trans-7-OH-IPAT: A Potential Sigma Receptor Imaging Agent for SPECT.

CHUMPRADIT, S.; KUNG, M.P.; GARNER, S. and KUNG, H.F.* Departments of Radiology and Pharmacology*, University of Pennsylvania, Philadelphia, PA 19104.

The sigma receptor displays a binding profile overlapping and similar to, yet distinct from the opiate receptor[1]. Administration of selective sigma agonists to laboratory animals induces psychotomimetic behavior. Many effective antipsychotic drugs, such as haloperidol, have high affinity for sigma binding sites. Development of sigma imaging agents is a subject of intensive interest in the past few years. Several new iodinated ligands, such as [^{125}I] PIPAG, a DTG analog (K_d ~ 10 nM), displays only moderate affinity for the sigma site[2-4]. In order to develop new sigma ligands for SPECT imaging, a tetralin derivative containing an iodopropenyl group, *trans*-7-OH-IPAT, **4**, was synthesized. Reductive amina-tion of 7-methoxy-2-tetralone with 2-propynylamine and sodium cyanoboronhydride in the presence of 5M hydrochloric acid/methanol gave compound **1** in good yield (49%). After addition of tri-(n-butyl)tin hydride using 2,2'-azobis(2-methylpropionitrile), AIBN, as the catalyst the reaction produced the desired tin adduct, **2**. The tin derivative, **2**, was iodinated by an oxidative destannylation reaction to give compound **3**, in 75% yield. Demethylation of **3** with boron tribromide yielded the desired final product, *trans*-7-OH-IPAT, **4**, (79 % yield). The sequence of reactions described above is not suitable for producing the starting material for radioiodination. An alternative reaction scheme was carried out for the preparation of the tin derivative,**6**. Compound **1**, was demethylated first with boron tribromide to give com-pound **4**. The tin derivative, **5**, was prepared by a similar addition reaction with tri-(n-butyl)tin hydride (yield 27%). The addition reaction produced a mixture of geometric isomers, *cis* and *trans* at 1:9 ratio. Only the *trans* isomer was characterized. The radioiodination with either ^{125}I or ^{123}I was carried out using hydrogen peroxide as the oxidant. Labeling yield for (R,S) [*I]*trans*-7-OH-IPAT, **4**, was 60-70% and the radiochemical purity was >98%. Using a chiral-cel-OD column and HPLC (hexanes/ethanol ; 90/10 at 1 ml/min) the racemic (R,S) [*I] *trans*-7-OH-IPAT, **4**, was resolved into peak A and B (retention time 12.7 and 14.9 min, respectively) (Fig. 1). Either the R- or S-isomer corresponds to the isomer A or B, respectively.

In vitro binding study showed that only the isomer A of [^{125}I]*trans*-7-OH-IPAT, **4**, displayed specific binding (Kd = 1.75 nM and Bmax = 254 fmol/mg of protein; rat cerebellar homogenates), and the peak B indicated no specific binding with the same tissue homogenates. Therefore, all of the biological evaluations were performed on isomer A of [^{123}I] or [^{125}I]*trans*-7-OH-IPAT, **4**. In vivo biodistribution study in rats suggested that the agent penetrated intact blood brain barrier (Table 1) and the regional distribution in brain appeared to favor specific regions, such as cerebellum, hypothalamus and hindbrain. The specific uptake can be blocked by pretreatment of the rats with a dose of haldol (a sigma ligand; 1 mg/kg) at 5 min before the iv injection of [^{123}I]*trans*-7-OH-IPAT-A, **4** (Fig. 2). Additional studies are needed to further characterize this potential sigma imaging agent.

References:

1. T. P. Su, Eur. J. Biochem. **1991**, 200, 633-642.
2. A. Kimes, A. Wilson, U. Scheffel, B. Campbell and E. London, J. Med. Chem. **1992**, 35 4683-4689.
3. B. de Costa, L. Radesca, C. Dominguez, L. Di Paolo and W. D. Bowen, J. Med. Chem. **1992**, 35, 2221-2230.
4. X.-S. He, W. Bowen, K. Lee, W. Williams, D. Weinberger and B. de Costa, J. Med. Chem. **1993**, 36, 566-571.

Acknowledgements: This work is supported by a grant awarded by NIH (NS-24538).

Scheme 1. Synthesis of (R,S)*trans*-7-OH-IPAT, **4**

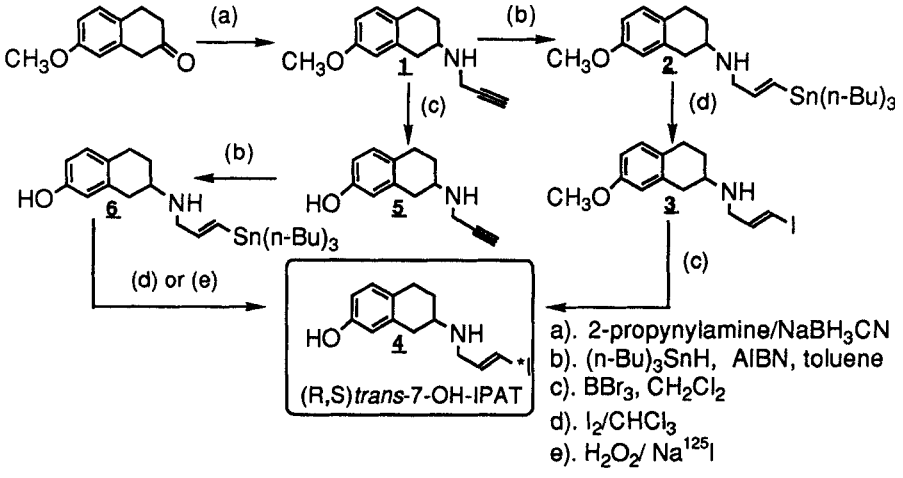


Fig 1. Separation of Stereoisomers by HPLC (Chiracel-OD Column)

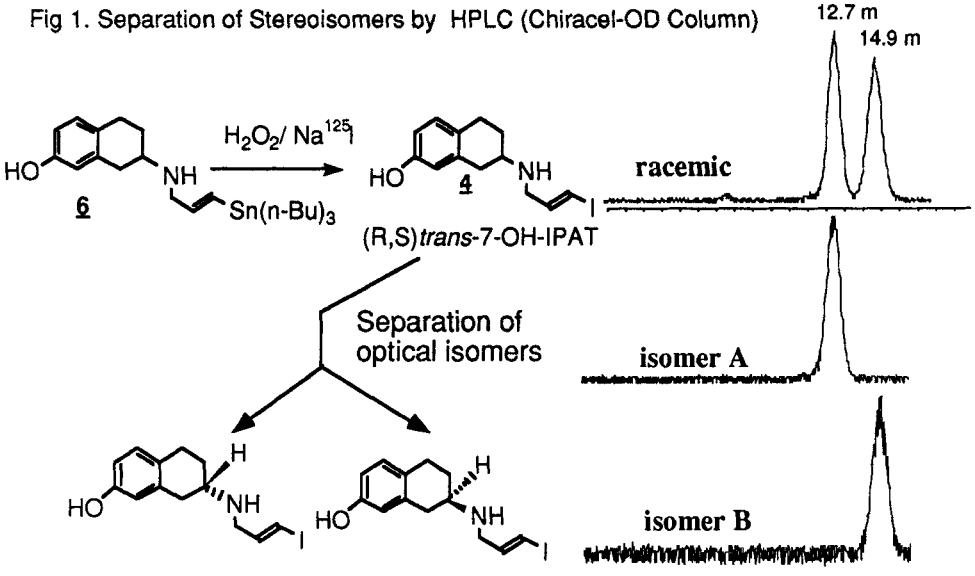


Table 1. In vivo biodistribution studies with [^{123}I]*trans*-7-OH-IPAT-A in rats (% dose/organ, average of 3 rats ± SD)

