 weeks intervals. Different doses of [^{11}C]YM-09151-2 were administrated to vary the receptor occupancy.

Scan procedure

PT 931 PET scanner (CTI, Tennessee) was employed to measure tissue radioactivity of the ligand administrated. The scanner has 8 mm (FWHM) of spatial resolution in the tomographic plain and 10 mm in the axial direction. Four rings BGO detectors provides seven images simultaneously which cover whole canine brain.

Repeated scannings were started at 30 seconds after administration and continued until 60 to 100 minutes with data acquisition time of 3 minutes during first 30 minutes and of 5 minutes thereafter. Arterial blood was taken periodically during the procedure to estimate 11C radioactivity in plasma. The fractions of [^{11}C]YM-09151-2 and its metabolites were measured with liquid chromatography.

Data analysis

Mean radioactivities for striatum and occipital cortex at 40 to 60 min after injection was obtained in the PET measurement. In each experiment, striatal minus occipital radioactivity was converted to pmol/ml unit with specific activity of the ligand administrated. The ratio of specific binding to free ligand (B/F) was also estimated from PET measurement assuming that occipital cortex has no dopaminergic neurons. B/F values were plotted against the concentration of specific binding ligands. The slope and the x-intercept was obtained for two dogs.

Results

Figure 1-a demonstrates [^{11}C]YM-09151-2 distribution in a canine brain obtained with PT-931 after administration of 44 nmol of the ligand (1.07μg/Kg). Figure 1-b shows [^{11}C]YM-09151-2 distribution when 388 nmol of [^{11}C]YM-09151-2 (9.09 μg/Kg) were given. No accumulation of the ligand to striatum was evident.

Radioactivities in striatum and occipital cortex were plotted against time after injection for three studies of different administration doses of [^{11}C]YM-09151-2 (44, 134 and 388 nmol) in Figure 2. Striatal radioactivity was 1.8 times higher than occipital one after 30 min when 44 nmol of the ligand was administrated. On the other hand, striatal and occipital radioactivities were same when 388 nmol were administrated. Assuming that occipital cortex has no dopaminergic projections and neurons, occipital radioactivity was considered to represent non- specific binding (free fraction) of [^{11}C]YM-09151-2. Specific binding (bound fraction) of the ligand to D_2 dopamine receptor (striatum minus cerebellum) reached constant at around 40 min and continued during the procedure(Figure 3-a). Figure 3-b shows total radioactivities and radioactivities of [^{11}C]YM-09151-2 in arterial plasma during

the procedure. The clearance was remarkably rapid and the radioactivities taken after 20 min were relatively constant.

The ratios of bound to free fraction in striatum were obtained for the PET measurement from 40 to 60 min in each study with different administration doses. The concentrations of [^{11}C]YM-09151-2 for bound fraction were calculated using values for specific radioactivity of [^{11}C]YM-09151-2 and striatum (Figure 4), D2 dopaminergic receptor density was 12.3 pmol/ml and K_D was 9.1 nM in one dog and 11.5 pmol/ml and 5.3 nM for another.

Discussion

Neural transmission of dopaminergic neurons in humans has been studied using positron emission tomography and [^{11}C]-N methyl spiperon[4], [^{11}C]Lacroplide.[5] We recently labeled a benzamide neuroleptic, YM-09151-2 with homnogenate of canine striatum, selectivity and competition between this ligand and dopaminergic agonists and antagonists had been studied.[8] Prior to the clinical PET study, we tested the analytical method and the feasibility using [^{11}C]YM-09151-2.

After the administration, the radioactivity in atriatum and occipital cortex reached stable at 30 to 40 min and continued to be constant during the procedure. Constant receptor bound fraction (total minus reference radioactivity, occipital cortex in this case) indicated that binding and release of ligands at specific binding sites are in equilibrium. This is consistent with the result that in canine striatum homogenate study [^{3}H]-YM-09151-2 (50-100 pmol/l) binding reached steady state equilibrium within 45-60 min.[8] As shown in Figure 4, a concentration of the ligand in plasma became constant at 20 min after injection. This might contribute to reach equilibrium early in striatum.

