SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-{[4-(4-[18F]FLUOROPHENYL)METHYL] PIPERAZIN-1-YL}-METHYL-1H-PYRROLO[2,3-B]PYRIDINE FOR IN VIVO STUDY OF DOPAMINE D4 RECEPTOR

D.Z. Yin [1], H.B. Tian[1], L. Zhang[1], L.H. Wang[1], C.F. Zhang[1], J.L. Li[1], W. Zhou[2], C.Y. Wu[1], Y.X. Wang

[1]Radiopharmaceutical research Center, Shanghai Institute of Nuclear Research, Chinese Academy of Sciences, P.O. Box 800-204, Shanghai, 201800, P. R. China. Email:ydzh1945@sohu.com
[2]Depatment of Nuclear Medicine , Hua-shang Hospital , University of fudan, Shanghai, 200040, P. R. China

KEY WORDS: Fluorine-18, Labeling, Dopamine D_4 receptor, 7-azaindole analog, Tissue Distribution

The dopamine D_4 receptor has been proposed as a target for atypical and novel antpsychotic drugs. The hypothesis is based on the regional distribution of the D_4 receptor and the relatively high affinity of clozapine for this receptor subtype [14]. The potential clinical importance of the D_4 receptor has inspired significant interests in its pathologic physiological role in neuropsychiatric disorders. But the study of this receptor subtype in vivo has so far been hindered by the lack of selective radioligands.

The 7-azaindole derivative (L-745,870) is a novel, high-affinity(Ki=0.43nM) and selective Dopamine D_4 R ligand. We synthesized and evaluated 3-{[4-(4-[^{18}F] fluorophenyl)methyl] piperazin-1-yl}-methyl-1*H*-pyrrolo[2,3-b]pyridine([^{18}F3]), which is an analog of L-745,870 specific binding D_4 receptor in vitro. The [^{18}F]3 was prepared by reductive alkylation of 3-(piperazin-1-yl)-methyl-1*H*-pyrrolo[2,3-b]pyridine with 4-[^{18}F] fluorobenzaldehyde. Radiochemical yield(decay-corrected)reaches 9.0-12.0% and specific activity more than 37GBq/μ mol. A higher uptake was observed in the frontal cortex, medulla and hippocampus(0.43%ID/g,0.38 ID/g,0.35 ID/g) compared to the striatum at 10min after injection. Tissue distribution study in rats demonstrated specific distribution of the [^{18}F]3 in brain regions, suggesting that this radioligand may be a suitable agent for in *vivo* study of dopamine D_4 receptor.

The metabolism study showed that there was no metabolite observed in rats brain. This result indicated that the brain distribution was derived from in *vivo* behavior of the [^{18}F]3.

DOPAMINE D_4 RECEPTOR ANTAGONIST 3-(4-[^{18}F]FLUOROBENZYL)-8-METHOXY-1,2,3,4-TETRAHYDROCHROMENO[3,4-C]PYRIDIN-5-ONE: RADIOSYNTHES AND *IN VIVO* CHARACTERIZATION IN RATS

H.B. Tian[1], D.Z. Yin[1], L. Zhang[1], L.H.Wang[1], C.F. Zhang[1], C.Y. Wu[2], Y.X.Wang[1]

[1]Radiopharmaceutical research Center, Shanghai Institute of Nuclear Research, Chinese Academy of Sciences, P.O. Box 800-204, Shanghai, 201800, P. R. China.
[2]Depatment of Nuclear Medicine , Hua-shang Hospital , University of fudan, Shanghai, 200040, P. R. China
*Address correspondence to: TIAN Hai-Bin, Radiopharmaceutical research Center, Shanghai Institute of Nuclear Research, The Chinese Academy of Sciences, P.O. Box 800-204, Shanghai, 201800, P. R. China.
Tel/Fax: +86 21 59552409. E-mail: thb1996@hotmail.com

Key Words: Fluorine-18, Dopamine D_4 receptor, PET, Tissue Distribution

The dopamine D_4 receptor (D_4R) is expressed in low density in various extrastriatal brain regions. With the discovery that clozapine targets the dopamine D_4 receptors, this receptor sites have strongly been suggested as target for antipsychotic drugs. But these studies may have significant limitations due to the lack of selective ligands suitable for labeling dopamine D_4 receptors in *vivo*. We synthesized novel ^{18}F-labeled a dopamine D_4 receptor antagonist (Ki =4.3 nM), 3-(4-[^{18}F]fluorobenzyl)-8-methoxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one ([^{18}F]**2**) , has exhibited nanomolar affnity and high selectivity. Radiosyntheses were accomplished by fluorine-18 labeled intermediate with 8-methoxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one (**1**) followed by HPLC purifications. The radiosynthesis of [^{18}F]**2** required approximatively 110 min with an overall radiochemical yield of 19.5%(decay-corrected) and with high specific activities (>37 GBq/mmol). Tissue distribution studies of the [^{18}F]**2** in rats showed he distribution pattern of radioactivity in the brain was frontal cortex and medulla , the region that has a high density of D_4 receptors. Pre-treatment with non-radioactive **2** (1 mg/kg) produced a significant reduction of radioactivity in all the regions. Analyses of blood extracts showed about 44% of total radioactivity in plasma represented unchanged radioligand at 60 min after injection as determined by HPLC, analysis of the rat brain extract shown that the composition of brain from rats 2,10,60 min after injection of [^{18}F]**2** consisted primarily of unchanged parent compound. These results indicate that [^{18}F]**2** may have some specific binding to the D_4 receptor. We are continuing to do the efforts in order to develop the suitable radioligand for D_4 receptors with PET.

DETERMINATION OF THE ARTERIAL INPUT FUNCTION OF THE MAO-A INHIBITOR [^{11}C]-HARMINE IN HUMAN SUBJECTS

A.A. Wilson, J. H. Meyer, A. Garcia, R. Guo, K. Singh, D. Hussey, S. Houle, and N. Ginovart

PET Centre, Centre for Addiction and Mental Health and Department of Psychiatry, University of Toronto, Toronto, M5T 1R8, Ontario, Canada e-mail aaw@camhpet.on.ca

Keywords: Carbon-11, MAO, PET, human metabolism, LOOP

[^{11}C]-Harmine is a radiotracer developed for the study of monoamine oxidase A (MAO-A) in the CNS by PET[1]. We are currently using [^{11}C]-harmine to study levels of MAO-A in major depressive episodes and for anti-depressant development. As there is no brain region which is devoid of MAO-A, a reference tissue model for quantifying MAO-A levels using [^{11}C]-harmine has not been developed. Quantification of [^{11}C]-harmine binding thus requires a full kinetic model using a time-dependant arterial input function which in turn requires analysis of [^{11}C]-harmine levels in the arterial plasma of subjects. We report here a robust method of plasma analysis of [^{11}C]-harmine based on the column capture and switching methods developed by Luthra and Hilton[2,3]

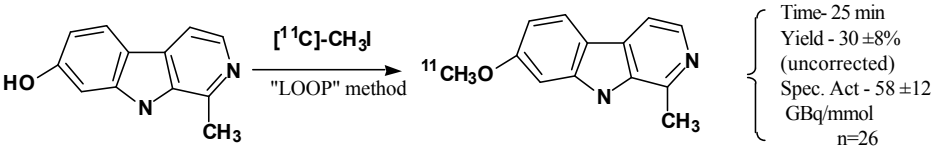
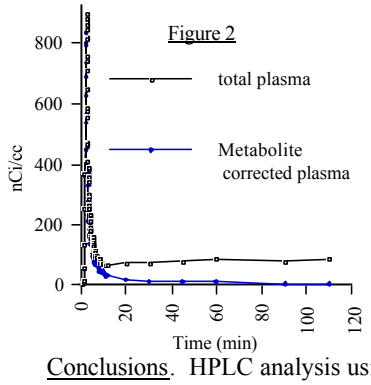


Figure 1. Radiosynthesis of [^{11}C]-Harmine.

