TECHNETIUM-99M LABELLED FLUOROBENZYLPEPIRIDINE AND IODINE-123 METAIODOBENZYLGUANIDINE FOR MAPPING MYOCARDIAL ADRENERGIC FUNCTION: A COMPARISON OF UPTAKE CHARACTERISTICS IN VASCULAR SMOOTH MUSCLE CELLS AND NEONATAL CARDIAC MYOCYTES, AND BIODISTRIBUTION STUDY IN RATS

S. Samnick [1], C. Scheuer [2], A. Schaefer [1], S. Richter [2], MD. Menger[2] and CM. Kirsch[1]

[1]Department of Nuclear Medicine and [2]Institute for Clinical and Experimental Surgery, Saarland University Medical Center, D-66421 Homburg/Saar, Germany. (E-mail contact: rassam@uniklinik-saarland.de)

Keywords: Cardiac neurotransmission, heart disorders, adrenoceptors, SPET

In developing ^{99m}Tc-based radioligands for in vivo study of cardiac adrenergic neurons, we compared the relative uptake of the new ^{99m}Tc-labelled compound ^{99m}Tc-FBPBAT with those of the SPET radiotracer ^{123}I-MIBG in rat vascular smooth muscle cells and neonatal ventricular cardiac myocytes as established *in vitro* models. Specificity of cellular uptake as well as the mechanisms underlying the uptake of both radiopharmaceuticals into the cardiovascular tissues were examined by pharmacological challenge experiments. Thereafter, cardiac and extracardiac accumulation of ^{99m}Tc-FBPBAT and ^{123}I-MIBG were assessed in intact rats and in rats pretreated with various - and -adrenoceptor drugs, and adrenergic reuptake blocking agents. The cellular uptake of ^{99m}Tc-FBPBAT and ^{123}I-MIBG was rapid and concentration dependent, more than 90 % of the total tissue radioactivity accumulation occuring within the first 5 min. Radioactivity concentration in cardiovascular tissues following a 60-min incubation at 37°C (pH 7.4) varied from 20 to 65% of the total activity per million cells (^{123}I-MIBG < ^{99m}Tc-FBPBAT). In comparison, tissue uptake of the cardiac perfusion radiotracers ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin remained relatively low (< 3%). Uptake of ^{99m}Tc-FBPBAT was obviously lower at 4°C and 20°C than at 37°C, while uptake of ^{123}I-MIBG showed only slight temperature dependence. Competitive inhibition experiments indicated that the uptake of ^{123}I-MIBG was predominantly mediated by the adrenergic uptake-I carrier, while the $_1$-adrenoceptors and in less instance $_1$-adrenoceptors were additionally involved in the uptake of ^{99m}Tc-FBPBAT into the cardiovascular tissues. Biodistribution in rats revealed a fast clearance of both radiopharmaceuticals from blood and a higher initial uptake of ^{99m}Tc-FBPBAT in the lung, which decreased rapidly with time (up to 55% after 60 min). On the other hand, significant uptakes and retentions of ^{99m}Tc-FBPBAT and ^{123}I-MIBG were observed in heart and spleen. Radioactivity accumulation in untreated rat myocardium 15 and 60 min following intravenous injection of ^{99m}Tc-FBPBAT accounted for 2.32 and 1.91 % of the injected dose per gram (i.d./g), respectively, compared with 3.10 and 2.21 % i.d./g in the *in vivo* experiment with ^{123}I-MIBG. Moreover, the heart uptake of ^{99m}Tc-FBPBAT was strongly inhibited by prazosin and metoprolol, more effectively than by desipramine and more potently than in lungs and other target organs, including kidney and spleen. In comparison, the myocardial uptake of ^{123}I-MIBG was only effectively lowered by pretreatment with the norepinephrine transporter inhibitor desipramine. Also, ^{99m}Tc-FBPBAT was excreted into urine and at less intensity by faeces. Urine analysis 6h p.i. revealed that more than 40% of the total excreted radioactivity was unmetabolized product. These results suggest that heart uptake of ^{99m}Tc-FBPBAT specifically reflects binding to cardiac adrenergic neurons. In contrast to ^{123}I-MIBG, the heart uptake of ^{99m}Tc-FBPBAT appears to be mediated predominantly via the $_1$/ß$_1$-adrenoceptor pathways. These data indicate that ^{99m}Tc-FBPBAT like ^{123}I-MIBG, exhibits interesting biological characteristics which hold promise for studies *in vivo* of myocardial sympathetic innervation by SPET.