Several techniques for quantitative assays of neuroreceptor densities have been reported. One of them is the dynamic approach which is similar to the three compartment analysis developed as an extension of the Sokoloff model and need measurement of time courses of the radioactivity in tissue and in plasma (Wong et al). This method fundamentally require high specific activity and high specific activity and high receptor binding affinity which allows measurement in trace amount of the ligand. When dissociation constant is negligibly small, this method can be simplified to the normalized graphical method.[7]

Another is the equilibrium approach which need to estimate the fraction of ligand bound to receptors under various tissue concentrations of unbound ligand. To establish the equilibrium of ligand-receptor interaction within a PET study, the ligand should have fast release rates from the receptor.

Considering the kinetic characteristics of [^{11}C]YM-09151-2 in canine brain, this ligand is not suitable for the normalized graphical method which is commonly employed for ^{11}C-NMSP and need assumption that dissociation is negligible. As shown in Figure 5, no straight portion was observed indicating that K_D is not small.

According to the equilibrium method, we estimated receptor density to be 11.5 pmol/ml and K_D to be 5.3 nM for [^{11}C]YM-09151-2. D_2 dopamine receptor density was close to the values estimated in human striatum with PET and other D_2-receptor binding ligands.

Although the equilibrium approach was successfully performed in canine, we need to know the efficacy of YM-09151-2. In the equilibrium method high dose of YM-09151-2 rather than trace dose should be administrated intravenously. The highest dose of YM-09151-2 administrated in the present series was 388 nmol, 0.15 mg (9.4 μg/Kg) which is 0.9 % of LD 50 for rat. No changes of blood pressure, body temperature, arterial PO_2 and PCO2 were observed.

Acknowledgment

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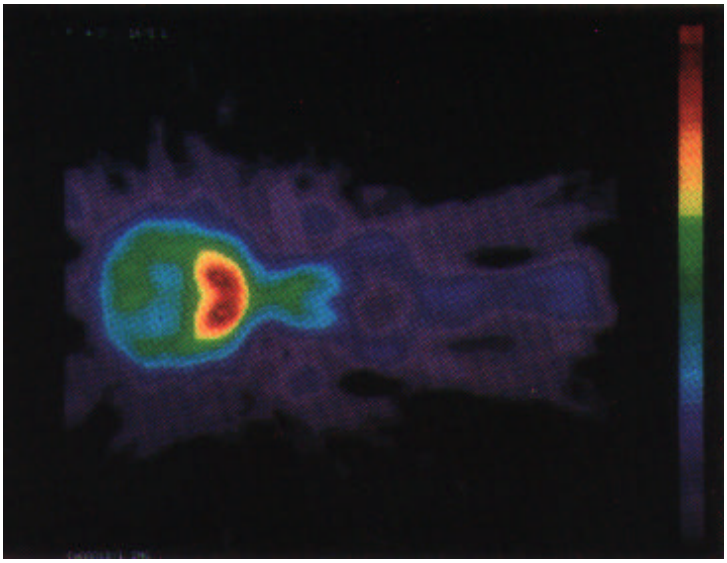
(a)



(b)