Methods. [^{11}C]-Harmine was synthesised as depicted in Fig. 1 using our previously reported "LOOP" method[4]. Subjects were administered [^{11}C]-harmine (370 MBq, IV) as a bolus injection. Early arterial blood radioactivity levels were counted using an ABSS system. Arterial blood samples were also withdrawn at predefined time, centrifuged, and whole unadulterated plasma injected onto a capture column packed with OASIS resin. Highly polar metabolites and plasma proteins were eluted with 1% CH_3CN in H_2O through a coincidence flow detector (Bioscan Flow-Count). Less polar metabolites and [^{11}C]-harmine were washed onto an HPLC column (Phenomenex AquaC18, 5µ) and resolved using 30%CH_3CN/70% H_2O + 0.1N A.F. pH4 as eluent.

Results. HPLC analysis of a plasma sample could be completed in less than 8 min allowing multiple time points to be analysed despite the short half life of carbon-11. [^{11}C]-Harmine was rapidly metabolised with >50% of the radioactivity in plasma attributable to polar metabolites after 20 min. No lipophilic metabolites were detected in any subject (n=15). Fig. 2 shows representative curves for total radioactivity and for metabolite corrected radioactivity in plasma. Using the latter as input function, kinetic analyses of [^{11}C]-harmine binding revealed that a 2 tissue compartment model with three parameter estimates provided reliable fits of the time-activity data in all subjects and all brain regions examined and allowed to estimate [^{11}C]-harmine kinetics parameters, distribution volume and binding potential values with accuracy.



Conclusions. HPLC analysis using column capture and switching techniques can provide a robust arterial input function for the kinetic modelling of [^{11}C]-harmine binding to MAO-A.

(1) Bergstrom, M, et al. Nucl Med Biol, 24, 287. (1997)
(2) Luthra, SK, et al. IXth Int. Symposium on Radiopharm. Chemistry, Paris, K15. (1992)
(3) Hilton, J, et al. Nucl Med Biol, 27, 627. (2000)
(4) Wilson, AA, et al. Nucl Med Biol, 27, 529. (2000)

METABOLISM OF DOPAMINE D_1 AGONIST *R*-(+)-[^{11}C]SKF 82957 PRODUCES A RADIOLABELED METABOLITE IN RAT BRAIN

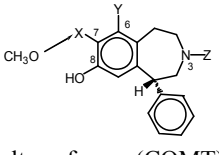
J.N. DaSilva[1,2,3], H. Cheung[1,2], A.A. Wilson[1] and S. Houle[1]

[1]Centre for Addiction and Mental Health PET Centre. [2]Department of Pharmacology, University of Toronto. [3]Cardiac PET Centre, University of Ottawa Heart Institute and Department of Cellular and Molecular Medicine, University of Ottawa, 40 Ruskin St., Ottawa, Ontario, Canada K1Y 4W7. E-mail: JDaSilva@ottawaheart.ca

Keywords: Dopamine D_1 receptor agonist, *R*-(+)-[^{11}C]SKF 82957, metabolism, HPLC, rat

Full dopamine D_1 receptor agonist *R*-(+)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (*R*-(+)-SKF 81297) was recently labeled by *N*-[^{11}C]methylation to produce D_1 agonist *R*-(+)-[^{11}C]SKF 82957 (1), which displays agonistic activity for adenylyl cyclase (2). Its in vivo rat evaluation demonstrated high binding selectivity for D_1 receptors, and low metabolism as measured by thin-layer chromatography (TLC) (1). Using the high-performance liquid chromatography (HPLC) procedure recently described by Hilton et al (3), *R*-(+)-[^{11}C]SKF 82957 metabolism was analyzed in rat plasma and brain extracts. By removing plasma proteins with a capture column after disruption with urea or acetic acid, this method allows reproducible analyses on a reverse phase column after column-switch that have higher resolution than TLC.

Using the same procedures, male Sprague-Dawley rat plasma and brain sample TLC analyses at 30 min post-injection of *R*-(+)-[^{11}C]SKF 82957 gave the same results as before (1). However, using the column-switch HPLC method, in addition to unchanged *R*-(+)-[^{11}C]SKF 82957, 4 more hydrophilic labeled metabolites were detected in plasma and one more lipophilic metabolite (~17% total radioactivity). Moreover, in rat brain homogenates, this labeled lipophilic metabolite was seen in similar amounts compared to unchanged tracer. In plasma from blood of control rats, addition of authentic *R*-(+)-[^{11}C]SKF 82957 produced only the lipophilic metabolite which increased in proportion with incubating time, whereas no extra peak was observed in the control brain mixture. These results demonstrate the presence of an enzyme in the blood that metabolizes *R*-(+)-[^{11}C]SKF 82957 into a lipophilic radioactive metabolite which crosses the blood-brain barrier (BBB).



X = OH Y = Cl Z = H *R*-(+)-SKF 81297
X = OH Y = Cl Z = CH_3 *R*-(+)-SKF 82957
X = Cl Y = H Z = CH_3 SCH 23390

Catechol-*O*-methyltransferase (COMT) enzyme has been shown to methylate the 3-hydroxy (not 4-OH group) of catecholamines such as dopamine, and is present in rat erythrocytes (4). It is possible that *R*-(+)-[^{11}C]SKF 82957 is also *O*-methylated by COMT at the 7-hydroxy position to produce the 7-methoxy derivative of *R*-(+)-[^{11}C]SKF 82957, which would be more lipophilic than SKF itself and would likely cross BBB. This process could explain the presence of the radioactive lipophilic metabolite peak in rat brain. In this benzazepine series, the presence of the 7,8-catechol has been shown to be essential for agonistic activity at D_1 receptors (2), while 7-X (Cl, Br or I) - 8-hydroxy derivatives are D_1 antagonists. We have previously reported that ~85% of the radioactivity signal in rat striatum can be blocked by the D_1 antagonist SCH 23390 (1), indicating that the radioactive lipophilic metabolite binds to D_1 receptors, possibly as an antagonist, and confounds *R*-(+)-[^{11}C]SKF 82957 binding and likely PET scanning quantification.

We acknowledge support by the Canadian Institutes of Health Research (MOP-13258).

1. DaSilva J N et al. *Nucl Med Biol* 1999; 26: 537-542.
2. Pfeiffer F R et al. *J Med Chem* 1982; 25: 352-358.
3. Hilton J et al. *Nucl Med Biol* 2000; 27: 627-630.
4. Männistö P T and Kaakkola S. *Pharmacol Rev* 1999; 51: 593-628.

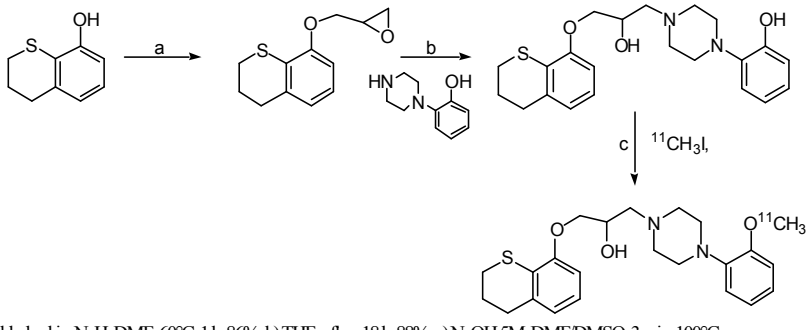
CARBON-11 LABELLING OF PRESYNAPTIC 5-HT$_{1A}$ RECEPTOR AGONIST 8{{3-[4-(2-METHOXYPHENYL)PIPERAZIN-1-YL]-2-HYDROXYPROPYL}OXY}THIOCHROMAN, AND ITS *IN VIVO* EVALUATION IN ANAESTHETISED RAT AND IN AWAKE CAT