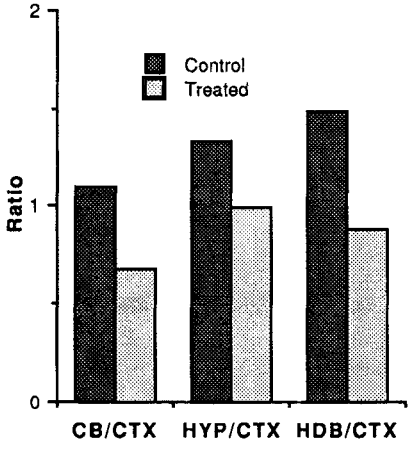
Organ	2 min	30 min	1 hour	2 hour
Blood	3.04 ± 0.40	2.11 ± 0.24	1.81 ± 0.53	1.19 ± 0.24
Heart	1.30 ± 0.07	0.23 ± 0.06	0.11 ± 0.02	0.06 ± 0.01
Muscle	58.51 ± 12.93	19.15 ± 2.81	11.32 ± 1.62	4.84 ± 0.95
Lung	18.32 ± 2.49	2.39 ± 0.67	0.88 ± 0.11	0.38 ± 0.02
Kidney	4.87 ± 1.73	3.05 ± 0.39	1.85 ± 0.50	0.87 ± 0.24
Spleen	0.21 ± 0.04	0.65 ± 0.16	0.23 ± 0.01	0.09 ± 0.01
Liver	4.14 ± 0.99	8.40 ± 1.03	10.65 ± 1.18	6.52 ± 1.14
Skin	6.21 ± 1.27	11.89 ± 1.76	7.71 ± 1.24	5.12 ± 0.69
Thyroid	0.17 ± 0.04	0.07 ± 0.01	0.06 ± 0.01	0.10 ± 0.04
Brain	2.35 ± 0.18	1.83 ± 0.28	0.65 ± 0.15	0.30 ± 0.04
Brain/Bld*	6.07 ± 0.86	6.75 ± 0.84	3.34 ± 1.63	1.90 ± 0.15

*% dose per gram of tissue;

Regional Biodistribution (% dose/gram):

Region	2 min	30 min	1 hour	2 hour
Cerebellum	1.272 ± 0.239	1.033 ± 0.255	0.378 ± 0.099	0.180 ± 0.032
Hypothalamus	1.462 ± 0.073	1.067 ± 0.073	0.486 ± 0.148	0.214 ± 0.038
Striatum	1.085 ± 0.237	0.983 ± 0.153	0.333 ± 0.065	0.219 ± 0.132
Hippocampus	1.259 ± 0.083	1.041 ± 0.105	0.424 ± 0.069	0.174 ± 0.027
Cortex	1.687 ± 0.456	1.067 ± 0.145	0.376 ± 0.092	0.158 ± 0.022
Pons/medulla	1.346 ± 0.225	1.234 ± 0.248	0.473 ± 0.147	0.239 ± 0.038

Fig 2. Comparison of regional brain distribution ratios of control and haldol treated rats (1 mg/kg; iv, 5 min prior to the injection of radioactive ligand) at 1 hr post iv injection of [^{123}I]*trans*-7-OH-IPAT-A. CB: cerebellum; CTX: cortex; HYP:hypothalamus; HDB: hindbrain.



^{11}C FORSKOLIN: A LIGAND FOR VISUALIZATION OF ADENYLATE CYCLASE-RELATED SECOND MESSENGER SYSTEM

Toru Sasaki [a], Atsuko Enta[b], Kohsuke Furukata[b] Tadashi Nozaki [b], Shin-ichi Ishii[a] and Michio Senda[a] [a]Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi, Tokyo and [b]Faculty of Hygienic Sciences, Kitasato University, Kitasato 1-15, Sagamihara, Kanagawa (Japan).

Signals of certain neurotransmitters and hormones are mediated by second messenger systems. One well-characterized second messenger system is the adenylate cyclase (AC)-related pathway. Forskolin, a diterpene isolated from *Coleus forskolii*, specifically binds to the a subunit of stimulatory guanidine nucleotide binding protein (Gsα) and AC, which it then activates. To visualize the AC-related second messenger system, we have previously reported the synthesis of [^{11}C]forskolin from 7-deacetylforskolin and [^{11}C]acetic acid using dicyclohexylcarbodiimide (DCC) (1,2). In this study, [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin were synthesized by acetylation of the respective deacetyl-precursors using [^{11}C]acetylchloride.

The deacetyl-precursors of [^{11}C]forskolin and its analogs, i.e., 7-deacetylforskolin, 7-deacetyl-1,9-dideoxyforskolin and 7-deacetyl-1-deoxyforskolin, were synthesized from forskolin, 1,9-dideoxyforskolin and 1-deoxyforskolin, respectively, by the method of Pfeuffer T. (3). Those deacetyl-precursors were acetylated with [^{11}C]acetylchloride in the presence of dimethylaminopyridine (See Fig 1). Five milligrams of deacetyl-precursors were dissolved in 0.5 mL of freshly distilled toluene, then 8 mg of dimethylaminopyridine was added. Thereafter

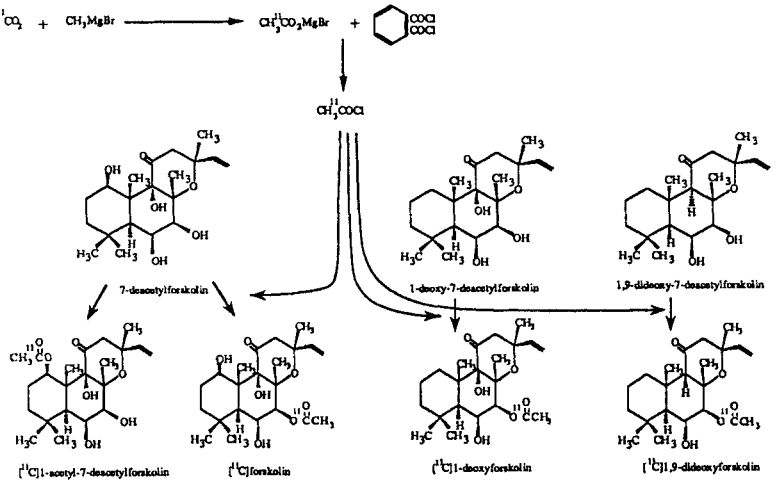


Fig. 1. Synthesis scheme of [^{11}C]forskolin and its analogs

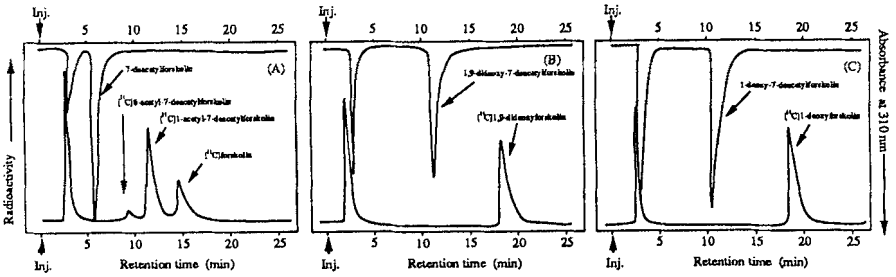


Fig. 2. Purification of [^{11}C]forskolin , [^{11}C]1-acetyl-7-deacetylforskolin (A), [^{11}C]1,9-dideoxyforskolin (B) and [^{11}C]1-deoxyforskolin (C) using a preparative HPLC system.

[^{11}C]acetylchloride, produced according to the procedures of Le Bars et al. (4), was introduced into the mixture at room temperature. The toluene was evaporated and the residue was dissolved in a small volume of acetonitrile:water=1:1 and purified on a HPLC system (reversed phase column; Megapak SIL-C_{18}, 10 mm I.D.x 250 mm; UV absorbance was monitored with a UV detector SPD-6AV) under elution with acetonitrile:water=1:1, at the flow rate of 5 mL/min for [^{11}C]forskolin and [^{11}C]1-acetyl-7-deacetylforskolin, and 8 mL/min for [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin (Fig. 2). The radiochemical yields of [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin, calculated from trapped [^{11}C]CO_2, were 4.1-5.7, 8.2-13.0, 14.5-15.2 and 17.5-18.0 %, respectively. The specific activities of purified [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin were about 37-55.5 GBq/mmol. The radiochemical purity of [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin were 98, 97, 99 and 99 %, respectively. The total amount of time required for the synthesis and purification of [^{11}C]forskolin and its analogs was about 35-45 min.

Of the four OH groups on 7-deacetylforskolin, 7-OH group was selectively acetylated by DCC method, as previously reported (1,2). However, because the DCC method comprises the process of obtaining [^{11}C]acetic acid free from water, it is not suitable for automation or remote operation. The present study showed the synthesis of [^{11}C]forskolin from 7-deacetylforskolin using [^{11}C]acetylchloride. However, this [^{11}C]acetylchloride method resulted in non-selective acetylation of the four OH groups on 7-deacetylforskolin; [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin and [^{11}C]6-acetyl-7-deacetylforskolin. Since the 1- and 9-OH groups on the forskolin structure are critical for specific binding to AC (active type), we considered [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin to be potential non-specific forskolin analogs. The results of the n-octanol/phosphate buffer partition ratio indicated that [^{11}C]1-acetyl-7-deacetylforskolin was most similar to [^{11}C]forskolin, but [^{11}C]1,9-dideoxyforskolin or [^{11}C]1-deoxyforskolin was different from [^{11}C]forskolin (Fig. 3). The [^{11}C]forskolin binding to the rat brain membranes was inhibited by loading of cold forskolin while the binding of [^{11}C]1-acetyl-7-deacetylforskolin was low and not influenced by forskolin loading (Fig. 4).