NEW PROMISING RADIOFLUORINATED DOPAMINE TRANSPORTER LIGANDS: SYNTHESIS, N.C.A RADIOFLUORINATION AND PRELIMINARY EVALUATION

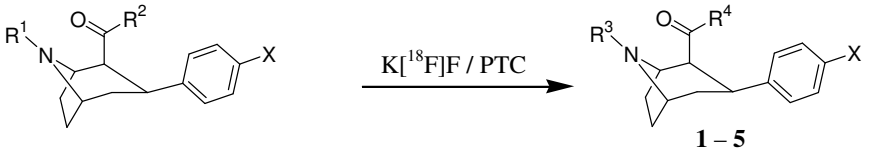
B. Krebs, D. Bier, W. Sihver, M. Holschbach, H.H. Coenen

Institut für Nuklearchemie, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany; e-mail address: b.krebs@fz-juelich.de

Keywords: Cocaine amide derivatives, [^{18}F]FP-CIT, DAT, PET

The dopamine transporter (DAT) is a widely accepted marker for the integrity of the presynaptic nigrostriatal dopaminergic system. DAT can be imaged usefully with suitable radioligands and neuroimaging techniques like single-photon emission tomography (SPECT) or positron emission tomography (PET). Presently the SPECT ligand [^{123}I]FP-CIT, known as DaTSCAN,[1] is the only commercial DAT radiotracer for routine use. Up to now there is no suitable radiofluorinated DAT ligand available for PET imaging although numerous investigations have been performed by different working groups.[2]

The present study describes the syntheses of potential fluorinated DAT ligands, those of suitable precursors for radiofluorination and their ^{18}F-labelling to give compounds **1** – **5** (Fig).



1 – 5

X	R^1	R^2	R^3	R^4	No.
I	Y-$(CH_2)_n$-	-O-CH_3	**[^{18}F]F**-$(CH_2)_n$-	-O-CH_3	**1**
CH_3	CH_3	Y-$(CH_2)_n$-O-	CH_3	**[^{18}F]F**-$(CH_2)_n$-O-	**2**
CH_3	CH_3	Y-$(CH_2)_n$-N-	CH_3	**[^{18}F]F**-$(CH_2)_n$-N-	**3**
CH_3	I-CH=CH-CH_2-	Y-$(CH_2)_n$-O-	*(E)*-I-CH=CH-CH_2-	**[^{18}F]F**-$(CH_2)_n$-O-	**4**
CH_3	I-CH=CH-CH_2-	Y-$(CH_2)_n$-N-	*(E)*-I-CH=CH-CH_2-	**[^{18}F]F**-$(CH_2)_n$-N-	**5**

Figure: Radiosynthesis of [^{18}F]DAT-ligands (PTC = phase transfer catalyst, Y = OTf, OTos, OMes)

The esters of cocaine derivatives possess a high selectivity and affinity ($K_i \leq 10$ nM) for the DAT.[2] Amides **3** and **5** represent a new promising class of DAT ligands. Results of binding studies and first preclinical *in vitro* and *ex vivo* studies of **3** and **5** in rodents will be presented.

In order to avoid a two-step radiosynthesis,[3] an automated and reliable one-step radiosynthesis of n.c.a. [^{18}F]FP-CIT **1** (n = 3) was established. In an earlier published study the radiochemical yield of such a procedure was in the range of 1 – 2 %.[4] A reliable increase to 7 % RCY was achieved by fine tuning of the reaction parameters temperature, time and solvent. The main side reaction during the labelling procedure was the elimination of the leaving group resulting in the formation of the N-allyl compound. This was unequivocally proven by mass spectroscopic identification of the unsaturated side product obtained in a carrier added synthesis. The optimum reaction conditions for the radiosynthesis of **1** consisted of performing the radiofluorination at a moderate temperature of 60 – 70 °C for 10 min using DMSO as a solvent. Other solvents such as CH_3CN or DMF as well as higher reaction temperatures resulted in a negligible RCY of **1**.

1.Nycomed Amersham plc *DaTSCAN* monograph 2000.
2.Singh S. *Chem Rev* 2000; 100: 925-1024.
3.Lundkvist C, Halldin C, Ginovart N, Swahn C G, Farde L. *Nucl Med Biol* 1997; 24: 621-627.
4.Chaly T, Dhawan V, Kazumata K, Antonini A, Margouleff C, Dahl J R, Belakhlef A, Margouleff D, Yee A, Wang S, Tamagnan G, Neumeyer J L, Eidelberg D. *Nucl Med Biol* 1996; 23: 999-1004.

