Progress Report 1994-1997

Development of Dopamine Receptor Radiopharmaceuticals For
The Study of Neurological and Psychiatric Disorders
DE-FG02-94ER61840

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We have made significant progress on the development of dopamine receptor radiopharmaceuticals during the period of June 1994 to December 1996. Our objectives in the grant application were three-fold: (1) Develop dopamine D-2 receptor antagonists, fallypride and desmethoxyfallypride; (2) Develop dopamine D-1 receptor antagonists based on SCH 38548, and (3) Initiate the development of dopamine D-2 receptor agonists based on PPHT. We have completed our investigations on the synthesis and radiosyntheses of fallypride and desmethoxyfallypride, *in vitro* and *in vivo* pharmacological experiments have been carried out and PET studies in non-human primates with ^{18}F -fallypride and ^{18}F -desmethoxyfallypride have also been conducted. We have accomplished the syntheses and radiosyntheses of various SCH 38548 derivatives, *in vitro* and *in vivo* pharmacological experiments have been accomplished and preliminary PET studies in non-human primates with ^{18}F -fluoropropylSCH 38548 have been carried out. Syntheses of the D-2 receptor agonists is currently being pursued. Various accomplishments during the funded years, briefly outlined in this document, have been disseminated by several publications in various journals and presentations in national and international meetings (Society of Nuclear Medicine, American Chemical Society, Society for Neuroscience and International Symposium on Radiopharmaceutical Chemistry).

We have no objection from a patent standpoint to the publication or dissemination of this material.

• Milestones in Research

1. Dopamine D-2 Receptor Antagonists

• Design, Syntheses and Radiosynthesis: We have developed two relatively selective and reversible radiotracers labeled with fluorine-18 for the study of dopamine D-2 receptors by PET (Mukherjee et al., *Nucl. Med. Biol.*, 1995; *Med. Chem. Res.*, 1995; Figure-1). ^{18}F -Fallypride is a high affinity radiotracer which provides excellent characteristics for purposes of studying the distribution of striatal and extrastriatal dopamine D-2 receptors. On the other hand, ^{18}F -desmethoxyfallypride is a moderate affinity radiotracer with properties similar to that of raclopride and may therefore be suitable for studying changes in the levels of endogenous dopamine. The radiosynthesis design of both the radiotracers is simple and employs a one-step nucleophilic substitution of a tosylate group with fluorine-18. Purification has been carried out on reverse phase chromatography to afford radiochemically pure tracers in 20-40% radiochemical yields in specific activities of 1-2 Ci/ μmol in approximately 60 minutes, reproducibly. Radiosyntheses of ^3H -fallypride and ^3H -desmethoxyfallypride is currently being completed in order to conduct a thorough *in vitro* pharmacological characterization using autoradiography (Yang and Mukherjee, manuscript in preparation).

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^{18}F -Fallypride

^{18}F -Desmethoxyfallypride

Figure 1. Dopamine D-2 Receptor Antagonists

• Pharmacology and PET Studies: We have characterized the binding of ^{18}F -fallypride to various brain regions in rats in controls and under various pharmacological challenges. Distribution of ^{18}F -fallypride correlated well with the reported distribution of dopamine D-2 receptors in the various brain regions. PET studies in rhesus monkeys confirmed the excellent characteristics of ^{18}F -fallypride as it bound to the

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dopaminergic regions quite rapidly and cleared from nonspecifically bound regions. Displacement studies of ^{18}F -fallypride with haloperidol were investigated in the rhesus monkeys (Mukherjee et al., *Nucl. Med. Biol.*, 1995). Ketanserin (which binds primarily to the serotonin 5HT-2 sites) was administered to the monkey had no significant effect on ^{18}F -fallypride binding whereas specifically-bound ^{18}F -fallypride was rapidly displaced with raclopride (which binds primarily at the dopamine D-2 sites). Similar kinetics of displacement were observed with haloperidol as well. Similarly, SCH 23390 which binds primarily to dopamine D-1 receptor sites had little effect on the binding of ^{18}F -fallypride PET (Mukherjee et al., *Med. Chem. Res.*, 1995). Currently, an analysis of the distribution of extrastriatal dopamine D-2 receptors using ^{18}F -fallypride is underway (Mukherjee et al., *Soc. Neurosci.*, 1996; manuscript in preparation). Studies on the distribution and kinetics of ^{18}F -desmethoxyfallypride in the monkey brains confirm its similarity to that of the binding properties of raclopride (Mukherjee et al., *Life Sci.*, 1996).