These studies indicated that [^{11}C]forskolin will be a useful imaging agent for the AC-related

second messenger system and [^{11}C]1-acetyl-7-deacetylforskolin might serve as a "non-specific forskolin analog" that possess similar structure to that of forskolin.

REFERENCES

1. Sasaki T, Ogihara-Umeda I, Nishigori H, Ikegami S, Senda M, Kitani K, Ogawa K, Nozaki T Rapid synthesis of ^{14}C forskolin by a route suitable for ^{11}C labeling. J Labelled Compounds Radiopharmaceuticals 1991; 29: 557-565.

2. Sasaki T, Yokota A, Ishiwata K, Senda M, Nozaki T, Nishigori H. Potentiality of C-11 forskolin for imaging of cAMP related second messenger system. J Nucl Med 1992; 33: 1025.

3. Pfeuffer T. Synthesis of forskolin-agarose affinity matrices. Methods Enzymology 1991; 195: 44-65.

4. Le Bars D, Luthra SK, Pike VW, Lu Duc C. The preparation of a carbon-11 labelled neurohormone-[^{11}C]melatonin. Appl Radiat Isot 1987; 38: 1073-1077.

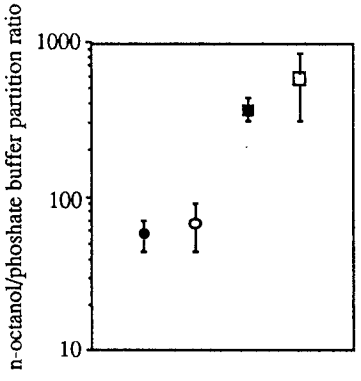


Fig. 3. Partition ratio of [^{11}C]forskolin (●), [^{11}C]1-acetyl-7-deacetylforskolin (○), [^{11}C]1,9-dideoxyforskolin (■)and [^{11}C]1-deoxyforskolin (□) in n-octanol/0.1 M phosphate buffer (pH 7.4).

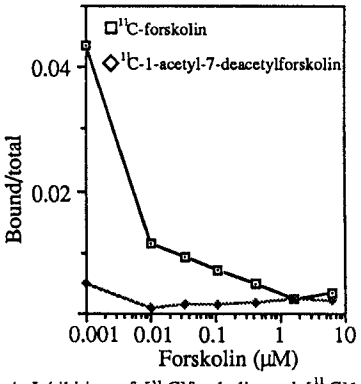


Fig. 4. Inhibition of [^{11}C]forskolin and [^{11}C]1-acetyl-7-deacetylforskolin binding to rat brain membranes by forskolin. Membranes (1 mg protein) were incubated with 37 kBq of [^{11}C]forskolin and [^{11}C]1-acetyl-7-deacetylforskolin in presence of various concentrations of forskolin.

PREPARATION OF [^{11}C] NNC 13-8199 AND [^{76}Br] NNC 13-8199, PARTIAL AGONISTS FOR THE STUDY OF BENZODIAZEPINE RECEPTORS USING PET.

Foged[1,2], C.; Halldin[1], C.; Loc'h[3], C.; Mazière[3], B.; Mazière[3], M.; Pauli[1], S.; Suhara[1], T.; Swahn[1], C-G.; Karlsson[1], P.; Hansen[2], H. C. and Farde[1], L. [1]Department of Psychiatry and Psychology, Karolinska Institute, Stockholm, Sweden, [2]Novo Nordisk A/S, Pharmaceuticals Division, Måløv, Denmark and [3]Service Hospitalier Frédéric Joliot, CEA, Orsay, France.

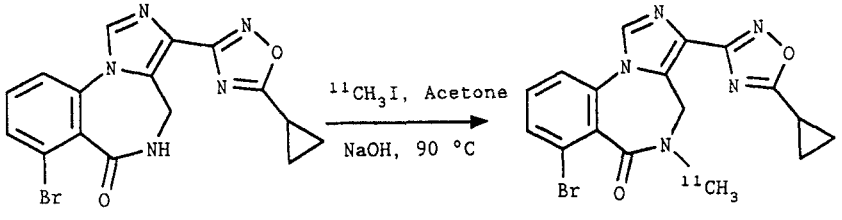
[^{11}C]Flumazenil has been the ligand of choice for several years to study benzodiazepine (BZ) receptor binding in monkey (1-2) and man (3-5) by PET. NNC 13-8199 (3-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-7-bromo-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a]benzodiazepine) is a new, partial agonist for the central BZ receptor. NNC 13-8199 inhibits the binding of [^{3}H]flunitrazepam in nanomolar concentration *in vitro* and is metabolically stable *in vivo*. The 5-cyclopropyl-1,2,4-oxadiazol-3-yl group in the 3 position is less sensitive to metabolism than the ethyl ester group in the widely used ligands flumazenil (6-7) and iomazenil (8). NNC 13-8199 contains both a N-methyl group and a bromine. This allows isotopic labelling with either ^{11}C or ^{76}Br. To examine brain kinetics, binding caracteristics and metabolism, an approach with alternative labelling may be useful.

[^{11}C]NNC 13-8199 was labelled by N-alkylation of the nitrogen of the amide group with [^{11}C]methyl iodide (Scheme 1). Reaction was performed in acetone at 90 °C for 4 minutes using sodium hydroxide as a base. Subsequent semi-preparative reversed-phase HPLC purification resulted in 25% radiochemical yield (from EOB and decay-corrected) with a total synthesis time of 30-35 minutes and a radiochemical purity of >99%. The specific radioactivity obtained at EOS was about 500 Ci/mmol.
[^{76}Br]NNC 13-8199 was prepared from the trimethyltin precursor and labelled with ^{76}Br by the chloramin-T method (Scheme 2).
Semi-preparative straight-phase HPLC purification resulted in 60% radiochemical yield and a radiochemcal purity of >98%.

PET examination of the Cynomolgus monkey brain after i.v. injection of [^{11}C]NNC 13-8199 showed high accumulation of radioactivity in regions known to have high density of BZ receptors, such as the occipital and frontal cortex. The ratio of radioactivity in the cortex to the pons was 3, 50 minutes after injection. HPLC analysis of plasma 60 minutes after injection, showed >98% unchanged compound, indicating a very slow metabolism in monkey. PET examination of the Baboon brain with [^{76}Br]NNC 13-8199 also demonstrated high accumulation of radioactivity in the occipital and frontal cortex. Radioactivity in all brain regions was rapidly reduced, almost to the level of the plasma, after i.v. injection of unlabelled flumazenil (1.4 mg/kg) 4 hours after injection of radioactivity, indicating that the brain's radioactivity represents binding to BZ receptors.

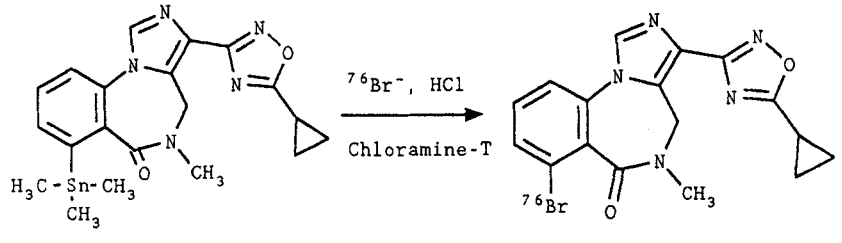
1. Mazière M., Hantraye P., Prenant C., Sastre J. and Comar D. — Appl. Radiat. Isot. 35: 973-976 (1984).
2. Hantraye P., Kaijima M., Prenant C., Giubert B., Sastre J., Crouzel M., Naquet R., Comar D. and Mazière M. — Neurosci. Lett. 48: 115-120 (1984).
3. Persson A., Ehrin E., Eriksson L., Farde L., Hedström CG., Litton J-E., Mindus P. and Sedvall G. — J. Psychiatr. Res. 19: 609-622 (1985).
4. Samson Y., Hantraye P., Baron J-C., Soussaline F., Comar D., Mazière M. — Eur. J. Pharmacol. 110: 247-251 (1985).
5. Persson A., Pauli S., Halldin C., Stone-Elander S., Farde L., Sjögren I. and Sedvall G. — Human Psychopharmacology 4, 21-31 (1989).
6. Halldin C., Stone-Elander S., Thorell J.O., Persson A. and Sedvall G. — Appl. Radioat. Isot. 39, 993-997 (1988).
7. Persson A., Pauli S., Swahn C.-G., Halldin C. and Sedvall G. — Human Psychopharmacology 4, 215-220 (1989).
8. Beer H.F., Blauenstein P.A., Hasler P.H., Delaloye B., Riccabona G., Bangerl I., Hunkeler W., Bonetti E.P., Pieri L.,Richard — J.G and Schubiger P.A.