Didier Le Bars[1,3*], Luc Zimmer[1,2], Guy Fournet[3], Benoît Joseph[3], Gérald Guillaumet[4]

[1]CERMEP, Biomedical Cyclotron, 59 Bd Pinel, 69003 Lyon, France (*email: lebars@univ-lyon1.fr)*
[2]Laboratoire de Neuropharmacologie et Neurochimie, INSERM U512, Université Claude Bernard - Lyon 1, France
[3]Laboratoire de Chimie Organique 1, Université Claude Bernard - Lyon 1, UMR-CNRS 5622, France
[4]Institut de Chimie Organique et Analytique, UMR-CNRS 6005, Université d'Orléans, France

Keywords: Serotonin 1A receptor; Carbon-11; PET; -Microprobe; rat; cat

8{{3-[4-(2-[^{11}C]methoxyphenyl)piperazin-1-yl]-2-hydroxypropyl}oxy}thiochroman is presynaptically agonist and postsynaptically antagonist of the serotonin 5-HT$_{1A}$ receptors. We labelled this molecule via O-methylation with [^{11}C]methyl iodide in good yield and specific activity.



a°epichlorhydrin, NaH, DMF, 60°C, 1 h, 86%; b) THF reflux, 18 h, 88%; c) NaOH 5M, DMF/DMSO, 3 min, 100°C.

For the biological evaluation of the radiolabelled compound, we adopted an original methodology which predicts the radiopharmacological characteristics of the new radioligand with a reduced number of animals, in contrary to classical pre-clinical evaluations. This approach includes (i) the use of a new -sensitive intracerebral probe (-Microprobe) implanted in brain of an anaesthetised rat and allowing to measure locally the kinetic of the new PET ligand, (ii) the PET scan on a cat maintained awake during the acquisition. The cerebellum was used as reference tissue because this region is practically devoid of 5-HT$_{1A}$ receptors and in the region of interest was the hippocampus, rich of 5-HT$_{1A}$ receptors.

Our results, obtained with two different but complementary methodologies, demonstrate the inability of the radiolabelled molecule to visualise the 5-HT$_{1A}$ receptors. A first explanation for the *in vivo* failure of the radiotracer may involve an insufficient brain penetration of this radioligand., explained by an inadequate lipophilicity or by a high peripheral protein binding. A second explanation may involve the pharmacological properties of the molecule, which is a partial agonist. This implies that the binding is limited to receptors coupled to a G-protein, representing only a variable fraction of the receptors available for binding to antagonists. Moreover, the binding of the agonist is generally transient with a rapid *in vivo* dissociation.

Since the discovery of a potent radiolabelled agonist would provide important insight into brain serotoninergic function, other 5-HT$_{1A}$ agonist thiochroman leads will be prepared for future investigations.

Accepted for publication Nucl. Med. Biol.

BIOLOGICAL EVALUATION OF ^{18}F-LABELED PIPERIDINYL, PIPERIDINEMETHYL AND PYRROLIDINYL ESTERS AS RADIOTRACERS FOR *IN VIVO* MEASUREMENT OF ACETYLCHOLINESTERASE

X. Shao[1], E. R. Butch[2], M. R. Kilbourn[1] and S. E. Snyder[1]

[1]Division of Nuclear Medicine, Department of Radiology, University of Michigan Medical Center, Ann Arbor, MI 48109 and [2]Department of Chemistry, Eastern Michigan University, Ypsilanti, MI 48197

Keywords: fluorine-18, acetylcholinesterase, Alzheimer's disease, PET

The activity of acetylcholinesterase (AChE) is an important marker for degeneration of the central cholinergic system. Clinical and postmortem studies provide evidence that the biochemical changes in brains of Alzheimer's disease (AD) patients include a decrease in AChE activity. *N*-[^{11}C]Methylpiperidinyl acetate (AMP) and propionate (PMP) are currently in routine clinical use as positron emission tomography (PET) radiotracers for studying AD. Based on our successes in using ^{11}C-labeled radiotracers for PET, a series of ^{18}F-Labeled PMP analogs have been studied. ^{18}F-labeled compounds would permit longer imaging times and allow the use of radiotracers with slower pharmacokinetics. Such may provide improved measurement of enzymatic activity in brain regions with high AChE concentration. The longer lifetime of ^{18}F also allows preparation of radiotracer batches for multiple patients and delivery of the tracer to other facilities, making the technique more widely available to clinical investigators.

We have previously reported two compounds, (*S*)-*N*-[^{18}F]fluoroethyl-2-piperidinemethyl acetate (**1**) and (*R*)-*N*-[^{18}F]fluoroethyl-3-pyrrolidinyl acetate (**2**) (1). In addition, a new compound, (*R*)-*N*-[^{18}F]fluoroethyl-3-piperidinyl acetate (**3**), was synthesized. The radiotracers were then evaluated for *in vivo* blood and brain metabolism in mice, brain pharmacokinetics in rats using microPET imaging and brain pharmacokinetics in monkeys (*M. nemistrina*) using PET imaging. All ^{18}F-labeled compounds were compared to [^{11}C]PMP.

Compound **1** showed rapid metabolism in mouse blood, relatively fast regional brain pharmacokinetics and poor discrimination between brain regions with different AChE concentration (striatum *versus* cortex).

Compound **3** showed slower blood metabolism and slower pharmacokinetics, but again poor discrimination between brain regions.

Compound **2** showed highly encouraging characteristics with an *in vivo* metabolism rate slower than [^{11}C]PMP, similar primate uptake and pharmacokinectics to [^{11}C]PMP, and good discrimination between brain regions. Further evaluation of this radiotracer as a ^{18}F-labeled alternative to PMP is wanted.

1. Shao X, Butch E. R, Kilbourn M. R, Snyder S. E. *Nucl. Med. Biol.* In press

RE-EVALUATION OF *IN VIVO* SELECTIVE BINDING OF [^{11}C]DOXEPIN TO HISTAMINE H_1 RECEPTORS IN ANIMALS

K. Ishiwata[1], K. Kawamura[1,2], W.F. Wang[1], H. Tsukada[3], N. Harada[3], H. Mochiduki[4], Y. Kimura[1], K. Ishii[1], R. Iwata[4] and K. Yanai[4]

[1]Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, 1-1 Naka-cho, Itabashi-ku, Tokyo, 173-0022, Japan; E-mail, ishiwata@ pet.tmig.or.jp; [2]SHI Accelerator Service LTD., Tokyo, Japan; [3]Hamamatsu Photonics K.K., Hamakita, Japan; [4]Tohoku University, Sendai, Japan.

Keywords: [^{11}C]doxepin, histamine H_1 receptor, brain, PET

[^{11}C]doxepin (Fig. 1) is a PET ligand for mapping histamine H_1 receptors in the brain. Clinically an age-related decrease of the binding of [^{11}C]doxepin to histamine H_1 receptors was clearly demonstrated (1), and the decline was much enhanced in the brain of Alzheimer's disease (2). The [^{11}C]doxepin PET is also a useful for evaluating receptor occupancy by antihitamines in the brain (3). The non-specific binding of [^{11}C]doxepin seems relatively high when the cerebellar binding was assumed to be the non-specific binding. Notwithstanding the clinical usefulness of [^{11}C]doxepin, the binding properties of [^{11}C]doxepin have not been evaluated sufficiently in animals. In the present study, we report on the specific binding of [^{11}C]doxepin to histamine H_1 receptors in the brains of four animal species.

The specific binding of [^{11}C]doxepine in the mouse brain was evaluated by tissue dissection in term of the dose-dependency and the blocking effects of several agonists: H_1-selective chloropheniramine and pyrilamine, H_2-selective zolantidine and H_3-selective thioperamide. The blocking effects of chloropheniramine were also evaluated in rats by tissue dissection, and rabbit and monkey by PET. Metabolites analysis was performed by HPLC.