COMPARISON OF [^{11}C](+)-MCN5652 AND S-([^{18}F]FLUOROMETHYL)-(+)-MCN5652 FOR PET IMAGING OF THE SEROTONIN TRANSPORTER

P. Brust[1], J. Zessin[2], O. Solin[3], J. Steinbach[1].

[1]Institut für Interdisziplinäre Isotopenforschung, Permoserstrasse 15, 04318 Leipzig, Germany, [2]Forschungszentrum Rossendorf, Institut für Bioanorganische und Radiopharmazeutische Chemie, PF 510119, 01314 Dresden, Germany,
[3]Laboratory of Radiochemistry, Department of Chemistry, POB 55, 00014 University of Helsinki, Finland.
e-mail: brust@iif-leipzig.de

Keywords: Serotonin transporter - Brain imaging - Positron Emission Tomography – Kinetic Modelling - Pig

S-([^{18}F]fluoromethyl)-(+)-McN5652 ([^{18}F](+)-FMe-McN5652) has recently been synthesized as a new potential radiotracer for positron emission tomography (PET) imaging of the serotonin transporter (SERT)(1). It is an analogue of [^{11}C](+)McN5652, which has already been used in clinical PET studies for SERT imaging. A comparison of the in vivo binding characteristics and kinetics of these two radiotracers is performed in the porcine brain.

PET images revealed that the highest accumulation of both radiotracers was found in the ventral midbrain, thalamus, olfactory lobe and pons which is consistent with the known density of the SERT. The specific binding was determined by subtracting the values of the inactive (-) enantiomers or of the occipital cortex from those obtained with [^{11}C](+)McN5652 or [^{18}F](+)-FMe-McN5652 in the time period between 75 and 115 min after radiotracer injection. The specific binding of the ^{18}F-labeled derivative was about 40 % higher than that of the ^{11}C-labeled derivative. A strong inhibition of the specific binding was observed for both radiotracers after pre-treatment with the highly selective SERT uptake inhibitor citalopram. [^{18}F](+)-FMe-McN5652 showed a faster kinetics than [^{11}C](+)McN5652. It reached the binding equilibrium during a study length of 120 min which was not the case for [^{11}C](+)McN5652. Rather uniform brain binding was observed after injection of the biologically inactive radiolabelled enantiomers or after pre-treatment with citalopram. The norepinephrine uptake inhibitor maprotilin did not show any inhibitory effect.

The blood-brain transfer of [^{18}F](+)-FMe-McN5652 is about 30 % higher and the peripheral metabolism somewhat slower than that of [^{11}C](+)McN5652. Using a one-tissue compartment model (K_1, k''_2) or a two-tissue compartment model (K_1 to k_4) with or without constraints, different parameters related to the binding to the SERT were calculated. For both radiotracers, a significant improvement of the fits was obtained with the two-tissue compartment model. In most cases higher absolute binding potential values and higher midbrain-occipital cortex ratios were obtained for [^{11}C](+)McN5652 than for [^{18}F](+)-FMe-McN5652. However, also the coefficient of variation was much higher for the ^{11}C-labelled derivative. The regional binding parameters of [^{11}C](+)-McN5652 and [^{18}F](+)-FMe-McN5652 are highly correlated among each other and with the SERT density as determined by in vitro binding of [^{3}H]citalopram. The K_1/k'_2 ratio calculated from studies after pre-treatment with citalopram was used as a constraint to correct for the free fraction and nonspecific binding of the radiotracers. It resulted in a considerable increase of the midbrain-occipital cortex ratios with higher values for [^{18}F](+)-FMe-McN5652 compared to [^{11}C](+)McN5652.

We conclude that [^{18}F](+)-FMe-McN5652 is a useful radiotracer for PET imaging of the SERT. It has slightly better features than [^{11}C](+)McN5652 for this purpose.

(1) Zessin et al. (2001) Nucl Med Biol 28: 857-863.

SEX DIFFERENCES IN THE UPTAKE OF [^{18}F]FMe-McN IN RAT BRAIN

P. Marjamäki[1], O. Eskola[2], M. Haaparanta[1], T. Grönroos[1], V. Fagerholm[3], J. Bergman[2], P. Lehikoinen[2], N. Savisto[2], J. Zessin[4], O. Solin[2,5]

[1]MediCity PET Laboratory, Turku PET Centre, Tykistökatu 6A, FIN-20520 Turku, Finland, [2]Radiopharmaceutical Chemistry Laboratory and Accelerator Laboratory, Turku PET Centre, Porthaninkatu 3, FIN-20500 Turku, Finland, [3]Department of Biology, Åbo Akademi University, Tykistökatu 6A, FIN-20520 Turku, Finland, [4]Institut für Bioanorganische und Radiopharmazeutische Chemie, Forschungszentrum Rossendorf, PF 510 119 01314 Dresden, Germany, [5]Radiochemistry laboratory, P.O. Box 55, FIN-00014 University of Helsinki, Finland, Contact e-mail: olof.solin@helsinki.fi