Scheme 1



NNC 13-8256 [^{11}C]NNC 13-8199

Scheme 2



Trimethyltin precursor [^{76}Br]NNC 13-8199

Enzyme Activated Trapping as a Mechanism for Developing PET Tracers of Neurotransmission. DEJESUS, O.T., MURALI, D., OAKES, T.R., KITCHEN R., HOLDEN, J.E. and NICKLES, R.J. Department of Medical Physics, University of Wisconsin Medical School, Madison, WI 53706, U.S.A.

The success and widespread use of [^{18}F]2-Fluoro-2-deoxyglucose (2-FDG) as a PET tracer for measurement of regional glucose metabolism is primarily due to simplification of data interpretation resulting from hexokinase-activated trapping of 2-FDG. We have been investigating a similar approach in the development of tracers for assessing dopamine neurotransmission. This approach involves the participation of two enzymes in consecutive steps in dopamine synthesis (Figure 1).

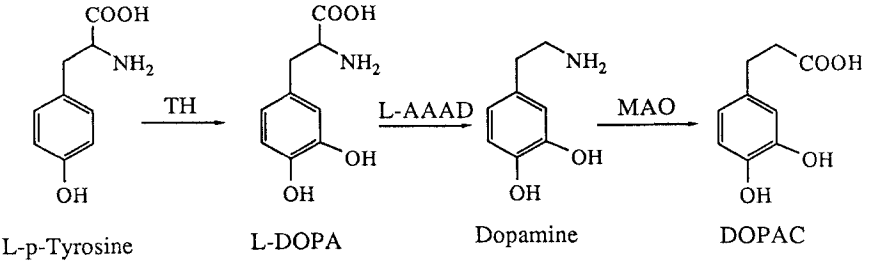


Figure 1. A simplified scheme of the metabolism of dopamine involving the enzymes TH=tyrosine hydroxylase, L-AAAD=L-aromatic amino acid decarboxylase, and MAO=monoamine oxidase.

This dual enzyme mechanism involves a tracer compound which is transformed by the first enzyme into an irreversible inhibitor of the second enzyme resulting not only in high specificity of localization but also long-term retention of radioactivity. The regional accumulation of radioactivity then is reflective of the substrate turnover rate of the first enzyme. We have targeted the enzyme pairs AAAD-MAO and TH-AAAD using the compounds β-fluoromethylene-m-tyrosine (FMMT) and α-fluoromethyl-p-tyrosine (FMPT) (Figure 2), respectively.

Our initial studies with [^{18}F]6-F-FMMT showed that, similar to 6-fluoro-L-DOPA (6-FD), 6-F-FMMT enters the dopamine synthetic pathway at the L-AAAD step and thus traces L-DOPA transport and decarboxylase activity (1). The decarboxylated product, 6-F-FMMTA, (Figure 2) is presumably not a substrate but a suicide inhibitor of MAO. The accumulation of trapped radioactivity may simplify assessment of regional cerebral L-AAAD activity by simplifying data interpretation. The challenge now is to demonstrate that these scans, which are indicative of the non-rate limiting and relatively non-neuronal specific L-AAAD activity, have improved diagnostic value over 6-FD scans.

Our second approach targets tyrosine hydroxylase (TH) using a TH-activated L-AAAD suicide inhibitor, ^{18}F-FMPT (Figure 2). TH activity measurement may be a more sensitive and selective measure of dopamine turnover since TH not only is the rate-limiting enzyme in catecholamine biosynthesis but also is highly neuronal- and substrate-specific.

The parent FMPT was originally prepared via a LDA-catalyzed reaction involving chloro-fluoromethane and a protected p-tyrosine derivative (2). We now prepare FMPT by the reaction of 4-methoxybenzyl chloride and fluoroacetonitrile via a Grignard intermediate followed by deprotection with refluxing HBr (3).

Direct radiofluorination of FMPT yields 3-[^{18}F]F-FMPT (4), a derivative which was found not to cross the blood brain barrier of the rhesus monkey (5). Since Coenen et al. (6) have shown that 2-fluoro-p-tyrosine (2-FT) is a good tracer of cerebral protein synthesis, synthetic

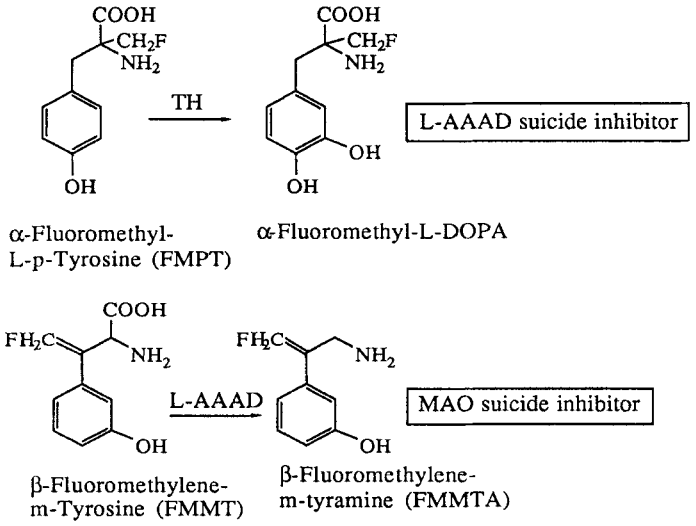


Figure 2. Mechanism of dual enzyme trapping of FMMT and FMPT in dopamine neurons.

methods to prepare 2-F-FMPT via electrophilic and nucleophilic approaches were undertaken. Electrophilic methods to prepare 2-F-FMPT require O-acetyl-FMPT in analogy to O-acetyltyrosine which was found by Coenen et al. (7) to give 2-FT:3-FT in a 84:16 ratio after reaction with acetylhypofluorite. Thus, radiofluorination of O-acetyl-FMPT with [^{18}F]acetylhypofluorite gives predominantly 2-[^{18}F]F-FMPT which is purified by HPLC.

In addition, a nucleophilic synthesis to prepare 2-[^{18}F]F-FMPT was developed based on the method of Lemaire et al. (8). Both (R) and (S) isomers of the synthon, 1-t-Boc-2-t-butyl-3-methyl-4-imidazolidone, were fluoromethylated at the 4-position using LDA-catalyzed carbanion reaction with chlorofluoromethane. After a second LDA-catalyzed carbanion reaction with 2-fluoro-4-methoxy-benzyl iodide, refluxing acid deprotection and HPLC purification, the product 2-F-FMPT is isolated.

Biological evaluation of 2-[^{18}F]F-FMPT as a PET tracer of dopamine neurotransmission is in progress.

References:

1. DeJesus O.T., Holden J.E., Endres C., Murali D., Oakes T.R., Shelton S., Uno H., Houser D., Freund L., Perlman S.B. and Nickles R.J. - Brain Res. 597:151(1992)
2. DeJesus O.T., Murali D., Kitchen R., Oakes T.R. and Nickles R.J. - J. Fluorine Chem., in press
3. Bey P. and Jung M. - U. S. Patent 4,342,780
4. DeJesus O.T., Murali D., Kitchen R., Endres C., Oakes T.R., Shelton S.E., Freund L., Houser D., Uno H., Holden J.E. and Nickles R.J. - submitted
5. Murali D., DeJesus O.T., Oakes T.R., Kitchen R., Holden J.E. and Nickles R.J. - J. Nucl. Med. 34: (1993) (abstract)
6. Coenen H.H., Kling P. and Stocklin G. - J. Nucl. Med. 30:1367 (1989)
7. Coenen H.H., Franken K., Kling P. and Stocklin G. - Appl. Radiat. Isot., Int. J. Appl. Instrum. Part A, 39:1243(1988)
8. Lemaire C., Plenevaux A., Damhut P., Guillaume M., Christiaens L. and Comar D. - Abstracts, 9th Int. Symp. Radiopharm. Chem., 1992.

Preparation and Evaluation of ^{76}Br-Metabromobenzylguanidine (^{76}Br-MBBG) as a Tool for PET Investigation of the Adrenergic Reuptake System.