The brain uptake of [^{11}C]doxepin in mice was slightly decreased by carrier-loading and by pre-treatment with H_1-selective antagonists (Fig. 2), but not by pre-treatment with H_2- and H_3-selective antagonists. Most radioactivity was detected as an unchanged form of [^{11}C]doxepin. The uptake was also slightly reduced by chloropheniramine in rats and a rabbit. However, the reduced fractions were only <20% in three species. In the monkey brain, the pre-treatment with chloropheniramine changed the time-activity curves (Fig. 3), which was not typical patterns in the radioligand-receptor binding under the blocking experiments; however, the distribution volume decreased in the cortical regions being rich in the receptors: baseline vs blocking, 60 vs 39 in frontal cortex; 56 vs 41 in cinglate cortex.

In conclusion, the specific binding of [^{11}C]doxepin to histamine H_1 receptor was low in animals.

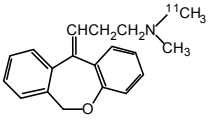


Fig. 1. [^{11}C]doxepin

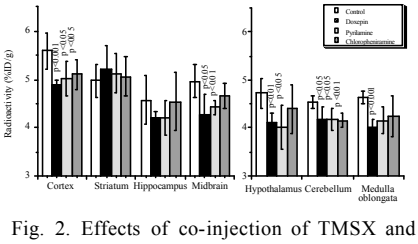


Fig. 2. Effects of co-injection of TMSX and histamine H_1 antagonists pyrilamine and chloropheniramine.

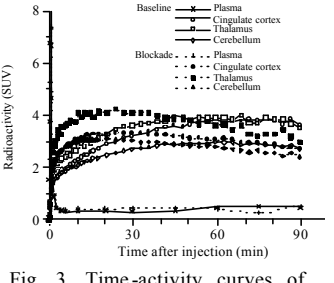


Fig. 3. Time-activity curves of [^{11}C]doxepin in the monkey brain.

References

1. Yanai K, Watanabe T, Meguro K, *et al., Neuroreport* 1992; **3:** 433–436.
2. Higuchi M, Yanai K, Okamura N, *et al., Neuroscience* 2000; **99:** 721–729.
3. Yanai K, Ryu JH, Watanabe T, et al., *Br J Pharmacol* 1995; **116:** 1649-1655.

COMPARISON OF TRITIATED AND RADIOIODINATED ZM241385 BINDING PROPERTIES AT CENTRAL ADENOSINE-A_{2A} RECEPTORS

H.H. Coenen, W. Sihver, D. Bier, A. Schulze, W. Wutz, M.H. Holschbach

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

Keywords: Adenosine-A_{2A} receptor ligand, ZM241385, SPECT

It is widely accepted that the adenosine-A_{2A} receptor (A_{2A}AR) is involved in neuroregulative processes of motor functions. Antagonistic interactions between A_{2A}ARs and dopamine D_2 receptors in the striatum may contribute to the antiparkinsonian potential of A_{2A}AR antagonists. In search for A_{2A}AR SPECT radioligands for the noninvasive visualization of these receptors for diagnosis and monitoring drug therapy, the potent non-xanthine antagonist 4-[2-(7-amino-2-furan-2-yl-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-ylamino)-ethyl]-phenol (ZM241385, **1**) (1) and its monoiodo-congener (iodo-ZM241385, **2**) were radioiodinated and the radioligands compared in rodent studies with tritiated ZM241385 in order to obtain information on *in vitro* binding and *in vivo* pharmacokinetics.

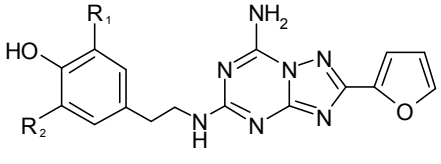


Figure: Structures of ZM241385, **1** / **1*** (R_1, R_2=H / R_1=H, R_2=^{3}H), iodo-ZM241385, **2** / **2*** (R_1=H, R_2=I, ^{131}I) and diiodo-ZM241385, **3** / **3*** (R_1, R_2=I / R_1=I, R_2=^{131}I), ***** radioactive analogue

Radioiodination of **1** with ^{131}I was performed by a modification of an earlier described labelling procedure (2). A similar method was used for the radiosynthesis of **3***, exept that **2** was used as the labelling precursor. Purification of the final products was performed with RP-HPLC, the radiochemical yields for both radioiodinations were > 80%, the radiochemical purity of both products exceeded 95%, and the specific activities were about 40 GBq / µmol (for n.c.a. radiolabelling) and 4 GBq / µmol (for c.a. labelling).

Performing *in vitro* autoradiography on rat brain slices revealed that the binding site with the highest density of A_{2A}ARs was localized in striatum having affinities of 0.4 nM, 2.2 nM and 21 nM for **1***, **2***, and **3***, respectively. While the parent compound **1*** showed only poor specific binding (~ 9 %) to cortical structures, the two radioiododerivatives did not bind at all in these regions. Thus, with regard to A_1/A_{2A}AR selectivity it can be concluded that the three ligands show similar high selectivities for the A_{2A}AR. Displacement studies with **1** as a competitor revealed high unspecific binding for both iodinated compounds, displacable radioactivity being only about 40 % and 25 % for **2*** and **3***, respectively.

Ex vivo studies in mice using n.c.a. **2*** showed a whole-brain uptake of ~ 0.2 % ID / g, 60 min p.i., the uptake in striatum being 4 times higher than in the rest of the brain. Only a small decrease of tracer uptake was observed by the application of the radiotracer with low specific activity.

In conclusion, radioiodo-ZM241385 is not a promising A_{2A}AR SPECT-ligand.

1.Poucher S M, Keddie J R, Singh P, Stoggall S M, Caulkett P W, Jones G, Coll M G. *Br J Pharmacol* 1995; 115: 1096-1102.
2.Palmer T M, Poucher S M, Jacobson K A, Stiles G L. *Mol Pharmacol* 1995; 48: 970-974.

PET STUDIES OF EPHEDRINE AND PSEUDOEPHEDRINE

Y.-S. Ding, M. Mueller, M. Schwaegler, D. Alexoff, F. Roesch, J.S. Fowler, N.D. Volkow

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

Keywords: ephedrine, ephedra, pseudoephedrine, PET

Ephedrine (EPH, common name “Ephedra”) and pseudoephedrine (PEPH) are widely available in asthma, ophthalmic, cold and allergy and weight-loss (so-called "fat-burning") products, and they are found in more than 100 pharmaceutical formulations. However, animal studies and human clinical evaluation of ephedrine neurotoxicity indicated that multiple doses of the dietary supplement ephedrine can cause severe hyperthermia and modest dopamine depletions in the brain. Moreover, because these drugs have reinforcing effects and are self-administered by laboratory animals there is concern that they may be diverted for abuse. Currently, a bill to ban the sale of all products that contain ephedrine is proposed, under the assumption that ephedrine, the chief active ingredient in many popular weight-loss, body-building and energy boosting products, has led to hundreds or thousands of deaths nationwide. The U.S. armed forces have now banned “ephedra” products from commissaries and military exchanges worldwide because it has been linked to heart attacks, strokes and seizures. Yet we know very little about the effects of these drugs in the human brain and the consequences of chronic treatment. It is therefore crucial to better understand how these two drugs behave in living systems.

Methods: We have currently labeled EPH and PEPH with carbon-11 by reacting the nor-precursor (compound without the N-methyl group) with [^{11}C]CH_3I, and initiated PET and MicroPET studies to determine their biodistribution and pharmacokinetics in the baboon and rodent brain. Binding specificity in vivo was accessed by blocking studies with the parent compound and specific blockers. Plasma assays for the presence of unchanged labeled tracers were carried out using both HPLC and solid phase extraction methods.