- **Substance Abuse (Amphetamine) Studies:** We have used ^{18}F -fallypride in the study of substance abuse drugs such as *d*-amphetamine. These studies have been carried out in collaborations with Dr. Lewis Seiden in the Department of Pharmacological and Physiological Sciences, who is one of the leading experts on neurotoxicity. We have carried out extensive studies with *d*-amphetamine and ^{18}F -fallypride. Endogenous dopamine was released at the synapse by administration of *d*-amphetamine pre- and post-injection of the administration of ^{18}F -fallypride. Intravenous administration of *d*-amphetamine in rhesus monkeys alters binding of ^{18}F -fallypride in PET studies. We have attempted to use this amphetamine-induced effect on specifically-bound ^{18}F -fallypride in order to evaluate a dose-response curve for AMPH. The monkeys were administered AMPH sulfate (dose range from 0.25 to 1.5 mg/kg, iv) after injection of ^{18}F -fallypride and the extent of change in specifically-bound ^{18}F -fallypride was measured (see copy of enclosed manuscript, Mukherjee et al., *Synapse*, under review).

- **Image Analysis and Kinetic Modeling:** In collaborations with Dr. Miles Wernick at the Illinois Institute of Technology, we have been working on image restoration techniques in order to enhance the recovery coefficients of the PET data (see copy of enclosed manuscript, Kao et al., *IEEE Transactions Medical Imaging*, under review). We have also commenced our collaborations both with Dr. Miles Wernick and Dr. James Holden at the University of Wisconsin-Madison on the kinetic modeling of PET data obtained in rhesus monkeys by using ^{18}F -fallypride and ^{18}F -desmethoxyfallypride.

- **Approval for Human Studies:** We are currently working on collecting the necessary information on fallypride in order to obtain approval of a Physician's sponsored Investigational New Drug (IND) application from the Food and Drug Administration for use of ^{18}F -fallypride in human subjects.

2. Dopamine D-1 Receptor Antagonists

^{18}F -Fluoropropyl SCH 38548

Figure 2. Dopamine D-1 Receptor Antagonists

- **Design, Syntheses and Radiosyntheses:** We have made significant progress in the development of D-1 receptor antagonists. We prepared *N*-alkyl, aryl, fluoroalkyl, fluoroaryl and iodoaryl derivatives of 7-chloro-8-hydroxy-3-methyl-1-(3'-aminophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 38548) as high affinity ligands for the dopamine D-1 receptor. Binding affinity studies of the compounds for dopamine D-1, D-2 and serotonin 5HT-2 receptor-sites in rat brain homogenates indicate a high degree of selectivity. The affinity of SCH 38548 for dopamine D-1 receptors was found to be 0.53 ± 0.46 nM. The fluoroethyl, fluoropropyl and fluorobenzyl derivatives showed approximately a 8-fold, 9-fold and 3-fold decrease in affinity for the D-1 sites compared to SCH 38548. We have succeeded in developing ^{18}F -fluoropropyl SCH 38548 (Figure 2) as one of the first successful fluorine-18 labeled radiotracer for the D-1

receptor. Radiosynthesis of ^{18}F -fluoropropyl SCH 38548 has been carried out in low yields. Optimization of the radiosynthesis is currently underway (Yang et al., *Nucl. Med. Biol.*, 1996).

- **Biodistribution Studies:** Biodistribution of ^{18}F -fluoropropyl SCH 38548 in rats showed specific uptake and retention of the radiotracer in the striata with striata to cerebellum ratios reaching 12 at 2 hr. post injection. Preliminary PET scans in rhesus monkeys indicate selective uptake of the radiotracer in the striata. After i.v. injection of ^{18}F -fluoropropyl SCH 38548 a rapid brain uptake of the tracer from blood was observed. Initial uptake in the striata and cerebellum was approximately 0.02% of injected dose/cc. Nonspecific uptake from the tissue surrounding the striata cleared slowly. The striata to cerebellum ratio increased from 1.20 at 3.5 minutes post-injection to approximately 2.5 at 120 minutes post-injection. The specific uptake of ^{18}F -fluoropropyl SCH 38548 in the striata was displaced by intravenous administration of SCH 24518 (Yang et al., *Nucl. Med. Biol.*, 1996). These results indicate that ^{18}F -fluoropropyl SCH 38548 is a potentially promising radiotracer for the D-1 receptor. Further pharmacological characterization of the binding of ^{18}F -fluoropropyl SCH 38548 is currently in progress.