Keywords: [^{18}F]FMe-McN, SERT, sex difference, metabolite

Serotonergic neurons of the mammalian brain are located in the mid- and hindbrain regions where they project to almost every area of the brain. The serotonergic system regulates diverse neural processes, such as integrative cognition, memory, arousal, mood, satiety and sexual behaviour. All of these functions are sensitive to the levels of ovarian hormones, estrogen and progesterone. In the rat brain the serotonergic system is more expressed in females than in males. This sexual dimorphism is not restricted to specific brain regions but suggests a general increase in serotonergic activity in the female rat brain.

The serotonin transporter (SERT) regulates the level of serotonin (5-HT) in the synaptic cleft by reuptake of 5-HT into presynaptic nerve endings. Drugs that inhibit SERT are important therapeutic agents in the treatment of psychiatric and neurologic diseases. We have recently synthesised (1) and evaluated (2) S-[^{18}F]fluoromethyl-(+)-McN5652 ([^{18}F]FMe-McN) as a radiotracer for imaging SERT. We now report on our studies on the sex difference in the brain uptake of [^{18}F]FMe-McN in rat. Female rats were at the phase of estrus or proestrus in the estrus cycle. The uptake of [^{18}F]FMe-McN in the brain of female (n=15) and male (n=17) rats was determined *ex vivo* from coronal brain sections with digital autoradiography. We have also studied the formation of radiolabelled metabolites of this tracer by radio thin layer chromatography (radio-TLC).

The tracer was injected *iv* and the rats were sacrificed at 120 min post injection. Radio-TLC-analyses showed that there was ~ 8% of unmetabolized [^{18}F]FMe-McN left in rat plasma at 120 min post injection. The uptake in the raphe nuclei, substantia nigra, hypothalamus, thalamus, locus coeruleus, amygdala, frontal cortex and cerebellum was analysed. The analysis showed that female rats had mean uptake values in regions rich in SERT varying from 0.65 to 0.77 %ID/g tissue. In males the corresponding values were 0.47 to 0.54. A statistical analysis demonstrated that females had significantly higher ($p<0.01$, T-test with equal variances) uptake values in all brain regions studied.

Our results show that [^{18}F]FMe-McN is a highly useful tracer for imaging sexual dimorphism of SERT in rat. Further studies are warranted in order to elucidate the interactions of gonadal hormones with SERT. The use of this tracer in human studies with positron emission tomography is planned.

1. Zessin J, Eskola O, Brust P, Bergman J, Steinbach J, Lehikoinen P, Solin O, Johannsen B. *Nucl Med Biol* 2001; 28:857-863.
2. Marjamäki P, Zessin J, Eskola O, Grönroos T, Haaparanta M, Bergman J, Lehikoinen P, Forsback S, Brust P, Steinbach J, Solin O. *Synapse* 2003; 47:45-53.

[^{11}C]SB207145, THE FIRST SELECTIVE PET LIGAND FOR DELINEATION OF 5-HT4 RECEPTORS IN THE BRAIN

A.D. Gee[1], J. Passchier[1], L. Martarello[1], D. Bender[2], C.A. Parker[1], J.C. Matthews[1], S. Ashworth[1], S. Knibb[1], J. Brown[1]

(1) GlaxoSmithKline, ACCI Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2GG, UK

(2) PET Centret, Bygning 10C, Aarhus Kommunehospital, 8000 Aarhus C, Denmark

Email: Antony.D.Gee@gsk.com

Keywords: serotonin, 5-HT4, brain

Introduction: In the CNS, the 5-HT4 receptor is believed to play a role in depression, memory impairment, and cognition but little is known about the *in vivo* pharmacology of this receptor. The availability of a selective 5-HT4 PET ligand could greatly facilitate new research into pathology believed to be related to this receptor. Although some *in vitro* radioligands are available, until recently no sucessful PET ligand has been published. Here we present the synthesis and *in vivo* evaluation in porcine brain of [^{11}C]SB207145 for the visualisation and quantification of 5-HT4 receptors using PET.

Methods: [^{11}C]SB207145 was produced by N-methylation of the corresponding desmethyl precursor with sodium hydride and [^{11}C]MeI in DMF at 110°C for 5 min followed by HPLC purification. The final product was obtained by *in vacuo* removal of organic solvents, followed by dissolving in 0.9% saline for injection.