Results: The distribution of [^{11}C]EPH and [^{11}C]PEPH in the baboon brain was heterogeneous with the highest uptake occurring in the basal ganglia (BG, average 0.025% of the injected dose/cc) and lowest in frontal cortex (FC) and cerebellum (CB). The BG/CB ratio was about 1.4-1.6 for both tracers. The time required to reach the peak brain uptake was approx. 30 min. The results of the assays for unchanged tracer in baboon plasma after IV injection of [^{11}C]EPH and [^{11}C]PEPH were similar, with 75-85% at 30 min and 60-70% at 60 min remaining unchanged. Pretreating baboons with unlabeled EPH or PEPH prior to tracer injection significantly altered the blood flow, resulting in increased uptake of the radiotracers as compared to the baseline.

Summary: The high uptake of EPH and PEPH in striatum (where the nucleus accumbens, which is the brain region associated with the reinforcing effects of abuse, is located) is compatible with their reinforcing effects in laboratory animals. However, their relative slow brain uptake as compared to that of cocaine or of methylphenidate (whose brain uptake peaked at < 5 min and at 8-10 min respectively) suggests that they will be less reinforcing than these stimulant drugs. We are currently also investigating their binding specificity in brain and peripheral organs. It has been speculated that ephedrine may cause deaths, heart attacks and strokes, which is consistent with our observation that these two drugs significantly alter blood flow in living systems. This places a sense of urgency to better understand their physiological role in humans. We believe these new probes, [^{11}C]EPH and [^{11}C]PEPH, will provide information vital to better understand their pharmacological properties as well as their potential side effects including those of abuse. These studies set the stage for future investigation of the drug effects in human. Supported by DOE-OBER, NINDS and NIDA.

RAT-PET MEASUREMENT OF D1-DOPAMINE RECEPTORS WITHOUT ANESTHESIA

K. Hatano[1], S. Momosaki [1], Y. Kawasumi[1], T. Kato[1], R. Hosoi[2], O. Inoue[2] and K. Ito[1]

[1]Department of Biofunctional Research, National Institute for Longevity Sciences, Obu, Aichi, Japan 474-8522 and [2]Department of Medical Physics, School of Allied Health Sciences, Faculty of Medicine, Osaka University, Suita, Osaka, Japan 565-0871. E-mail contact: hatanok@nils.go.jp (K.H.).

Keywords: PET, Dopamine Receptor, SCH23390, Rat, Rodents

We previously reported age-related decline D1 as well as D2-dopamine receptor binding in rat brain indicating the technique to be reliable and sensitive enough to examine physiological alternation in living animals (1). To date, however, rodent PET study including ours are usually performed under anesthesia which could include pharmacological effect to modify receptor binding directly or indirectly. The aim of this study is to establish the procedure to acquire PET images of conscious rats and, therefore, to examine the effect of various anesthetic drugs on D1-dopamine receptor quantification.

Using originally developed fixation apparatus male F344/N rats (6 months old) were trained to remain in the scanning atmosphere for 3 hours. This training process was repeated twice a day for 2 weeks. About 10 MBq of [^{11}C]SCH23390, selective D1-dopamine receptor ligand, was intravenously injected to the trained rat and data was dynamically acquired for 64 min by SHR-2000 animal PET camera (Hamamatsu Photonics, Japan). The animal was continuously monitored by a video camera. Striatal and cerebellar ROI were placed on the PET images thus obtained and the time-activity curve was analyzed by Logan plot to obtain binding potential (BP). Animals under chloral hydrate, ketamine and petobarbital anesthesia were examined following the same procedure for comparison.

The PET measurements of conscious rats were successful as the trained rats scarcely moved during the scanning and the obtained BP showed small standard deviation (12% of mean value). Even after the careful training process, possibility of alternation of neuroreceptor measures by the fixation stress still could not be excluded. But the stable BP value in the conscious rats at least suggests their acclimation to the atmosphere. Three anesthetic drugs were proved to modify D1-receptor binding. BP under chloral hydrate or ketamine was significantly higher than that of conscious rats, whereas pentobarbital treated rats showed decreased estimate. The present procedure could be valuable for studies with pharmacological challenge or physiological interventions that could be obscured by anesthetizing drug treatments.

References

1. Suzuki M, Hatano K, Sakiyama Y, Kawasumi Y, Kato T, Ito K. *Synapse* 2001;41:285-293.

SIMPLE CBF ESTIMATION FOR RAT EXPERIMENTAL MODEL WITH RADIOIODINATED IMP

Y. Magata[1], T. Temma[2], T. Mukai[3], H. Kitano[3], M. Ogawa[1] and H. Saji[2]

[1]Laboratory of Genome Bio-Photonics, Photon Medical Research Center, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan
[2]Department of Patho-Functional Bioanalysis, Graduate School of Pharmaceutical Sciences, Kyoto University, Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan
[3]Department of Nuclear Medicine and Diagnostic Imaging, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8501, Japan.
e-mail contact to magata@hama-med.ac.jp

Keywords: IMP, CBF, Rat, H_2O, Brain

It has been possible to induce point mutation for an arbitrary gene as embryological engineering and molecular biology has greatly advanced to make many kinds of animal models in order to investigate mechanism of several diseases and bring new pharmaceuticals. It is important to obtain CBF value as well as other brain functional parameters such as CMRglu, receptor density. [^{14}C]iodoantipyrine is usually used for quantitative determination of CBF in small animal models[1]. However, very complicated technique is necessary to yield CBF value with [^{14}C]iodoantipyrine. Then, a simple index of CBF value is required. In this paper, DUR post-injection of [^{125}I]IMP was estimated to be an index of CBF value in normal rat brain in comparison with quantitative CBF value using [^{15}O]H_2O and [^{125}I]IMP.

PET studies were performed with a PET scanner for animals (SHR-7700L, Hamamatsu Photonics, Hamamatsu, Japan). The rats were anesthetized with i.p. pentobarbital injection. A catheter was inserted into the left femoral artery for blood sampling. Dynamic PET scan was initiated after i.v. injection of saline solution including [^{15}O]H_2O and [^{125}I]IMP. Arterial blood sampling was performed for 5 min post-injection of radiopharmaceuticals. Immediately after blood sampling period, the rat brain was removed to measure the concentration of [^{125}I]IMP radioactivity.

The CBF value derived with [^{15}O]H_2O was calculated by ARG method [2]. The ARG method [3] was used to calculate CBF value with [^{125}I]IMP. The arterial concentration of [^{125}I]IMP radioactivity was corrected by octanol extraction rate for non-lipophilic metabolite of [^{125}I]IMP [4]. DUR was obtained by multiplying % injected dose per gram brain by body weight.

A good correlation was observed between CBF value by the ARG method with [^{15}O]H_2O and that by the ARG method with [^{125}I]IMP ($r=0.81$). Moreover, a good correlation between DUR post-injection of [^{125}I]IMP and CBF value with [^{125}I]IMP was shown ($r= 0.75$).

The ARG method with [^{15}O]H_2O for the CBF values was employed as a golden standard method. It was confirmed that the CBF value with [^{125}I]IMP was well correlated to the true CBF value obtained by [^{15}O]H_2O. Also, it was indicated that DUR of [^{125}I]IMP was useful as a simple indicator of the CBF value at 5 min after injection without the arterial blood sampling and the metabolite correction.

References

[1] O. Sakurada, et al. Measurement of local cerebral blood flow with iodo [^{14}C] antipyrine, Am J Physiol 234 (1978) H59-66.
[2] M. E. Raichle, et al. Brain blood flow measured with intravenous $H_2{}^{15}O$. II. Implementation and validation, J Nucl Med 24 (1983) 790-798.
[3] H. Iida, et al. Quantitative mapping of regional cerebral blood flow using iodine-123-IMP and SPECT, J Nucl Med 35 (1994) 2019-2030.
[4] T. Nagata, et al. [^{125}I]iomazenil binding in the brains of spontaneously epileptic rats: an ex vivo quantitative autoradiographic study, Nucl Med Biol 22 (1995) 445-449.