LOC'H, C.; MARDON. K.; VALETTE, H.; BRUTESCO, C.; FUSEAU, C.; MERLET, P.; SYROTA, A.; and MAZIERE, B. Service Hospitalier Frédéric Joliot, CEA, F-91406 Orsay, France.

False adrenergic neurotransmitters and catecholamine derivatives are known to be stored, released and transported by the same mechanism as norepinephrine (NE) (1). For PET studies, such compounds (6-fluorodopamine, 6-fluoronorepinephrine, fluorometaraminol, hydroxyephedrine have been labelled with ^{18}F or ^{11}C. For SPECT studies, meta-iodobenzylguanidine (MIBG), a functional analogue of NE has been labelled with ^{123}I. For PET assessment of catecholamine reuptake, ^{76}Br-meta-bromobenzylguanidine (^{76}Br-MBBG) was prepared and used in biodistribution studies to evaluate its potential for the clinical investigation of heart disease and for the localisation of pheochromocytomas.

Cold MBBG was prepared using the same method described for MIBG (2). A mixture of Cyanamid and 3-bromobenzylamine hydrochloride was heated at 105°C for 10 h (fig 1). After cooling, the solid was dissolved in 5 mL water and 3-bromobenzylguanidine bicarbonate was precipitated by adding $NaHCO_3$. The MBBG hemisulfate, prepared by adding H_2SO_4 to a MBBG bicarbonate solution, was crystallised on cooling to room temperature.

^{76}Br-MBBG was prepared from the iodinated analogue (MIBG) and ^{76}Br-NH_4Br using a Cu^+ assisted halogen exchange reaction (3, 4), (fig 2). The exchange between nca ^{76}Br and the cold iodine of MIBG was performed at 165°C for 60 min in presence of $CuSO_4$ and an excess of reducing agents (gentisic acid, ascorbic acid and citric acid). Polar by-products and unreacted ^{76}Br-NH_4 were eliminated by solid phase extraction on a C18 cartridge. ^{76}Br-MBBG eluted by 3 mL of water-methanol (80-20, v/v), was purified by RP-HPLC with a mixture of 0.01 mol/L NaH_2PO_4, CH_3CN (85/15,v/v) as eluent. ^{76}Br-MBBG was produced in a 80% radiochemical yield with a specific radioactivity of 20 GBq/μmol.

In rats, biodistribution kinetic studies showed a high specific uptake of ^{76}Br-MBBG in heart tissue with its maximum of 5% ID/g at 2 h post injection; whereas 4 h p.i. the maximum of the heart to lung concentration ratio of 8 was observed. Pharmacological blocking studies have shown that the uptake of ^{76}Br-MBBG was reduced to the following values: 37% after desipramine (uptake-1 inhibitor) (DMI), 88% after dexamethasone (uptake-2 inhibitor) (DXM) and 16% after chemical destruction of neurones by 6-hydroxydopamine (6OHD) (figure 1). In nude mice, which were transplanted with pheochromocytomas (PC12), a very high uptake of ^{76}Br-MBBG(80±7 % ID/g) was observed 8 h after injection. From these results, it appears that ^{76}Br-MBBG has the potential of being developed as a useful radiotracer to study, by PET, the heart catecholamine system and as an interesting agent for the localisation of various adrenergic tumours.

1. Kopin I.J., Circulation, 82: 646 (1992)
2. Wieland D.M., Wu J.L., Brown L.E., et al, J. Nucl. Med. 21: 349 (1980)
3. Mertens J., Vanryckeghem W., Gysemans M., et al, Eur. J. Nucl. Med. 13: 380 (1987)
4. Loc'h C., Guillaume M., Stulzaft O., et al, J. Lab. Comp. Radipharm. 32: 291 (1993)

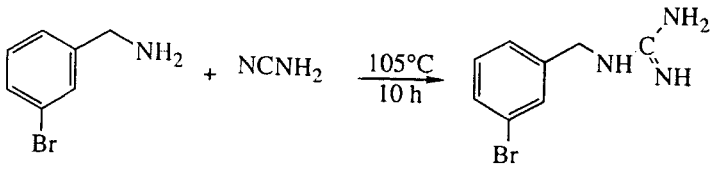


Fig 1

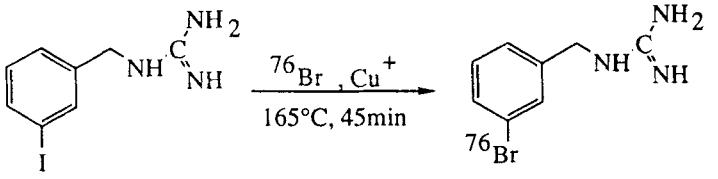


Fig 2

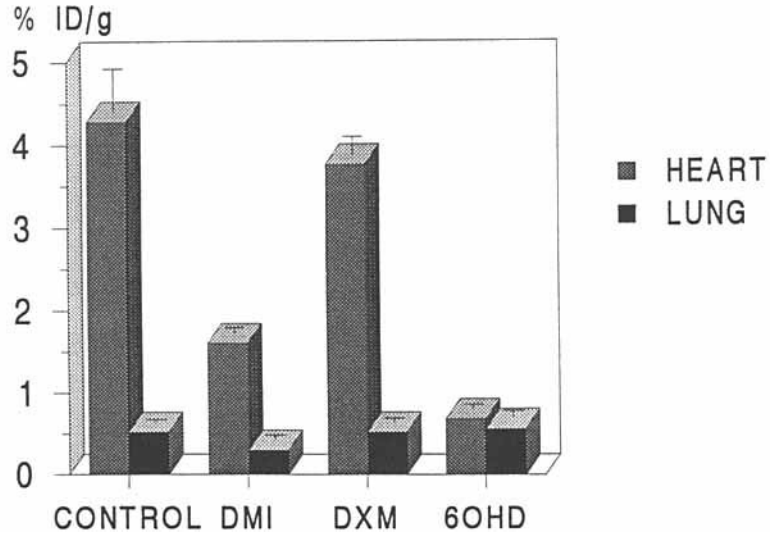


Fig 3

MOLECULAR DETERMINANTS OF SELECTIVITY AT THE VESAMICOL RECEPTOR.

Efange,S.M.N.* & Parsons, S.M. * University of Minnesota, Minneapolis, MN 55455 & University of California, Santa Barbara, CA 93106.

The discovery of the vesamicol receptor (VR), a presynaptic cholinergic marker, provides new opportunities for the development of novel radiotracers for mapping cholinergic innervation. However, 2-(4-phenylpiperidinyl)-cyclohexanol (vesamicol), the prototypical VR ligand is nonselective, exhibiting α-adrenoceptor activity and nanomolar affinity for the sigma receptor (SR). In the attempt to develop selective VR ligands, two major classes of vesamicol analogs, Ring C-deficient and Ring C-modified, have emerged (1,2). Radiotracers such as IBVM, 4-HIPP, and MIBT have been derived from these analogs (3-5). Although these compounds exhibit high affinity for the VR *in vitro,* their selectivity for the VR *in vivo* is clearly varied. A better understanding of the structural attributes which determine selectivity for the VR would be invaluable in the development of novel and selective ligands for this receptor.

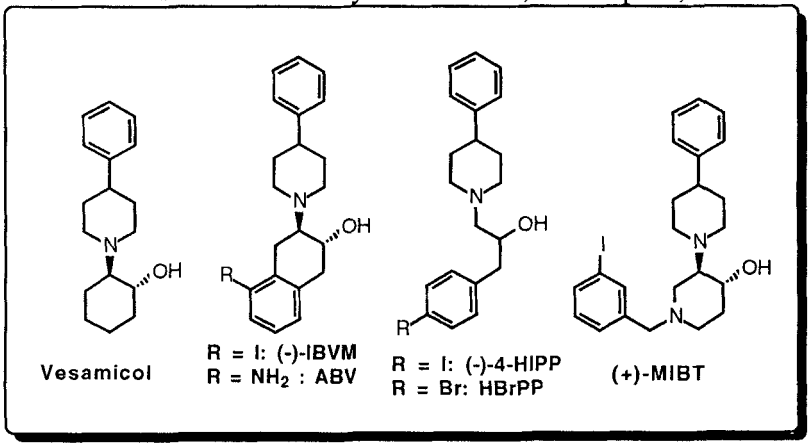
In this study, initiated to gain a better understanding of the structural determinants of VR/SR selectivity, the affinities of a number of vesamicol analogs at the vesamicol and sigma receptors were compared. In contrast to the VR, binding of vesamicol analogs at the SR was not stereospecific. Vesamicol and some ring C-modified analogs such as MIBT showed nanomolar affinity for both receptors. In addition, all Ring C-deficient analogs, regardless of VR affinity, showed high SR affinity. On the other hand, the benzofused ring-C modified analog ABV, a potent VR ligand, showed poor affinity for the sigma receptor, and was therefore the most selective VR ligand.