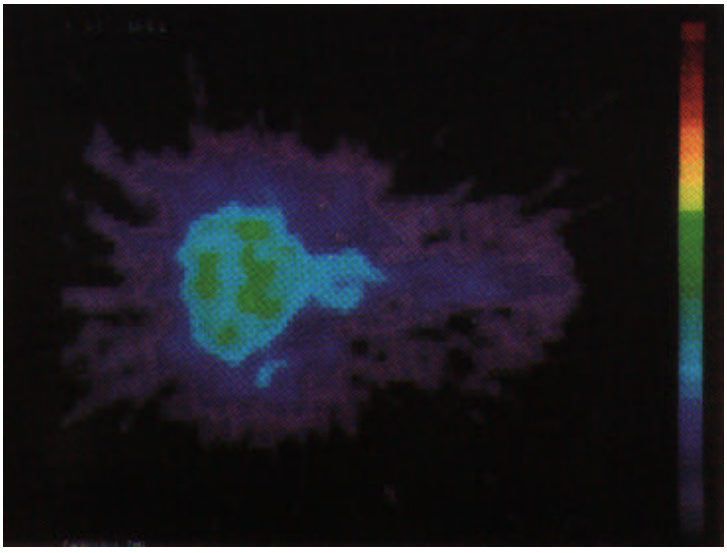
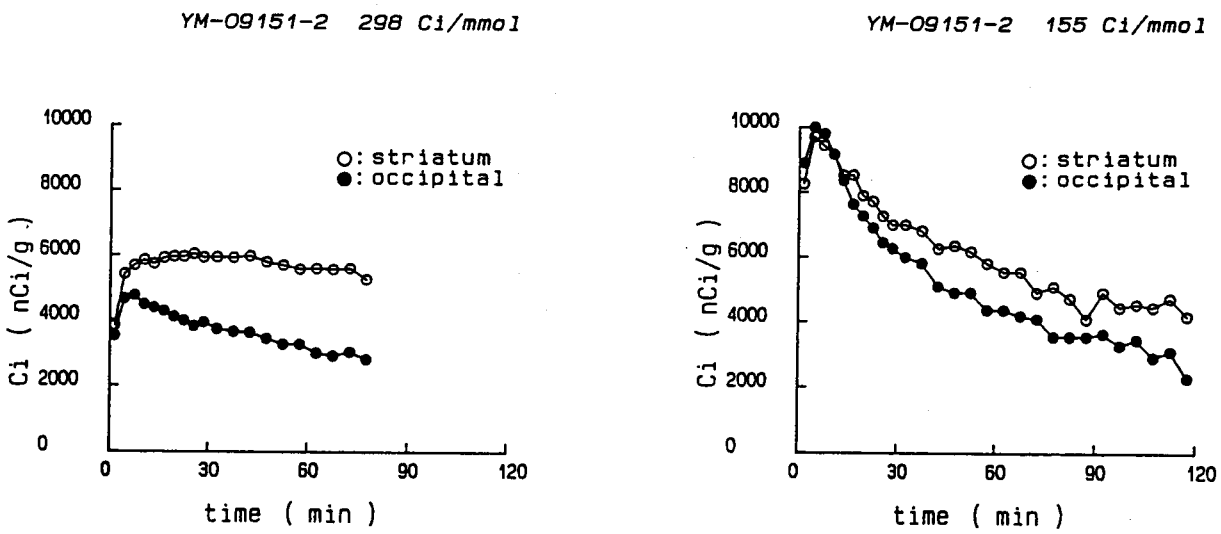


Fig. 1. PET images of a canine brain after administration of [^{11}C]YM-09151-2 (44 nmol (a) and 388 nmol (b)). In Figure 1.a, specific bindings of the ligand to striatal D_2 receptor was visible whereas in Figure 1-b no accumulation of the ligand in striatum because the receptors were occupied by non-radioactive YM-09151-2.



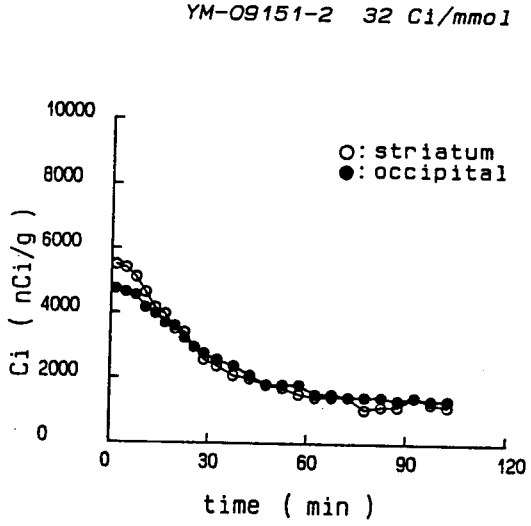


Fig. 2. Time-radioactivity curves of striatum and cerebellum in three studies with different administrated dose of [^{11}C]YM-09151-2.