BIOLOGICAL PROPERTIES OF [^{18}F]FFMZ (2'-FLUOROFLUMAZENIL)

Y.S. Chang,[1,2] J.M. Jeong,[1] Y.H. Yoon,[1] H.W. Kim,[1] Y.J. Kim,[1] D.Y. Chi,[3] D.S. Lee,[1] J.K. Chung,[1] M.C. Lee.[1]

[1]Department of Nuclear Medicine, college of Medicine, Seoul National University, 28 Yungun-dong, Chongro-ku, Seoul 110-744, Korea (jmjng@snu.ac.kr); [2]Pharmacy, Ewha Women's University, Seoul, Korea.; [3]Chemistry, Inha University, Incheon, Korea

Keywords: [^{18}F]FFMZ, stability, autoradiography, binding assay

Flumazenil is a well-known antagonist of the benzodiazepine receptor. [^{11}C]Flumazenil is commonly used for mapping the central benzodiazepine receptor using positron emission tomography (PET). However it has several disadvantages due to the short half-life of ^{11}C. To overcome the problem, [^{18}F]fluoroethylflumazenil using two step synthesis procedure has been developed.[1] Further improvement in the synthesis procedure was achieved by synthesizing a new derivative [^{18}F]FFMZ (2'-fluoroflumazenil) which employs a new tosylated precursor for one-step labelling reaction [2]. In this study, we investigated the biological properties of [^{18}F]FFMZ in rat brain.

[^{18}F]FFMZ was synthesized by an automatic labeling system employing a one-step reaction [2]. Stability test: [^{18}F]FFMZ (25 μL) was incubated with human and mice sera (325 μL) at 37°C for 1 hr. Absolute ethanol (775 μL) was added to the mixtures to precipitate serum proteins and centrifuged (3,000 rpm) for 5 min. The supernatants were analyzed using analytical HPLC. Phosphoimaging: [^{18}F]FFMZ was intravenously injected to male Sprague-Dawley rats through the tail vein with or without cold flumazenil. The rats were sacrificed by cervical dislocation 5, 10 and 20 min after administration. The brains were quickly removed and then frozen in a cryostat microtome. Coronal sections (20 μm) were obtained on the slide glasses and were exposed to the imaging plates for about 12 hours. The exposed imaging plate was analyzed using a Bio-imaging analyser. Binding assay: The cerebral cortex and cerebellum were obtained from rat brains and then membrane fractions were prepared according to the reported method [3]. Protein concentration of membranes was determined by Lowry's method using bovine serum albumin as a standard. The membrane fractions were incubated with [^{18}F]FFMZ. Binding reactions were terminated by filtration through Whatman GF/B glass fiber filters under reduced pressure. Filters were washed and counted with a gamma well-counter. Non-Specific binding was determined in the presence of cold-flumazenil.

[^{18}F]FFMZ was successfully synthesized with radiochemical yields of 68±20% and specific activity of 5.92 ×10^9 MBq/mol. Although [^{18}F]FFMZ was stable in the human serum for 1 hr, it was rapidly hydrolyzed in the mouse serum. The intact [^{18}F]FFMZ was 61%, 27% and 8% at 10, 30 and 60 min, respectively. In the phosphoimaging results, high uptakes in the cortex, thalamus and cerebellum, that could be blocked by coinjection of cold flumazenil, were observed. These results are consistent with the distribution of benzodiazepine receptor reported in the literature. Specific binding of [^{18}F]FFMZ was proportional with protein concentrations in the range of 0.1~1.6 mg/1 mL protein. Specific binding was saturable and the derived Kd and Bmax values were 0.7 nM and 1.1 pmol/mg-protein, respectively.

A new flumazenil derivative [^{18}F]FFMZ is a promising PET agent for central benzodiazepine receptor imaging with a convenient labeling procedure and a specific binding property.

References

1. Leveque P, Labar D, Gallez B. *Nucl Med Biol* 2001;28:809-814
2. Yoon YH, Jeong JM, Kim HW, et al. *Nucl Med Biol* 2003; 30: in press.
3. Giannaccini G, Betti L, Correani M, et al. *Neurochem Int* 1997;31:781-787

PREPARATION AND ANIMAL STUDIES OF ^{18}F-MPPF AS SEROTONIN IMAGING AGENT

C.Y. Wu[1,2], X.T. Lin[2], C.X. Lu[1], F.P. Xue[2], Z.W. Zhang[2], P. Liu[2], J. Zhao[2], Y.H. Quan[2], Z.S. Xu[2], H.Q. Zhu[2], X.D. Liu[2]

1. Jiangsu Institute of Nuclear Medicine, National Laboratory of Nuclear Medicine, Wuxi, Jiangsu, 214063, P.R.China Email: wcyzjl@163.com
2. Department of nuclear medicine, Huashan hospital, Fudan university, Shanghai, 200040, P.R.Cina

Keywords: serotonin receptor, ^{18}F-MPPF, pharmacology

Objective Develop an 18-Fluorine labeled serotonin receptor imaging agent for evaluating changes of serotonin receptor in the brain.

Methods MPPF and its MPP-NO_2 precursor were synthesized by us. ^{18}F-MPPF was successfully prepared by oil heating and microwave heating. Partition coefficient and stability were determined. Animal studies have been performed in rats.

Results The structure of MPPF and MPPNO_2 were confirmed by IR, NMR and MS. Radiochemical purity of ^{18}F-MPPF was over 95% after being purified by preparative HPLC, ^{18}F-MPPF was stable for 2-3hr at room temperature. The partition ratio in octanol and buffer were 29.5 and 52.5 at pH 7.00 and 7.40 respectively. Clearance curve of ^{18}F-MPPF in blood showed that ^{18}F-MPPF can be distributed rapidly. $T_{1/2\,a}$ =4.52min, $T_{1/2\,ß}$ =52.62min. Biodistribution in rats displayed high initial uptake in rat brain (1.00 %ID/organ at 2 min postinjection). The uptakes of ^{18}F-MPPF in hippocampus were 0.862, 0.196, and 0.048 %ID/g at 2 min, 30min, and 60min, respectively. The ratios of hippocampus to cerebellum were 1.90, 3.20 and 2.60 at 2min, 30min and 60min, respectively. The ratios of hippocampus to cerebellum were decreased to 1.04 and 1.30 at 30min postinjection after being pretreated with WAY100635 and 8-OH-DPAT, respectively. Liver and kidney were the major excretion organs.

Conclusions These data suggest that ^{18}F-MPPF exhibited favorable stability and biological properties. It was worth further studying.

EXPERIMENTAL STUDIES ON THE VARIATION OF DAT, 5-HTT AND 5-HT IN THE BRAIN USING A MODEL OF DEPRESSION IN RATS

C.Y. Wu[1,2], X.T. Lin[2], C.X. Lu[1], Q.F. Jiang[1], S.F. Wang[1], T.X. Zhang[1], Z.P. Chen[1], R.G. Fu[1], X.M. Li[1], M.D. Zhang[1], J.Q. Zhu[1]

1. Jiangsu Institute of Nuclear Medicine, National Laboratory of Nuclear Medicine, Wuxi, Jiangsu, 214063, P.R.China Email: wcyzjl@163.com
2. Department of nuclear medicine, Huashan hospital, Fudan university, Shanghai, 200040, P.R.C

Keywords: Depression disorder, disease model, DAT, 5-HTT, 5-HT

Objective To investigate the variation of dopamine transporter (DAT), serotonin transporter (5-HTT) and serotonin receptor (5-HT) in the brain of depression model of rats.

Methods The depression model of rats was produced by separation and chronic unpredictable mild stress. The behavior of the rats was observed in an open-field test. Histochemistry was used to compare the changes of morphological and number of hippocampal neurons in the depressive rats and control rats. Biodistribution of ^{125}-FPCIT, ^{125}I-ADAM and ^{125}I-MPPI in rat brain were used to detect the levels of DAT, 5-HTT and 5-HT.