Previous structure-activity studies (1) suggest that the 2-(4-phenylpiperidinyl)ethanol fragment is responsible for molecular recognition at the vesamicol receptor. Since this fragment also contains the 4-phenylpiperidyl moiety, the presumptive sigma receptor pharmacophore (6), we conclude that the binding of vesamicol analogs to both receptors is not wholly unexpected. Secondly, since the most selective VR ligand was derived from the most conformationally restrained backbone, we suggest that conformational mobility is a key determinant of VR/SR selectivity, and increased rigidity results in higher VR/SR selectivity.

Supported by the AHAF & NIH/NINDS.

1) Rogers et al., J. Med.Chem. 32:1217 (1989); 2) Efange et al, J. Med. Chem. 34: 2639 (1991); 3) Jung et al.,33:2065 (1990); 4) Efange et al., Nucl. Med. Biol. 19:337 (1992); 5) Efange et al., J. Med. Chem., in press.; 6) Largent et al., Mol. Pharmacol. 32:772 (1987).

Supporting data for MOLECULAR DETERMINANTS OF SELECTIVITY AT THE VESAMICOL RECEPTOR. S.M.N. EFANGE & S. M. PARSONS. University of Minnesota, Minneapolis, MN 55455.



Potency of analogs at Vesamicol (A) and Sigma (B) Receptors

Table A:

Compound	IC_{50} (nM)
Haloperidol	43*
(+)-Vesamicol	500**
(-)-Vesamicol	20**
(dl)-ABV	100**
(+)-HBrPP	328±108
(-)-HBrPP	36±5
(dl)-MIBT	26±11

Table B:

Compound	Percent Inhibition of [^{3}H]DTG binding at		
	10^{-9}M	**10^{-7}M**	**10^{-5}M**
(+)-vesamicol	8	72	100
(-)-vesamicol	0	71	100
(dl)-ABV	NT	45	99
(+)-HBrPP	NT	97	100
(-)-HBrPP	NT	97	100
(dl)-MIBT	K_i = 5.9 nM		
Haloperidol	K_i = 1.3 nM		

* Altar & Marien, Synapse 2:486 (1988).

** Ref. 1.

A Radiotracer for Mapping Acetylcholinesterase(AchE) in The Brain *In Vivo*: Evaluation in A Rat Model of Alzheimer's Disease.

IRIE, T.; FUKUSHI, K.; NAMBA*, H.; IYO**, M.; NAGATSUKA***, S. Division of Clinical Research, National Institute of Radiological Sciences, Chiba, *Department of Neurosurgery, Chiba Cancer Center Hospital, Chiba, **National Institute of Mental Health, National Center of Neurology and Psychiatry, Chiba, ***Tokai Research Laboratories, Daiichi Pure Chemicals Co., Ibaragi, Japan

Although extensive losses of cholinergic enzymes have been reported in the cerebral cortex of the patients with Alzheimer's disease, there is no *in vivo* method for evaluating activity of cholinergic enzymes in the brain. At the previous meeting (1), we have introduced the design and synthesis of lipophilic acetylcholine radio-analogs, N-[^{14}C/^{11}C]methyl-3- or 4-piperidyl esters for mapping AchE in the brain, and also have shown that they crossed the blood-brain barrier, and were hydrolyzed mainly by the acetylcholinesterase (AchE), thus trapped in the brain, using mice. The results of mice prompted us to examine whether they responded to changes of enzymatic activity. Here we chose three N-[^{14}C]methyl-4-piperidyl esters(acetate; MP4A, propionate; MP4P, isobutyrate; MP4IB, Fig.1) to simplify the problem of enantiomers arising in the case of 3-piperidyl esters, and evaluated their response against changes in AchE activity using rats with a lesion in the nucleus basalis magnocellularis (NBM), an animal model of Alzheimer's disease.

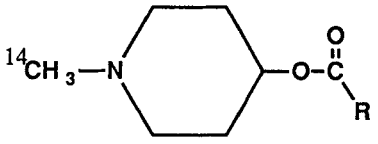
Selectivity and reactivity of three tracers for enzymatic hydrolysis by AchE were examined *in vitro* using rat brain homogenates (striatum and cerebral cortex) and a specific AchE inhibitor, BW2854c51 (Table 1). Both MP4A and MP4P had high selectivity, more than 80 %, but MP4IB showed lower selectivity in cortex, about 45 %. The reactivity varied widely among three traces, MP4A>MP4P>MP4IB, and the order and relative differences were similar to the previous result in mouse brain.

The response study was performed by a following method. Rats were anesthetized and a lesion was made in NBM with an injection of 10 μg of ibotenic acid. Two weeks after NBM lesion, the animals were injected intravenously with either MP4A, MP4P or MP4IB and decapitated 30 min later. The brains were removed, and the cerebral cortex and striatum were dissected. AchE activity of the homogenized tissue was measured with the method of Ellman et al. and uptake of tracers was measured from radioactivity of the tissue. In other animals, the brains were sectioned for autoradiographs (*ex vivo*) and histochemical staining for AchE.

AchE activity of the cerebral cortex in the side of NBM lesion was reduced to about 50% of that in the unlesioned side, and uptake of MP4A and MP4P in the cerebral cortex of the lesioned side decreased to 81% and 73% of the unlesioned side, respectively (Table 2). Uptake of MP4IB, which had low selectivity against AchE, did not decrease by NBM lesion, though the lesions were not so deep as MP4A and MP4P series. No side-to-side differences in AchE activity and tracer uptake were observed in the striatum. Since the NBM lesion model used in the present study does not produce significant changes in cortical blood flow (2), the results indicate that changes in uptake of MP4A and MP4P are attributed to changes in AchE activity. In autoradiographic study, the pattern of uptake of MP4A and MP4P was found to be quite similar to that of AchE staining.

MP4A and MP4P were found to have a potential for measuring AchE activity in the brain. We are preparing experiments using ^{11}C labeled tracers for PET study.

Fig. 1 N-[^{14}C]methyl-4-piperidyl esters



MP4A : R=CH_3
MP4P : R=CH_2CH_3
MP4IB : R=$CH(CH_3)_2$

Table 1 Selectivity and reactivity in enzymatic hydrolysis in rat brain homogenate

	Cerebral cortex		Striatum	
	total rate*	selectivity** for AchE(%)	total rate*	selectivity** for AchE(%)
MP4A	0.43	87	18.5	99.5
MP4P	0.14	80	4.2	98
MP4IB	0.05	45	0.26	83

* fraction per min for brain tissue concentration of 1 g / 1 ml

** % rate by AchE to total rate

Table 2 Ratios (lesioned side / unlesioned side)

	Cerebral cortex		Striatum	
	AchE activity	tracer uptake	AchE activity	tracer uptake
MP4A	0.55 ± 0.07	0.81 ± 0.05	0.98 ± 0.07	0.98 ± 0.04
MP4P	0.50 ± 0.06	0.73 ± 0.05	0.98 ± 0.06	0.99 ± 0.02
MP4IB	0.71 ± 0.06	0.97 ± 0.03	0.94 ± 0.08	0.99 ± 0.07

(Mean ± SD)

(1) Irie T., Fukushi K., Akimoto Y. et al., 9th International Symposium on Radiopharmaceutical Chemistry, *Abstract* 488(1992)
(2) Namba H., Irie T., Fukushi K. et al., Neuroscience Research,12,463-467(1991)

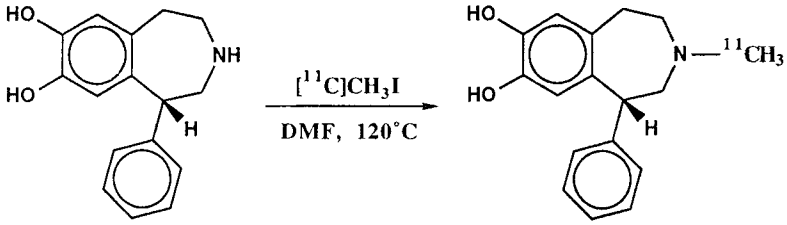
Synthesis of [^{11}C]SKF 75670 as a Potential Dopamine D_1 Receptor Agonist Imaging Agent for PET.