3. Dopamine D-2 Receptor Agonists

- We are investigating the development of radiotracers based on agonists that will allow the *in vivo* assessment of the high affinity state of the dopamine D-2 receptors in order to evaluate the functional state of these receptors. We have attempted developing agonist radiotracers based on the D-2 receptor selective agonist, 2-(*N*-phenethyl-*N*-propyl)-amino-5-hydroxytetralin (PPHT, Figure 3). Since PPHT has been shown to tolerate various functional groups as well as bulk at the para-position in the free phenyl group, we initiated preparation of derivatives by incorporating an amino group at this position. We have prepared several derivatives of PPHT and PPHT- NH_2 . The *N*-fluoroethyl derivative of PPHT- NH_2 had only moderate affinities ($\text{IC}_{50} = 0.31 \mu\text{M}$ at the D-2 receptor-sites, using ^3H -spiperone and rat striata). Preliminary radiolabeling experiments have been carried out by reacting PPHT- NH_2 with ^{18}F -fluoroethyl iodide in the presence of *tert*-butyl lithium for 15 min at 80°C followed by HPLC purification to provide radiolabeled PPHT- $\text{NHCH}_2\text{CH}_2^{18}\text{F}$. However, the low affinity of the radiotracer for the D-2 receptors was found to limit any regional localization of the radiotracer in the rat brain and therefore unsuitable for development (Yang and Mukherjee, *J. Nuc. Med.*, 1995; *J. label. Compds. Radiopharm.*, 1995).

Figure 3.

- In order to increase the affinity of the radiotracers, we are currently investigating alternate ways that would enhance the binding affinity of the fluorinated PPHT derivatives. We have now developed a new class of compounds which contain the cyclohexylethyl group instead of the phenethyl group which is found in PPHT. The first compound, ZYY-339 (Figure 3) has a very high affinity ($\text{IC}_{50} = 1.30 \text{ pM}$) for the D-2 receptors. This class of compounds are currently being pursued for radiotracer development.

• Accomplishments in Education and Training

The following graduate students have been involved with work related to the grant and whose names appear in papers and presentations along with the principal investigator:

Xiaohu Ouyang, B.S. (M.S., University of Chicago), 1992 - 1995, Master's Degree in Medical Physics at The University of Chicago, June 1995, with emphasis in Radiopharmaceutical Chemistry. Mentor: Dr. J. Mukherjee; Thesis title: Synthesis and evaluation of thienylcyclohexylpiperidine derivatives for the study of NMDA receptor-linked calcium ion-channel. Mr. Ouyang is currently Research Associate in Amgen Pharmaceuticals.

Jeffery Yap, B.S., 1988 - Present, Research on knowledge-based principal component analysis of dynamic nuclear medicine image data. Dr. J. Mukherjee is a member of his Ph.D. Dissertation Committee. Mr. Yap is in his final stages of the Ph.D. thesis in the Graduate Program in Medical Physics. Mr. Yap is currently Postdoctoral fellow at Hammersmith Hospital.

Chienmin Kao, B.S., 1992 - Present, Research on signal recovery in image reconstruction. Dr. J. Mukherjee is a member of his Ph.D. Dissertation Committee. Mr. Kao in his final stages of the Ph.D. thesis in the Graduate Program in Medical Physics. Mr. Kao plans to continue as Research Associate at The University of Chicago.

James Roemer, B.S., 1993-1995, Research and Training in PET technology, animal studies and in the analysis of PET data. Mr. Roemer is currently pursuing a graduate degree in Computer Science at DePaul University, Chicago.

• Research Services

During the 1994-1996 funding years this grant award has made possible the successful operation of the radiopharmaceutical laboratories in the Radiotracer Program in the Franklin McLean Memorial Research Institute, Department of Radiology at the University of Chicago. The radiopharmaceutical laboratories provide collaborative support to a number of investigators in the University who pursue radiotracer methods.

• Publications During this Grant Period (June 1994-December 1996)

Journal Articles

Mukherjee, J., Yang, Z.Y., Das, M.K. and Brown, T.: Fluorinated benzamide neuroleptics 3.

Development of (S)-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3[F-18]-fluoropropyl)-2,3-dimethoxy benzamide as an improved dopamine D-2 receptor tracer. *Nucl. Med. Biol.*, **22**:283-296, 1995.

Mukherjee, J., Yang, Z.Y., Brown, T., Jiang, M., Kapp, O., Chen, C-T. and Cooper, M.:

Potential anomalies of dopamine D-2 receptor : Challenges for non-invasive imaging studies by positron emission tomography. *Med. Chem. Res.*, **5**: 174-192, 1995.