Results The open-field behaviors of depressive rats were significantly decreased compared with control. The number of hippocampal neurons was significantly decreased and the activity of acidic phosphorase(ACP) was significantly increased compared with control. Table1 showed the variations of DAT, 5-HTT and 5-HT in depressive rat brain and control. The uptake of ^{125}I-FPCIT and ^{125}I-MPPI in depressive rats had not significantly decreased compared with control. The uptake ratio of ^{125}I-ADAM in hippocampus and hypothalamus to cerebellum had significantly decreased compared with control

Conclusions These data suggest that chronic depression could result in the decrease level of neurotransmitter. The variation of 5-HTT in a depression model of rats brain may be more sensitive than those of DAT and 5-HT.

Uptake ratio of different regions to cerebellum

ratio	^{125}I-FPCIT		^{125}I-ADAM		^{125}I-MPPI	
	control	depression	Control	depression	control	depression
ME/CB	1.467	1.463	2.218	1.982	1.450	1.373
FL/CB	1.888	1.858	3.088	3.104	1.701	1.676
PL/CB	1.732	1.656	2.281	2.147	1.618	1.578
ST/CB	4.032	3.833	2.597	2.414	1.141	1.053
HP/CB	1.605	1.515	2.723	2.168	2.707	2.754
HY/CB	1.926	1.972	3.461	2.910	1.196	1.177

ME: medulla, FL: frontal lobe, PL: parietal lobe, ST: striatum, HP: hippocampus, HY: hypothalamus

NICOTINIC ACETYLCHOLINE RECEPTOR DISTRIBUTION IN-VITRO AND IN-VIVO IN NORMAL AND DISEASE STATE HUMAN BRAIN USING 5-[125 OR 123I]-A-85380

J. Owens[1], S. Pimlott[1], S. Colloby[2], J.Fenwick[2], M. Piggott[3], L.E. Greally[3], J.A Court[3], E.K. Perry[3], J. O'Brien[2], D. Wyper[4],

1. West of Scotland Radionuclide Dispensary, North Glasgow University Hospitals NHS Trust, Western Infirmary, Dumbarton Rd, Glasgow, G11 6NT, UK. Email: j.owens@clinmed.gla.ac.uk.
2. Institute for Ageing and Health, Wolfson Research Centre, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne NE4 6BE, UK
3. Newcastle General Hospital, MRC/University of Newcastle Centre Development in Clinical Brain Ageing, MRC Building, Westgate Road, Newcastle upon-Tyne NE4 6BE, UK
4. Department of Clinical Physics, South Glasgow University Hospitals NHS Trust, Southern General Hospital, Govan Road, Glasgow G51 4TF, UK

Keywords: 5-I-A85380, nicotinic receptor, Dementia, SPECT

5-I-A85380 (5-IA) is a pyridyl ether based nicotinic acetylcholine receptor (nAChR) marker, binding predominantly to [alpha]4[beta]2 subtype. Radiolabelled 5IA was prepared from the corresponding trimethylstannyl compound by electrophilic iododestannylation. The radioiodination was achieved in good isolated yield (66.8 % ± 4.75, n = 3) and after careful formulation with reasonable stability.

An *in vitro* autoradiography study describes the distribution of 5-[125I]IA binding in post mortem brain tissue from normal elderly subjects and cases with age-associated dementias of both neurodegenerative and vascular type. 5-[125I]IA binding in normal brain tissue was found to be consistent with the reported distribution of other high affinity nicotinic ligands such as [3H]epibatidine. In addition to high thalamic and moderate striatal and temporal cortex density, moderate 5-[125I]IA binding was also seen in white matter tracts, indicating the presence of nAChRs along nerve fibre tracts. In Parkinson's disease (PD) loss of striatal 5-[125I]IA binding closely parallels the loss of nigrostriatal dopaminergic markers previously observed. In dementia with Lewy bodies (DLB) reduced striatal 5-[125I]IA binding density, comparable to that in PD, maybe a marker of early degeneration in nigrostriatal inputs, while in Alzheimer's disease (AD) reduced striatal 5-[125I]IA binding could be related to reduced cortical inputs. The reductions of nAChRs seen in AD, DLB and PD were not apparent in vascular dementia (VaD). These results indicate 5-IA may be useful in differentiating primary degenerative dementia from VaD.

In vivo imaging with 5-[123I]IA has been carried out in AD and DLB subjects as well as an age matched control group. Images obtained 4 hours after administration of 185 MBq of 5-[123I]IA provide a map of [alpha]4[beta]2 nAChR density. These initial studies indicate a reduction in nAChR density in the AD subjects compared to controls.

In conclusion 5-IA is clearly a useful ligand for both *in vitro* and *in vivo* single photon emission tomography human studies investigating disease symptoms and progression.

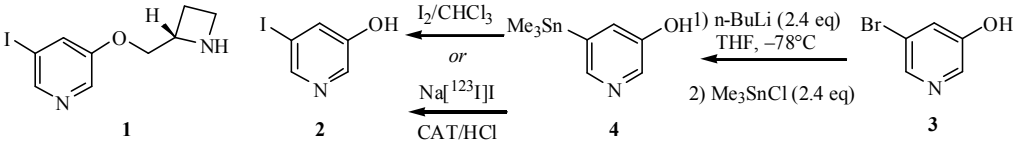
5-IODO-3-PYRIDINOL (5-IP), PUTATIVE METABOLITE OF (*S*)-5-[^{123}I]IODO-3-(2-AZETIDINYL)METHOXYPYRIDINE (5-IA)

R.M. Baldwin[1*], E. Brenner,[1] G. Tamagnan,[1] M.S. Al-Tikriti,[1] L. Amici,[1] S.S. Zoghbi,[1,2]

[1] Yale University School of Medicine, VA HCS Psychiatry (116A2), West Haven CT 06516 USA, and [2] NIMH/NIH, Bldg 1, B3-10, Bethesda, MD 20892 USA. Email ronald.baldwin@yale.edu

Keywords: Iodine-123; metabolism; nicotinic acetylcholine receptor; pharmacokinetics

(*S*)-5-[^{123}I]Iodo-3-(2-azetidinylmethoxy)pyridine ([^{123}I]5-IA, **1**) is a radiotracer being investigated for imaging the nicotinic acetylcholinergic receptor in vivo by SPECT imaging, with applications in psychiatric and neurodegenerative disorders (*1*). Plasma analysis has revealed a less lipophilic metabolite fraction, which is not ionic iodide (*2*). The purpose of the experiments reported here was to test the hypothesis that at least one metabolite of [^{123}I]5-IA is 5-iodo-3-pyridinol (**2**), designated 5-IP.



Authentic 5-IP was synthesized (without optimization) in two steps from 5-bromo-3-pyridinol (**3**). Treatment with 2.4 molar equivalents of n-butyl lithium followed by trimethylstannyl chloride gave 33% of the stannyl derivative **4**, accompanied by 5-n-butyl-3-pyridinol (11%), 3-pyridinol (24%), and 5,5'-bis-3-pyridinol (29%). Iodination with elemental iodine in chloroform at room temperature and flash chromatography gave 88% yield of 5-IP **2**, mp 202-204 C. Reaction of **4** with Na[^{123}I]I in the presence of Chloramine-T and HCl (pH 3) gave [^{123}I]**2** in 12% labeling yield.