DaSILVA, J.N.; WILSON, A.A.; SEEMAN†, P.; and HOULE, S. PET Centre, Clarke Institute of Psychiatry. Department of Psychiatry and Pharmacology†, University of Toronto, 250 College St., Toronto, Ontario, Canada, M5T 1R8.

Alterations of post-synaptic dopamine (DA) receptors (mostly D_1 and D_2) have been associated with psychiatric and neurological disorders, such as Schizophrenia, Parkinson's and Huntington's diseases (1,2). The two main DA receptor subtypes (D_1 and D_2) differ in their pharmacological and biochemical characteristics. Dopamine can excite or inhibit neurons: D_1 receptors stimulate adenylate cyclase activity, whereas D_2 receptors inhibit it. These two receptors can enhance or oppose each other's action for many psychomotor mechanisms, i.e. D_1 is functionally linked to D_2, opposing for some effects and synergistic for others. D_1 and D_2 receptor sites exist in two interconvertible states exhibiting either high or low affinity for agonists, while antagonists do not differentiate between these two affinity states (3,4). Dopamine and DA agonists show higher binding affinity for the high state compared to the low state of D_1 and D_2 (DA: D_1^{High}, Ki = 0.5 nM; D_1^{Low}, Ki = 580 nM; D_2^{High}, Ki = 7.5 nM; D_2^{Low}, Ki = 4300 nM) (4). There may be an elevated density of DA receptors in the high-affinity state in brains suffering from psychiatric or neurological disorders, compared to normals. In fact, the density of D_1 and D_2 dopamine receptors (B_{max}) may be the same, except that more receptors may be in the high-affinity state. The development of a high affinity D_1 agonist radioligand could thus be more appropriate than a D_1 antagonist radiotracer to study and better estimate the density of post-synaptic D_1 receptors in their high affinity state in these disorders.

SKF 38393, a widely used agonist for D_1, and its N-methylated analog (SKF 75670) bind selectively with high affinity to D_1 (SKF 38393, D_1 Ki = 18 nM, D_2, Ki = 9300 nM; SKF 75670, D_1 Ki = 1.9 nM, D_2, Ki = 1130 nM) (5). We report here the synthesis of the active enantiomer (R(+)) of [^{11}C]SKF 75670 as a potential dopamine D_1 receptor agonist radiotracer for studying and imaging the functions of the dopaminergic system using PET.

Scheme 1. Synthesis of [^{11}C]SKF 75670.



SKF-38393 **[^{11}C]SKF-75670**

R(+)-[^{11}C]SKF 75670 was prepared by N-[^{11}C]methylation of the active enantiomer of R(+)-SKF 38393 with [^{11}C]methyl iodide (Scheme 1). Maximum [^{11}C]methyl iodide was trapped in a reaction vessel containing R(+)-SKF 38393 free base (1 mg; prepared from R(+)-SKF 38393 hydrochloride, Research Biochemicals Inc.: ammonia 25%, CH_2Cl_2 extraction, Na_2SO_4 drying, evaporation) in DMF (200 µL) at -72°C. The reaction mixture was sealed and heated at 120°C for 5 min. [^{11}C]SKF 75670 was then purified by semi-preparative HPLC with ~37% radiochemical yield (decay-corrected, based on [^{11}C]CH_3I, unoptimized). After evaporation of the HPLC solvent, the residue was dissolved in sterile saline and filtered to provide an injectable formulation within 30 min from EOB. Lower radiochemical yield (~23%, decay-corrected, based on [^{11}C]CH_3I) was obtained by generating the free base *in situ* with tetrabutylammonium hydroxide (0.8 equiv., 1 M in methanol) (6) in DMF (200 µL), followed by [^{11}C]CH_3I trapping at -72°C, then heating at 120°C for 5 min, and HPLC purification. The identity of [^{11}C]SKF 75670 was confirmed by comparison of semi-preparative and analytical HPLC retention times, which were identical to those of authentic SKF 75670 (gift from SmithKline Beecham Pharm., King of Prussia, PA, U.S.A.).

Biodistribution studies including regional brain distribution in mice are in progress to evaluate the *in vivo* potential of [^{11}C]SKF 75670 as a high affinity D_1 agonist radiotracer to study the dopaminergic system using PET.

Acknowledgements. This work is supported by a grant from the Ontario Ministry of Health.

1. Seeman P., Bzowej N.H., Guan H.C., Bergeron C., Reynolds G.P., Bird E.D., Riederer P., Jellinger K. and Tourtelotte W.W. — Neuropsychopharmacology 1: 5 (1987)
2. Joyce J.N., Lexow N., Bird E. and Winokur A. — Synapse 2: 546 (1988)
3. Clark D. and White F.J. — Synapse 1: 347 (1987)
4. Seeman P. and Niznik H.B. — I.S.I. Atlas of Sci.: Pharmacology 2: 161(1988)
5. Andersen P.H. and Jansen J.A. — Eur. J. Pharmacol. 188: 335 (1990)
6. Chakraborty P.K., Gildersleeve D.L., Toorongian S.A., Kilbourn M.R., Schwaiger M. and Wieland D.M. — J. Label. Compd. Radiopharm. 32: 172 (1993)

N-Alkylated Analogues of [F-18]Benperidol as Dopaminergic D-2 Receptor Ligands.

MOERLEIN, S.M.[a]; PERLMUTTER. J.S.[a,b]; BOCKHORST, J.L.[a]; PARKINSON, D.[c]; and WELCH, M.J.[a]. [a]Edward Mallinckrodt Institute of Radiology, [b]Department of Neurology and Neurosurgery, and [c]Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

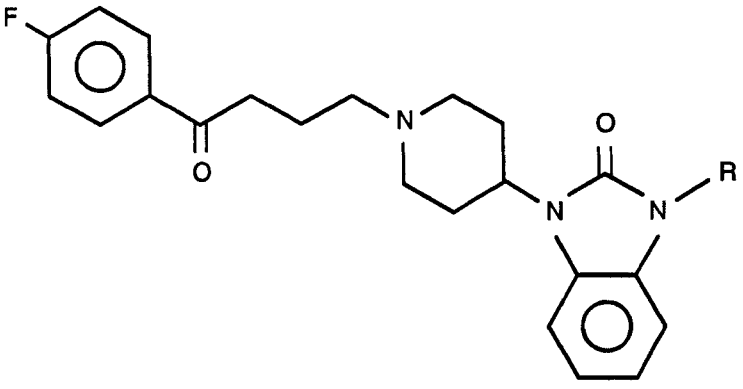
Benperidol (BP) is a butyrophenone neuroleptic that binds to dopaminergic D-2 receptors with high affinity and high selectivity, and analogues of this ligand have been labeled with fluorine-18 and carbon-11 for use as PET radiopharmaceuticals[1,2]. Because N-alkylation has been shown to influence the receptor-binding localization of the D-2 ligand spiperone[3], we have synthesized various alkylated derivatives of [^{18}F]benperidol to identify a PET tracer with optimal *in vivo* kinetics. It was anticipated that attachment of alkyl substituents to the amide nitrogen would not significantly alter D-2 receptor-binding affinity, but would increase ligand lipophilicity. Our goal is to tailor ligand lipophilicity to maximize brain extraction while avoiding excessive nonspecific binding.

Benperidol derivatives NMB, NEB, NPB and NBB were prepared with amido methyl, ethyl, n-propyl and n-butyl substituents, respectively. The log octanol/water partition coefficients for these structures were calculated to range from 3.2 for BP to 5.3 for NBB. The ligands were synthesized in 60-80% yield by heating BP with excess alkyl halide in the presence of tetrabutyl ammonium hydroxide. Final products were purified using silica gel and recrystallization of the HCl salts. All compounds were characterized using ^{1}H-NMR, MS, elemental analysis, TLC and melting point.

The ligands were evaluated for D-2 receptor-binding affinity using *in vitro* binding assays. Displacement experiments using [^{3}H]spiperone and primate caudate tissue indicated that the benperidol derivatives had affinity constants K_i in the range 2.4 - 7.0 nM. These data suggest that N-alkylation does not significantly change the binding affinity of benperidol derivatives for D-2 receptors.

The fluorine-18 labeled radioligands were prepared in high specific activity using a synthetic sequence similar to that reported for other butyrophenones[4,5]. The labeling procedure involved microwave-facilitated aromatic nucleophilic [^{18}F]fluorination of (p-nitrophenyl)cyclopropyl ketone. The resulting ([^{18}F]fluorophenyl)cyclopropyl ketone was converted to the corresponding ω-chloro propyl derivative by refluxing in methanolic hydrochloric acid. This intermediate was then used to alkylate the respective benzimidazolinyl piperidine substrate to yield [^{18}F]BP, [^{18}F]NMB, [^{18}F]NEB, [^{18}F]NPB or [^{18}F]NBB, which were purified using preparative HPLC. The radiotracers were prepared in 5-10% radiochemical yield and specific activities exceeding 1000 Ci/mmol within an overall preparation time of 110-120 min.

