Supporting Information

In vitro Ligand Binding Kinetics Explains the Pharmacokinetics of [^{18}F]FE-PE2I in Dopamine Transporter PET Imaging

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Methods

The PE2I and FE-PE2I samples were kindly donated by PharmaSynth AS. The buffer components were purchased from either Sigma-Aldrich or Lach-ner. The ScintiSafe 3 scintillation cocktail was used for counting the samples. Whatman GF/B filters were used for the filtration assays. The Wallac 1219 Rackbeta liquid scintillation counter was used to measure the radioactivity of the samples. The inhibition constant of FE-PE2I was calculated from the corresponding IC_{50} value using the Cheng–Prusoff equation,[1] and 4.8 nM and 3 nM were used as K_d and concentration of radioligand,[2] respectively. All kinetic curves of reporter-ligand binding followed the pseudo-first-order rate equation and were fitted to a one-phase exponential association function. The radioligand binding rate constant versus unlabeled ligand concentration plots were analyzed as previously described.[3] The data were analyzed with the GraphPad Prism 4 software package.

Ligand binding. Striata from two-month old male C57BL/6 mice were rapidly dissected, weighed, frozen, and stored at −80 °C. The tissue was suspended in an ice-cold buffer (120 mM NaCl, 5 mM KCl, 30 mM HEPES, pH 7.4), sonicated using an ultrasound homogenizer (Bandelin SONOPLUS; 3×15 sec), and centrifuged at 30,000 *g* for 15 min at 0 °C. The supernatant was discarded, and the remaining pellet was resuspended in an ice-cold buffer and homogenized again. This procedure was repeated three more times to yield a final membrane suspension, which was divided into aliquots and stored at −80 °C until needed. The final membrane concentration was approximately 4 mg of wet tissue per mL. [^{3}H]PE2I, with a specific activity of 69.4 Ci/mmol, was used at a final concentration of 3 nM throughout the displacement and kinetic experiments. Inhibition constants were determined by radioligand displacement assays. The membrane suspension (150 µL) was preincubated for 10 min at 25 °C with the unlabeled ligand solution (50 µL) at various concentrations before adding 15 nM [^{3}H]PE2I (50 µl, final concentration 3 nM) and incubating for 1 h at 25 °C. The kinetic experiments were conducted by adding 9 nM [^{3}H]PE2I (0.5 mL) and unlabeled ligand solution (at various concentrations) to the membrane suspension (1 mL) in a thermostated cuvette maintained at 25 °C. Aliquots were taken at predetermined time intervals. The reaction was terminated by the addition of an ice-cold buffer (20 mM potassium phosphate, 100 mM NaCl, pH 7.4), filtration through GF/B filters (presoaked in 0.3% polyethylene imine solution) and washing with an ice-cold buffer (5 mL). The filters were air dried and

transferred into scintillation vials, and then the scintillation cocktail was added. The vials were then shaken for 12 h before measuring the radioactivity using a liquid scintillation counter.

Kinetic Analysis A systematic kinetic study of the FE-PE2I interaction with DAT sites in striatum membrane fragments was performed by using the method of kinetic analysis previously described.[3,4] In these experiments, the time-course of [^{3}H]PE2I binding with membrane fragments was monitored in the presence of various concentrations of non-labeled FE-PE2I (Figure 1), and the dependence of the observed rate constants k_{obs} upon FE-PE2I concentration was analyzed (Figure 1). The k_{obs} values increased in the presence of FE-P2I, and this hyperbolic plot provided evidence that FE-PE2I is also able to initiate the slow isomerization of the ligand-DAT complex, as shown in Scheme 3. From the same k_{obs} versus [FE-PE2I] plot (Figure 1), the kinetic parameters K_L, k_i and k_{-i} were calculated for this ligand by using the rate equation

$$k_{obs} = \frac{k_i[L]}{K_L+[L]} + C \qquad \text{Eq. 2}$$

where k_{obs} is the observed rate constant of a binding reaction, k_i is the on-rate constant of the isomerization of unlabeled ligand and DAT, [L] is the concentration of the ligand, K_L is the equilibrium constant of initial binding and C can be calculated as follows

$$C = \frac{k_i^*[L^*]}{K_L^*+[L^*]} + \frac{k_{-i}^*+k_{-i}}{2} \qquad \text{Eq. 3}$$

where k_i* is the on-rate constant of isomerization of radioligand-DAT complex, k_{-i}* and k_{-i} are the off-rate constants of unlabeled ligand and radioligand, respectively, [L*] is the concentration of the radioligand, and K_L* is the equilibrium constant of initial binding of the radioligand as previously described.[3]

(1) Yung-Chi, C.; Prusoff, W. H. Relationship between the Inhibition Constant (KI) and the Concentration of Inhibitor Which Causes 50 per Cent Inhibition (I50) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22* (23), 3099–3108.

(2) Kukk, S.; Stepanov, V.; Järv, J. Thermal Stability of Dopamine Transporters. *J. Membr. Biol.* **2015**, *248* (4), 775–781.

(3) Kukk, S.; Järv, J. Differentiating between Drugs with Short and Long Residence Times. *Medchemcomm* **2016**, *7* (8), 1654–1656.

(4) Kukk, S.; Järv, J. Small Structural Changes at the N -Position of the Tropane Core Control the Mechanism of Nortropane Derivatives Binding to Dopamine Transporter. *ChemistrySelect* **2018**, *3* (23), 6581–6584.