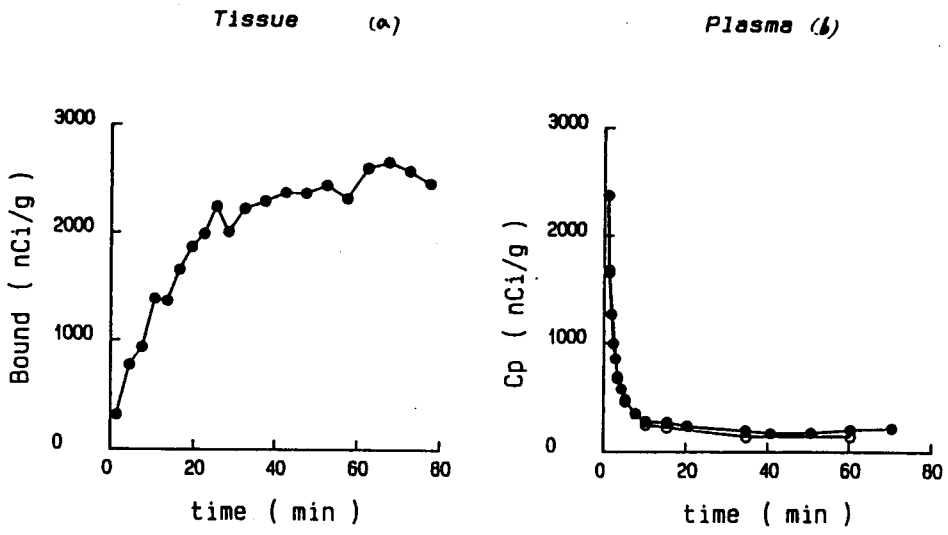


Fig. 3. Receptor-bound fraction of [^{11}C]YM-09151-2 (a) and total ^{11}C (•) and plasma [^{11}C]YM-09151-2 measured with HPLC (o) (b). Both reached the equilibrium at around 40 min after administration.

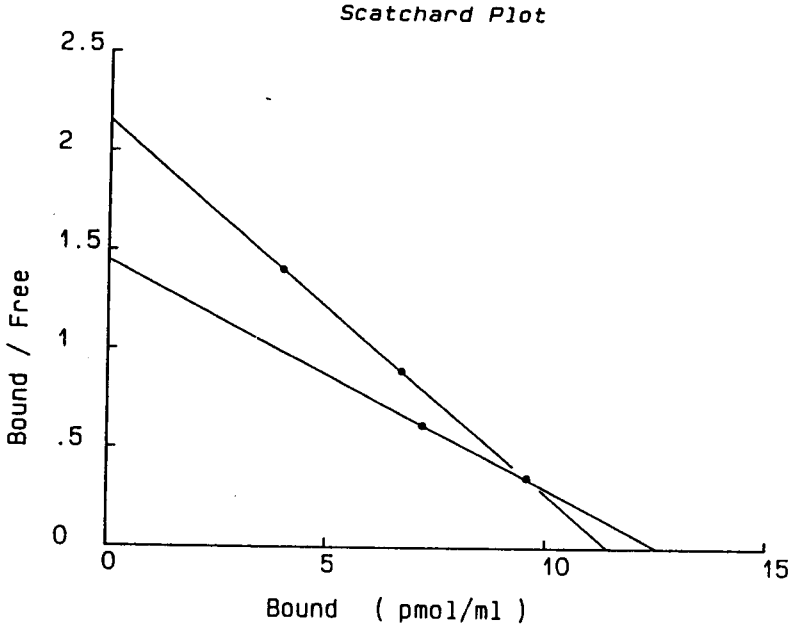


Fig. 4. Scatchard plots of [^{11}C]YM-09151-2 in striatum. The x-intercepts indicate D_2-dopamine receptor density and the slopes are $-1/K_D$, where K_D is a dissociation constant of the ligand to the receptor.

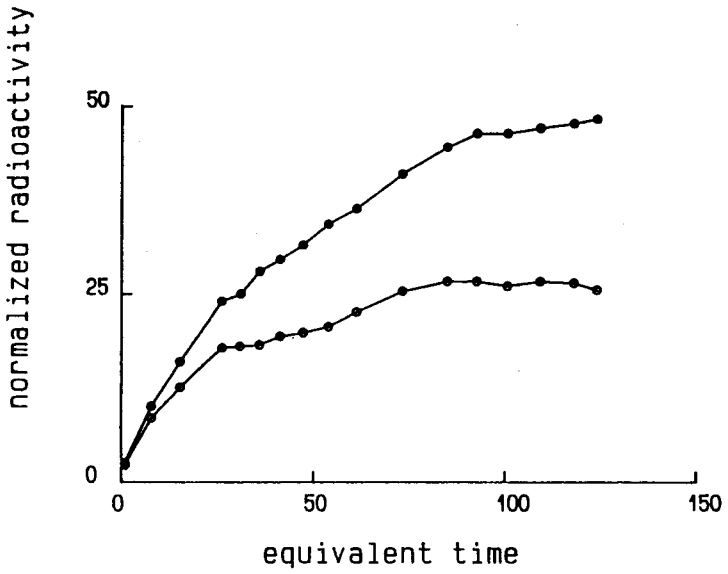


Fig. 5. Graphical analysis of [^{11}C]YM-09151-2 in canine striatum (•) and occipital cortex (o). No straight portion of the plots was found during the study indicating that release rate is not negligibly small.