Retention time t_R (min) of [^{123}I]5-IA and metabolites compared to 5-IP

	HPLC System A	HPLC System B
Plasma Parent 5-IA (**1**)	11.7	3.5–6.5
Plasma Metabolite(s)	3.6–6.2	8.7
5-IP (**2** or [^{123}I]**2**)	3.2	8.6

Plasma metabolites isolated from human subjects undergoing quantitative SPECT scans were compared with the authentic 5-IP and 5-IA in two HPLC systems, both using a reverse phase C-18 column (Waters Novapak C-18, 3.9 x 150 mm) at 1.0 mL/min, with in-line UV (280 nm) and radioactive detectors (Table). In system A (MeOH/H_2O/Et_3N; 65/35/0.2 v/v/v), a basic system used for primary plasma analysis (*2*), parent [^{123}I]5-IA was resolved at 11.7 min and radioactive metabolites eluted in a broad band at 3.6–6.2 min; 5-IP also eluted in this range (t_R 3.2 min). In system B (MeOH/80 mM NaOAc buffer pH 5; 50/50 v/v), an acidic system, the order of elution was reversed, with 5-IP at 8.5 min; pure 5-IA eluted at 5.6 min, but was not resolved from a broad band at 3.5–6.5 min. The resolved radiometabolite fraction behaved similarly to 5-IP, eluting at the same time as authentic standard. Although these results are consistent with 5-IP being one metabolite, it is clear that it is not the only one. Additional experiments will be necessary to resolve and identify individual metabolites more clearly.

Supported in part by NIDA & NCI P50 DA84733 and VA REAP (Depression).

REFERENCES

1. Fujita M, Seibyl JP, Vaupel DB, Tamagnan G, et al. *Eur. J. Nucl. Med.* (2002) **29**: 183-190.
2. Zoghbi SS, Tamagnan G, Fujita M, Baldwin RM, et al. *Nucl. Med. Biol.* (2001) **28**: 91-96.

EVALUATION OF (R)-[^{11}C]VERAPAMIL AS PET TRACER OF P-GLYCOPROTEIN FUNCTION IN THE BLOOD BRAIN BARRIER: KINETICS AND METABOLISM IN THE RAT

G. Luurtsema[1], R.C. Schuit[1], C.F.M. Molthoff[1], A.D. Windhorst[2], R.Boellaard[1], K. Takkenkamp[1], A.A. Lammertsma[1], E.J.F. Franssen[1]

[1] PET Center, VU University Medical Center, De Boelelaan 1117,
1007 MB Amsterdam, The Netherlands
[2] Radionuclide Center VU, De Boelelaan 1085c, 1081 HV Amsterdam, The Netherlands

Key words: (R)-[^{11}C]verapamil, P-gp, metabolism, animal PET, Blood brain barrier

Introduction: P-glycoprotein (P-gp) in the blood brain barrier (BBB) is involved in the bioavailability and pharmacodynamics of various drugs. (R)-[^{11}C]verapamil has been developed for imaging P-gp in the BBB using PET. For quantification of P-gp function *in vivo*, a mathematical model describing kinetics of uptake and clearance of verapamil is needed. The purpose of the present study was to study kinetics and both plasma and tissue metabolism in Wistar rats.

Methods: Wistar rats were anaesthetised and injected i.v. with 37-100 MBq of (R)-[^{11}C]verapamil. Dynamic PET scans (60 minutes) were performed using a high resolution research tomograph (HRRT) PET scanner. Several arterial blood samples were collected in order to derive an input-curve. In separate groups of animals, metabolite analyses in plasma, brain and liver tissue were performed at 5, 10, 30 and 60 minutes post injection. For the sample preparation, a Sep-Pak vac tC18 column was used. Polar radioactive metabolites were eluted after a washing step with water. The remaining parent compound and its apolar metabolites were eluted from the Sep-Pak column with 1.5 ml methanol. The elution fraction was analyzed with a radio HPLC with online and off-line radioactivity detection.

Results: PET data showed high uptake in lungs, fast clearance from blood, and low uptake in the brain. The radioactive metabolites formed in the liver were: [^{11}C]D-617, [^{11}C]D-702, [^{11}C]D-703 and an as yet unidentified [^{11}C]polar metabolite fraction. These metabolites were also present in plasma, but at lower concentrations. Except for the [^{11}C]polar fraction, no lipophilic radioactive metabolites were detected in brain tissue.

Conclusion: The present study provides initial biological data needed for the development of the tracer kinetic model to quantify P-gp function.

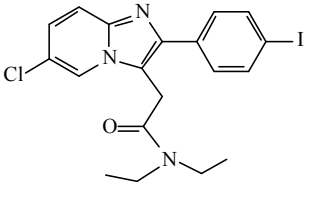
IN VITRO AND *EX VIVO* PHARMACOLOGICAL EVALUATION OF THE PERIPHERAL BENZODIAZEPINE RECEPTOR RADIOLIGAND [^{125}I]-CLINDE IN AN ANIMAL MODEL OF EXCITOTOXIC NEURONAL DEATH

A. Katsifis[2], M. Bottlaender[1], F. Mattner[2], M. Ottaviani[1], C. Coulon[1], C. Loc'h[1], H. Valette[1]

[1]Service Hospitalier Frédéric Joliot, Département de Recherche Médicale, CEA/DSV, 4 Place du Général Leclerc, 91401 Orsay, France. [2]Radiopharmaceutiacals Division, ANSTO, New Illawarra Road Lucas Heights, NSW, 2234 Australia. E-mail: akx@ansto.gov.au

Key words : Imidazopyridines, PBR, Neurodegeneration, SPECT, PET

In the CNS, the peripheral benzodiazepine receptors (PBRs) have increased levels in neurodegenerative diseases such as Alzheimer and Huntington's diseases. The PBR density in both astrocytes and microglia is also significantly increased in inflammation and metabolic stress. A number of halogenated imidazopyridines have been prepared with high affinity and selectivity for the PBRs, a number of which can be radiolabelled with either radioiodine as well as carbon-11. The aim of this study was to evaluate N,N-diethyl-6-chloro-(4'-iodophenyl)imidazo(1,2-a)pyridine-3-acetamide labelled with iodine-125 (^{125}I-CLINDE) **1,** in a prototypic animal model of excitotoxic neuronal death.



1

Radioiodination of **1** was achieved by destannylation of the tributyltin precursor in the presence of peracetic acid. Purification by C-18 reverse phase HPLC gave the desired product in 70-85% radiochemical yields and in greater than 98% radiochemical purity.

In vitro binding of [^{125}I]-CLINDE to mitochondrial membrane homogenates from rat brain cortex, demonstrated high affinity for PBRs, Kd = 3.84 nM with a Bmax = 0.34 pmol/mg protein.

In vivo brain distribution of [^{125}I]-CLINDE was studied in a rat model of activated microglia induced by unilateral intra-striatal injection of quinolinic acid. Rats (n=4) were anaesthetised and held in a stereotaxic frame. The tips of Hamilton syringes were stereotaxically placed in right and left striata. The excitotoxin, quinolinic acid (150 nmol in 1 µL) was injected over 1 min in the right striatum while vehicle (1 µL) was injected in the left striatum.

One week later, rats received i.v. 2 MBq (100 µL) [^{125}I]-CLINDE. Animals were sacrificed by decapitation, 30 min post injection. Striata, anterior and posterior cortices and cerebellum were dissected. The radioactivity in these structures was counted in a counter.

In normal rats, the uptakes in the striatum and the cortex were 0.1-0.12 %ID/g tissue and 0.18 %ID/g tissue in the cerebellum. In quinolinic lesionned rats, in the left side (sham) of the brain, the uptake in all structures was homogeneously increased (0.18-0.20 %ID/g tissue). In the right side, the uptake was dramatically increased in the lesionned striatum (0.35 %ID/g tissue) whilst in all other structures, the uptake was similar to the opposite side (0.18-0.20 %ID/g tissue). Similar findings were observed by *ex vivo* autoradiography in rats in the same excitotoxic model.

These results indicate that CLINDE, labelled with either iodine-123 or carbon-11 is a promising radioligand for imaging activated microglia using SPECT or PET.

This work was supported in part by the "Ministère Français des Affaires Etrangères".