[^{11}C]SB207145 was evaluated in anaesthetized Yorkshire pigs under baseline conditions and following coinjection with masses of SB207145 ranging from 0.5µg/kg to 500µg/kg. PET scans were acquired over a 90 minute period post iv tracer injection using a Siemens EXACT HR PET camera. Region of interest derived time-activity curves were generated for striatum, cortices, and cerebellum. Blood samples were obtained from the femoral artery to determine the input function. The rate of metabolism in plasma was established using radio-HPLC.

Results: [^{11}C]SB207145 was synthesised with a radiochemical purity of 93-99%, a specific activity of 12-70 GBq/µmol, and the unlabelled precursor as the only detectable chemical impurity. [^{11}C]SB207145 showed rapid entry into the brain with the highest retention in the striatum, followed by cortical regions and lowest retention in cerebellum. Mass doses > 50µg/kg of cooinjected SB207145 produced almost complete displacement of the specific signal, with significant displacement observed at 5µg/kg and a small displacement at 0.5µg/kg. Radio-HPLC analysis revealed that [^{11}C]SB207145 was rapidly metabolised to more hydrophilic labelled fractions in arterial plasma with the parent representing approximately 5 % of the total plasma radioactivity at 10 min post tracer administration. Data fom *ex vivo* studies in rats with [^{3}H]SB207145 suggests that these labelled metabolites do not cross the blood-brain barrier.

Conclusion: [^{11}C]SB207145 shows a distribution in porcine brain, consistent with reported 5-HT4 receptor densities as determined by tissue section autoradiography in animals and man. The high sub nanomolar affinity (Kd 0.2 nM) of SB207145 means that doses as low as 0.5 ug/kg may produce occupancies of the order of 10% and hence high specific activities are likely to be necessary for this radioligand. To our knowledge, [^{11}C]SB207145 is the first PET ligand to image 5-HT4 receptors in the brain. Currently, the behaviour of [^{11}C]SB207145 in healthy human volunteers is under investigation to demonstrate its utility in man.

COMPARISION OF THREE F-18 LABELED PET LIGANDS FOR THE SEROTONIN TRANSPORTER: RADIOSYNTHESIS AND IN VIVO IMAGING STUDIES IN BABOON

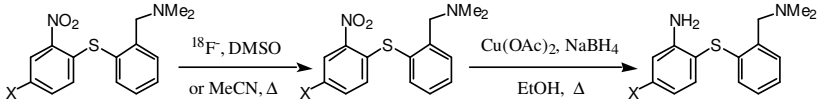
Y. Huang, Z. Zhu, S.A Bae, D.R., Hwang, R. Narendran, V. Phan, D. Erritzoe, P.S. Talbot, M. Laruelle

Department of Psychiatry, Columbia University, New York, NY, USA

Key words: Serotonin transporter, PET, Radioligand

The development of F-18 labeled PET radioligands for the serotonin transporter (SERT) has been an active area of research[1]. We have previously reported [^{18}F]AFM as a suitable PET tracer for SERT[2]. And another tracer in the same series, [^{18}F]AFA, has also been described[3]. Here we report the synthesis of a third F-18 labeled ligand for SERT, [^{18}F]AFE {[^{18}F]-(2-(2-dimethylaminomethylthio-phenyl)-5-fluoroethylphenylamine)}. An improved preparation of [^{18}F]AFM and [^{18}F]AFA, as well as comparison of these three F-18 labeled SERT ligands in the same baboon, will also be described.

The three F-18 labeled ligands, [^{18}F]AFA, [^{18}F]AFM, and [^{18}F]AFE, were prepared from their respective precursors by a nucleophilic displacement with [^{18}F]fluoride, followed by reduction of the nitro functionality with $Cu(OAc)_2$ or $SnCl_2$ and $NaBH_4$ in ethanol, as depicted in the scheme below. All three ligands were prepared in >10% radiochemical yield in the two-step synthetic sequence. The final products have >95% radiochemical purity.