Ouyang, X-H., **Mukherjee, J.** and Yang, Z.Y.: Syntheses, radiosyntheses and biological evaluation of thienylcyclohexyl piperidine derivatives as potential radiotracers for the N-methyl-D-aspartate receptor-linked calcium ionophore. *Nucl. Med. Biol.*, **23**: 315-324, 1996.

Mukherjee, J., Yang, Z.Y., Brown, T., Roemer, J. and Cooper, M.: ¹⁸F-Desmethoxyfallypride: A fluorine-18 radiotracer with properties similar to carbon-11 raclopride for PET studies of dopamine D2 receptors. *Life Sciences*, **59**: 669-678, 1996.

Yang, Z.Y., Perry, B.D. and **Mukherjee, J.**: Fluorinated Benzazepines 1. Syntheses, radiosyntheses and biological evaluation of a series of substituted benzazepines as selective and high affinity radiotracers for positron emission tomographic studies of dopamine D-1 receptors. *Nucl. Med. Biol.*, **23**: 793-805, 1996.

Kao, C-M., Yap, J.T., **Mukherjee, J.** and Wernick, M.N.: Improving region-of-interest quantitation in dynamic PET studies by low-order approximation and pre-reconstruction sinogram restoration. *IEEE Transactions in Medical Imaging*, (under review).

Mukherjee, J., Yang, Z.Y., Lew, R., Brown, T., Kronmal, S., Cooper, M. and Seiden, L.S.: Evaluation of *d*-amphetamine effects on the binding of dopamine D-2 receptor radioligand, [F-18]fallypride in non-human primates using positron emission tomography. *Synapse*, (under review).

Mukherjee, J., Das, M.K., Yang, Z-Y. and Lew, R.: Evaluation of the binding of ¹⁸F-fluoxetine (¹⁸F-Prozac) as a potential marker for serotonin uptake sites in the rodent brain. *Life Sciences*, (under review).

Book Chapter

Mukherjee, J., Yang, Z.Y. and Das, M.K.: Development of fluorine-18 radiopharmaceuticals for dopamine neuroreceptors. In: "Chemists Views of Imaging Centers", A.M. Emran, Ed., Plenum press, New York, 1995, pp 265-275.

Patents

"Dopamine Receptor Antagonists", Mukherjee, J. and Yang, Z.Y., United States Patent, 08/421,557, Filed in April 1995.

Manuscripts in Preparation

- Mukherjee, J.**, Yang, Z.Y., Brown, T., Ouyang, X. and Cooper, M.: Evaluation of extrastriatal dopamine D-2 receptor binding in the rodent and non-human primate brains using the high affinity radioligand, [F-18]fallypride. *Eur. J. Pharmacol.*, (to be submitted).
- Yang, Z-Y. and **Mukherjee, J.**: Paradoxical effects of the *N*-cyclopropylmethyl group in substituted benzamides: Design of potential dopamine D-3 receptor agents. *Med. Chem. Res.*, (to be submitted).
- Mukherjee, J.** and Yang, Z.Y.: Design and synthesis of 2-(*N*-cyclohexylethyl-*N*-propyl)-amino-5-hydroxytetralin and fluorinated analogs as high affinity dopamine D-2 receptor agonists for use as potential PET radiotracers. *J. Med. Chem.*, (to be submitted).
- Brown, T., **Mukherjee, J.**, Yang, Z.Y., Chen, C.T. and Cooper, M.: Radiation dosimetry of the dopamine D-2 receptor PET radiotracer, (*S*)-*N*-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-[¹⁸F]fluoropropyl)-2,3-dimethoxybenzamide (¹⁸F-Fallypride). *J. Nucl. Med.*, (to be submitted).
- Yang, Z.Y. and **Mukherjee, J.**: Synthesis of ³H-fallypride, a high affinity fluorinated dopamine D-2 receptor radiopharmaceutical. *J. Label. Compds. Radiopharm.*, (to be submitted).