1. X = NO_2
2. X = CH_2Cl
3. X = CH_2CH_2OTs
4. X = ^{18}F
5. X = $CH_2{}^{18}F$
6. X = $CH_2CH_2{}^{18}F$
7. [^{18}F]AFA: X = ^{18}F
8. [^{18}F]AFM: X = $CH_2{}^{18}F$
9. [^{18}F]AFE: X = $CH_2CH_2{}^{18}F$

In vitro binding studies indicated that AFM has a higher affinity for SERT than AFA or AFE (Ki 0.42 nM for AFM, 1.46 and 1.88 nM for AFA and AFE). Lipophilicity of the three ligands is similar (log P of 2.53, 2.44, and 2.38 for [^{18}F]AFA, [^{18}F]AFM, and [^{18}F]AFE). PET imaging studies were conducted in the same baboon to compare the in vivo behavior of the three radioligands. Results from these experiments indicated that all three ligands display uptake pattern consistent with the rank order of SERT distribution in the baboon brain. Brain kinetics of [^{18}F]AFA and [^{18}F]AFE is very similar, with peak uptake in the thalamus at 15 to 35 min after tracer injection, while [^{18}F]AFM has a slower brain kinetics, with peak uptake occurring at 40 to 60 min post-injection. For [^{18}F]AFA and [^{18}F]AFE, thalamus to cerebellum activity ratio plateau at ~2.5 at 90 min post-injection, while this ratio for [^{18}F]AFM reaches ~3.2 at 90 min and still increasing at the end of the three-hour scan. Kinetic analysis using a two-compartment model returned equilibrium specific-to-nonspecific partition coefficient {V_3", calculated as [($V_{T\ ROI}$ / $V_{T\ Ref}$) – 1]} of 1.29, 0.60, 0.35, 0.11 and 0.10 for [^{18}F]AFA; 1.30, 0.61, 0.47, 0.19 and 0.21 for [^{18}F]AFE in the thalamus, striatum, hippocampus, temporal cortex and cingulate cortex, compared with 2.27, 1,13, 0.65, 0.53 and 0.34 for [^{18}F]AFM in the same regions..

In conslusion, we have synthesized three F-18 labeled PET radioligands for SERT. All three ligands appear to be appropriate PET ligands for the in vivo imaging of SERT in baboons, with [^{18}F]AFM gives much higher specific binding than [^{18}F]AFA or [^{18}F]AFE in all baboon brain regions.

References:

1. Marjamaki P, Zessin J, Eskola O, Gronroos T et al. *Synapse* 2003; 47:45-53
2. Huang Y, Hwang DR, Narendran R et al. *Neuroimage* 2002; 16:S2
3. Shiue GG, Fang P, Greenburg JH, Shiue CY. *J Nucl Med* 2002; 43:165p

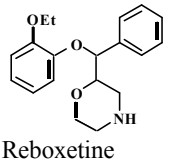
SYNTHESIS AND EVALUATION OF A NEW NOREPINEPHRINE TRANSPORTER PET LIGAND IN NON-HUMAN PRIMATES

K. S. Lin, Y.-S. Ding

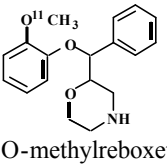
Brookhaven National Laboratory, Chemistry Department, Bldg 555, Upton, NY, USA 11973.

Keywords: norepinephrine transporter, brain, PET

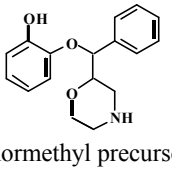
Research on dopamine (DA) and serotonin (SER) systems related to various CNS disorders has benefited from the availability of suitable radioligands. The norepinephrine transporter (NET) has long been recognized in relation to the pathophysiology and treatment of ADHD, substance abuse and depressive disorders. However, brain imaging of NET has been hampered by the lack of suitable radioligands. The fact that all three transporters (NET, DAT and SERT) are involved in various neurological and psychiatric diseases places a sense of urgency to develop new NET ligands so that we will be able to tease out the roles of individual transporters underlying specific CNS disorders. Reboxetine, (RS)-2-[(RS)- (2 ethoxyphenoxy)-benzyl]morpholine, is a specific NET inhibitor with a high affinity and high selectivity (IC_{50} DAT/NET = 4000) and it has been approved for the treatment of depressive illness in several European countries. The purpose of this study is to synthesize and evaluate a C-11 labeled analogue of reboxetine ([^{11}C]O-methyl-reboxetine) for PET imaging studies of NET in non-human primates.



Reboxetine



[^{11}C]O-methylreboxetine



normethyl precursor

Methods: Initially, O-methylreboxetine was synthesized according to synthetic procedures reported previously. Attempts to prepare the desired O-demethylated precursor via demethylation of O-methylreboxetine were not successful. A new nine-step synthetic procedure was thus developed to prepare the normethyl precursor, which was then used to synthesize [^{11}C]O-methyl-reboxetine. With tracer in hand, PET imaging studies were carried out in baboon.

Results: [^{11}C]O-methylreboxetine was obtained in high radiochemical yield (89%) and high specific activity by reacting the precursor with [^{11}C]CH_3I in the presence of base. The reaction conditions were highly selective for O-methylation over N-methylation, and no significant amount of N-methylated by-product was observed. PET studies in baboon revealed high uptakes in the regions of brain with the highest uptake in thalamus (TH), modest in cerebellum (CB) and striatum (ST), and lowest in frontal cortex (FC). Pretreatment with nisoxetine, a specific NET blocker, significantly reduced the binding in TH and CB, but not in ST, suggesting the specific binding of [^{11}C]O-methylreboxetine towards NET. Baboon plasma at various time points after injection of the radiotracer was assayed for unchanged radiotracer by HPLC and solid phase extraction methods. Both methods consistently indicated that approx. 55, 40, 32, and 22% remained as unchanged radiotracer in plasma at 5, 10, 30 and 60 min, respectively.

Summary: The relative regional distribution of the radioactivity after injection of [^{11}C]O-methylreboxetine in baboon brain is consistent with the known distribution of NET. The fact that tracer uptakes were blocked by nisoxetine in TH and CB, but not ST, suggests that [^{11}C]O-methylreboxetine specifically binds to NET in the brain and it may be a useful scientific tool to provide the specific and functional maps of NET in the brain. Since reboxetine is a racemic mixture of the R,R(-) and S,S(+) enantiomers and the S,S(+) enanatiomer is more potent in norepnephrine re-uptake inhibition, comparative studies in vivo with PET using individual C-11 labeled [^{11}C]O-methylreboxetine would be important and they are currently under investigation in our lab. These studies will allow a better understanding of the role that NET plays in living systems, and set the stage for drug development and future examination of ADHD, substance abuse, depression and anxiety disorders. Supported by DOE-OBER and NIH (NS-15380).

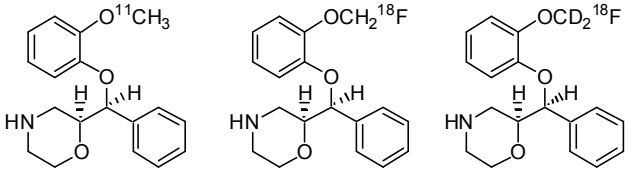
PREPARATION AND PET EVALUATION OF [^{18}F]FMPBM-D_2 – A PROMISING BRAIN NOREPINEPHRINE TRANSPORTER (NET) RADIOLIGAND

M. Schou[1], C. Halldin[1], V.W. Pike[2], J. Sovago[1], B. Gulyas[1], E. Shchukin[1], D. Mozley[3], D. Dobson[3], D.P. Johnson[3], R.B. Innis[2] and L. Farde[1]

[1]Karolinska Institutet, Dept. of Clinical Neuroscience, Psychiatry Section, Karolinska Hospital, S-17176 Stockholm, Sweden. [2]Molecular Imaging Branch, NIMH, Bethesda, MD 20892, USA. [3]Lilly, Indianapolis, USA. E-mail contact address: christer.halldin@ks.se

Keywords: Fluorine-18, NET, PET, FMPBM-D_2, Brain

No useful radioligand for imaging the brain norepinephrine transporter (NET) *in vivo* with positron emission tomography (PET) currently exists. PET evaluation of the selective NET inhibitor, (*S,S*)-[^{11}C]2-(-(2-methoxyphenoxy)benzyl)morpholine ([^{11}C]MPBM or [^{11}C]Me-NER; **1**), *in vivo* revealed that specific binding increased continuously until the end of the experiment (1). In order to extend PET examination time and possibly reach equilibrium, we prepared a novel fluoromethoxy analog of MPBM, namely (*S,S*)-[^{18}F]2-(-(2-fluoromethoxy-phenoxy)benzyl)morpholine ([^{18}F]FMPBM, **2**). To try to prevent rapid defluorination of the radioligand, we also prepared the *di*-deuterated analog, [^{18}F]FMPBM-D_2 (**3**).



1 **2** **3**

O-Desmethyl-MPBM and *N-Boc-O-desmethyl*-MPBM were prepared and treated with no-carrier-added (NCA) [^{18}F]bromofluoromethane, [^{18}F]fluoromethylene triflate or their *di*-deuterated analogs to yield [^{18}F]FMPBM or [^{18}F]FMPBM-D_2. Each radioligand was injected i.v. into Cynomolgus monkeys and examined during baseline and pretreatment (desipramine, 5 mg/kg) conditions with PET. Striatum was used as a reference region for free radioligand and non-specific binding. Radioactive metabolites in plasma were measured by HPLC.