Presentations

- Mukherjee, J.**, Yang, Z-Y., Brown, T., Roemer, J. and Cooper, M.: Evaluation of d-amphetamine effects on dopamine D-2 receptor radiotracers, [F-18]fallypride and [F-18]desmethoxyfallypride. Presented at the The Society of Nuclear Medicine 41st Annual Meeting, June 5-8, 1994, Orlando, Florida. (*J. Nucl. Med.*, **35**:139P, 1994).
- Mukherjee, J.** and Yang, Z-Y.: Is quaternary piperazine N-oxide an aromatic ring activator for nucleophilic substitution reactions with [F-18]fluoride? Presented at the The Society of Nuclear Medicine 41st Annual Meeting, June 5-8, 1994, Orlando, Florida (*J. Nucl. Med.*, **35**:252P, 1994).
- Yang, Z-Y. and **Mukherjee, J.**: Development of Fluorinated Dopamine Receptor Agonists as Potential Radiotracers for Functional Assessment of the Receptors. Presented at the *XIth International Symposium on Radiopharmaceutical Chemistry*, Vancouver, Canada, August 13-17, 1995 (*J. Label. Compds. Radiopharm.* **37**:6-7, 1995).
- Mukherjee, J.**, Yang, Z-Y., Brown, T., Ouyang, X. and Cooper, M.: Biodistribution Study of the Dopamine D-2 Receptor PET Radiotracer, ¹⁸F-Fallypride. Presented at the *XIth International Symposium on Radiopharmaceutical Chemistry*, Vancouver, Canada, August 13-17, 1995 (*J. Label. Compds. Radiopharm.* **37**:8-9, 1995).
- Mukherjee, J.**, Yang, Z-Y., Ouyang, X., Brown, T., Lew, R. and Cooper, M.: Measurement and use of d-amphetamine effects in vivo by PET. Presented at the "*PET and Neuroscience Symposium*", August 23, 1995, American Chemical Society, Chicago, USA.
- Mukherjee, J.** and Yang, Z-Y.: Development of radiotracers for the assessment of functional state of dopamine D2-like receptors. Presented at the "*1995 International Chemical Congress of Pacific Basin Societies*", December 17-22, 1995, Honolulu, Hawaii, USA.
- Mukherjee, J.**, Yang, Z-Y., Brown, T. and Cooper, M.: Evaluation of a dose-response curve of d-amphetamine as a function of change in [F-18]fallypride binding at dopamine receptors. Presented at The Society of Nuclear Medicine 42nd Annual Meeting, June 12-15, 1995, Minneapolis, MN (*J. Nucl. Med.*, **36**:255P, 1995).
- Yang, Z-Y. and **Mukherjee, J.**: Development of agonists as radiotracers for the assessment of the functional state of dopamine D-2 receptors. Presented at The Society of Nuclear Medicine 42nd Annual Meeting, June 12-15, 1995, Minneapolis, MN (*J. Nucl. Med.*, **36**:152P, 1995).
- Ouyang, X., Yang, Z-Y. and **Mukherjee, J.**: Towards a positron-radiotracer for the ionophore associated with the NMDA receptor. Presented at The Society of Nuclear Medicine 42nd Annual Meeting, June 12-15, 1995, Minneapolis, MN (*J. Nucl. Med.*, **36**:150P, 1995).
- Wernick, M.N., Wang, G., Kao, C-M., Yap, J.T., **Mukherjee, J.**, Cooper, M. and Chen, C.T.: Reconstruction methods for dynamic PET image sequences. Presented at the *IEEE Nuclear Science*

Symposium and Medical Imaging Conference San Francisco, USA, October 21-28, 1995 (*IEEE 1995 Nuclear Science Symposium and Medical Imaging Conference Record*, 3:1718-1722, 1996).

Yang, Z-Y. and Mukherjee, J.: Paradoxical effects of the N-cyclopropylmethyl group in substituted benzamides: design of potential dopamine D-3 receptor agents. Presented at the Society of Nuclear Medicine 43rd Annual Meeting, June 3-6, 1996, Denver, Colorado (*J. Nucl. Med.*, 37: 193P, 1996).

Mukherjee, J. and Yang, Z-Y.: [F-18]Fluorinated substituted benzamide analog of YM-43611 as a potential radiotracer for dopamine D-4 receptors. Presented at the Society of Nuclear Medicine 43rd Annual Meeting, June 3-6, 1996, Denver, Colorado (*J. Nucl. Med.*, 37: 50P, 1996).

Blend, M.J., Friedman, M., O'Dowd, T., Tantayakom, S., Rubas, B., Levi, H., Penny, B.C. and Mukherjee, J.: Improved flood source uniformity using chelation of In-111 in a liquid filled plexiglass tank. Presented at the Society of Nuclear Medicine 43rd Annual Meeting, June 3-6, 1996, Denver, Colorado (*J. Nucl. Med.*, 37: 211P, 1996).

Mukherjee, J., Yang, Z.Y., Brown, T., Roemer, J. and Cooper, M.: Evaluation of extrastriatal dopamine receptors by using [F-18]fallypride. Presented at the Society for Neuroscience 26th Annual Meeting, Washington D.C., November 16-21, 1996 (*Soc. Neurosci. Abstr.*, 22: 498, 1996; abstract #202.6).