NCA [^{18}F]FMPBM and [^{18}F]FMPBM-D_2 were obtained in quantitative radiochemical incorporation yield from labeled precursors and were radiochemically stable for > 5 h. High bone uptake of radioactivity was observed after injection of [^{18}F]FMPBM, whereas bone uptake was much lower with [^{18}F]FMPBM-D_2. After injection of [^{18}F]FMPBM-D_2, radioactivity in brain peaked at 12 min (3.6% injected dose). PET images were consistent with high uptake of radioactivity into supposed NET-rich regions. Radioactivity levels in thalamus, mesencephalon, lower brainstem and temporal cortex were 1.3-, 1.6-, 1.5-, and 1.6-fold higher than in striatum by 183 min. In the pretreatment experiment, these ratios were reduced markedly by (e.g. to 1.03 in mesencephalon). Labeled metabolites in plasma were more polar than the parent radioligand (60% parent at 100 min). The lower bone uptake of radioactivity arising from [^{18}F]FMPBM-D_2, compared to [^{18}F]FMPBM, is similar to the effect of *di*-deuteration observed for another radioligand (2). These preliminary results indicate that [^{18}F]FMPBM-D_2 has potential to be a useful radioligand for imaging NET *in vivo*.

1. Halldin C, Schou M, Sovago J *et al.*, *Mol. Imaging Biol.,* 2002, 4, S52.
2. Hamill TG, Burns HD, Eng W *et al.*, *Mol. Imaging Biol.,* 2002, 4, S34.

NEW APPROACHES TO MONITORING GENE/CELL THERAPY

Y. Fujibayashi, T. Furukawa, S. Takamatsu and Y. Yonekura

Biomedical Imaging Research Center, Fukui Medical University, 23-3 Shimoaizuki, Matsuoka, Yoshida, Fukui, 910-1193, JAPAN
e-mail: yfuji@fmsrsa.fukui-med.ac.jp

Key words: molecular imaging, PET, reporter gene, ES cell, regenerative medicine

Gene / cell therapy is a new methodology which introduces a desired function into target cells / tissue. In gene therapy, functional gene is introduced into existing cells to express protein, and in cell therapy, stem cells such as ES cells having ability to provide a desired function are transplanted into target tissue. In both cases, noninvasive detection of gene- / cell- specific mRNA or protein is of great importance for the evaluation of therapeutic efficacy, in the process of developmental research as well as clinical practice.

For the detection of gene expression at mRNA level, antisense OLIGO concept allows us to design an appropriate probe for any type of therapeutic gene, based on the sequence of target gene. However, radiolabeling of antisense OLIGOs suitable for in vivo detection is quite demanding; it requires high specific activity, radiolabeling method with short-lived gamma emitter, simple labeling method (hopefully without purification), preserved binding ability to specific sequence, and so on. In our Lab, DNA OLIGO with amino-linked multi-chelating sites (MCS-probe) has been developed for this purpose. MCS-probe could be labeled by simple mixing with In-111. In-111-MCS-probe (antisense) showed sequence specific binding to sense DNA, and high uptake in cells expressing target mRNA in *in vitro* and *in vivo*.

For the detection of gene expression at protein level, two approaches are possible; detection of therapeutic protein itself, or use of reporter gene system. The former requires specific radioligand for each protein. Pair of radiolabeled acyclovir derivative and HSV-TK is a good example of this category. The latter approach uses a “general radioligand - reporter gene” system, which requires an additional introduction of artificial gene merely to report gene expression.

In stem cell transplant therapy, viability of the cells as well as functional differentiation is essential for successful therapy. Assessment of cell viability is very important, especially for troubleshooting in the case of unsuccessful outcome. For this purpose, we have developed ES cell lines transfected with new reporter gene(s) for PET imaging. In general, reporter protein should not be physiologically active, immnogenic, in existence in the target area, secretory nor big in size. In addition, PET-ligand should be safe for use in human and able to cross the cell membrane (preferably blood-brain barrier (BBB) also). We selected a ligand binding domain of estrogen receptor (ER) or that of a mutant-estrogen receptor (Mer: tamoxifen receptor). The former has a high affinity to estradiol, and the latter, altered specificity (Tamoxifen>> estradiol). Both F-18-estradiol and F-18-tamoxifen are estabilished PET ligands for clinical practice. We established two types of ES cell transfectants; one with stable expression and another with inducible expression of the reporter gene. We confirmed that these ES cell transfectants still expressed Oct-3/4 gene, a marker of pluripotency. Using these cells, ligand binding ability in vitro as well as in vivo was studed.

These tactics will bring new information and approaches in the field of molecular / cellular medicine.