Preliminary imaging experiments compared the tracer kinetics of [^{18}F]BP with [^{18}F]NMB in baboons. Both ligands localized *in vivo* within D-2 receptor-rich tissues, with putamen-to-cerebellum ratios of 14-16 at 285 minutes. [^{18}F]NMB cleared slowly from putamen whereas [^{18}F]BP did not clear at all; the accumulation of [^{18}F]NMB in putamen was 2.4 times that of [^{18}F]BP at 285 minutes. The free fraction and *in vivo* metabolites were very similar for the two tracers. These results suggest that N-alkylation produces subtle, yet important, changes in the *in vivo* localization of the butyrophenone.



	R	log P	K_i(nM)
BP	H	3.2	0.3 ± 0.1
NMB	CH_3	3.7	3.6 ± 1.0
NEB	CH_2CH_3	4.2	7.0 ± 0.5
NPB	$CH_2CH_2CH_3$	4.8	4.8 ± 0.3
NBB	$CH_2CH_2CH_2CH_3$	5.3	2.4 ± 0.2

Acknowledgements

This work was supported in part by NIH grants 1R29N526788 and HL13851.

References

(1) Arnett, C.D.; Shiue, C-Y,; Wolf, A.P.; et al. Comparison of three ^{18}F-labeled butyrophenone neuroleptic drugs in the baboon using positron emission tomography. J. Neurochem. 44, 835 (1985).

(2) Suehiro, M.; Dannals, R.F.; Scheffel, U.; et al. In vivo labeling of the dopamine D2 receptor with N-^{11}C-methyl-benperidol. J. Nucl. Med. 31, 2015 (1990).

(3) Moerlein, S.M.; Laufer, P.; Stöcklin, G. Effect of lipophilicity on the *in vivo* localization of radiolabelled spiperone analogues. Int. J. Nucl. Med. Biol. 12, 353 (1985).

(4) Hwang, D-R.; Moerlein, S.M.; Dence, C.S.; Welch, M.J. Microwave-facilitated synthesis of [^{18}F]spiperone. J. Lab. Comp. Radiopharm. 26, 391 (1989).

(5) Shiue, C-Y.; Fowler, J.S.; Wolf, A.P.; et al. Syntheses and specific activity determinations of no-carrier-added fluorine-18-labeled neuroleptic drugs. J. Nucl. Med. 26, 181 (1985).

Production of [^{11}C]raclopride with high specific activity for clinical use

BAUER, B.; and WAGNER, R. Max-Planck-Institut für neurologische Forschung, Gleueler Str. 50, D-50931 Köln, FRG

We started to produce [^{11}C]raclopride for routine clinical application by the synthetic procedure according to FARDE 1988, HALLDIN 1991 and MAZIERE 1992 (1-3).
For the one-pot production of [^{11}C]methyl iodide we used 200 µl of a solution of lithium aluminium hydride in THF. The reagent solutions were prepared by use of a glove box flushed with argon 5.0 . Portions of 10 - 15 mg lithium aluminium hydride (Aldrich or Merck) were placed into 1 ml glass vessels (Alltech) with screw caps and teflon coated silicon septa. THF (distilled over Na/benzophenone) was added and the suspensions magnetically stirred overnight. These reagent samples can be stored at least three months under argon atmosphere without noticeable decrease of specific activity of the produced [^{11}C]raclopride.

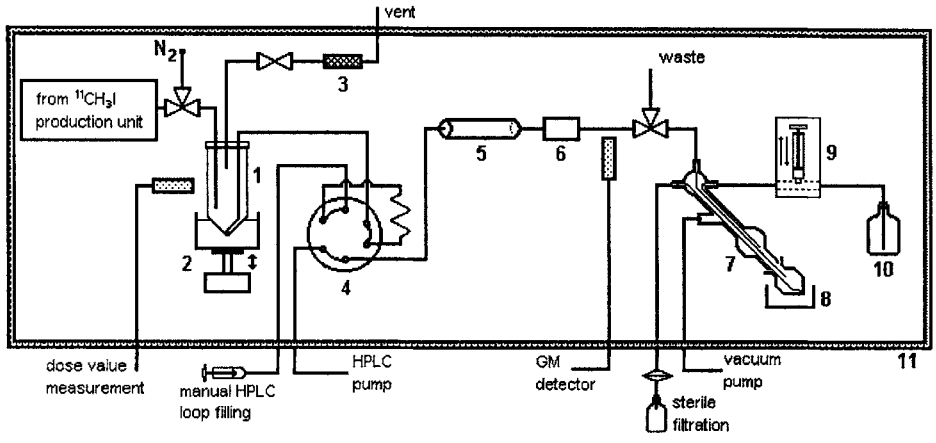
Preparation of [^{11}C]raclopride (Fig. 1): A solution containing 2.5 - 3 mg of desmethylraclopride (S-enantiomer), 300 µl DMSO (Aldrich, 99+%, anhydrous, stored over molecular sieve 0.3 nm) and 6 µl 5M NaOH was used for trapping of [^{11}C]methyl iodide. 3 - 10 min after the addition of the base, stirring and contact to oxygen the solution developped a green colour. Without coloration we could not induce formation of the desired product. At 6 min EOB [^{11}C]methyl iodide was bubbled into this solution during 2 - 3.5 min. Transformation of the [^{11}C]methyl iodide to [^{11}C]raclopride was 36 - 42%. After 5 min at 80°C 600 µl of eluent (0.01M H_3PO_4/CH_3CN = 70:30) were added and the resulting yellowish solution was injected onto a semipreparative HPLC-column (µ-Bondapak C18, 300 x 7.8 mm). Routinely 60 - 70% of the activity of the reaction mixture consisted of the desired product. The HPLC fraction collected between 8.5 and 10.5 min (flow rate 6 ml/min) is evaporated and the residue is redissolved in 8 ml of physiological saline. Filtration through 0.22 µm Millipore filter yields 7.2 - 7.6 ml (determined by weighing) of sterile and pyrogen free [^{11}C]raclopride.

Yields: 69 - 82 mCi are produced from a 40 min, 35 µA, 17 MeV proton bombardment. The synthesis time is 40 - 45 min from EOB, radiochemical purity greater than 99% and specific activity (EOS) 1200 - 2000 mCi/µmol. Radiochemical yields range from 28 - 34% (decay corrected, based on [^{11}C]CO_2).

Analysis: HPLC analysis was performed with a Waters µ-Bondapak C18 column (300 x 3.9 mm), eluent 0.01M H_3PO_4/CH_3CN = 69:31 and a flow rate of 2.0 ml/min (Fig. 2). Retention times were: desmethylraclopride 3.7 - 3.9 min, raclopride 5.7 - 6.2 min (UV wavelength 230 nm) and sometimes a non radioactive contaminant at 4.9 - 5.2 min up to max. 10% of the raclopride mass peak. The desmethylraclopride peak represented 2 - 13% of the mass of the corresponding raclopride mass peak. Radioactive contaminants were observed in the range of 0.5 - 1% at a retention time below 3 min. Initially our experiments showed increased by-product formation in this region due to overheating of the reaction mixture during evaporation of solvent by a hot blower (150 - 200°C). After subtitution of the hot blower for a water bath (85°C) the contaminants could be reduced to below 1% .

References

1. Farde L., Pauli S., Hall H., Eriksson L., Halldin C., Högberg T., Nilsson L., Sjögren I. and Stone-Elander S. - Psychopharmacology 94: 471(1988)
2. Halldin C., Farde L., Högberg T., Hall H., Ström P., Ohlberger A. and Solin O. - Nucl.Med.Biol.18: 871(1991)
3. Maziere B., Coenen H.H., Halldin C., Nagren K. and Pike V.W. - Nucl.Med.Biol.19: 497(1992)



1: conical glass reaction vessel equipped with screw cap and teflon coated silicon septum
2: oil bath (80°C) on electrically operated elevator
3: molecular sieve
4: electrically operated HPLC injection valve with 1ml sample loop
5: µBondapak HPLC column
6: UV detector (220nm)
7: rotary evaporator
8: water bath (75-85°C)
9: motor driven syringe
10: 8ml 0.9% NaCl solution
11: lead shielded box

Figure 1: schematic view of [^{11}C] raclopride production

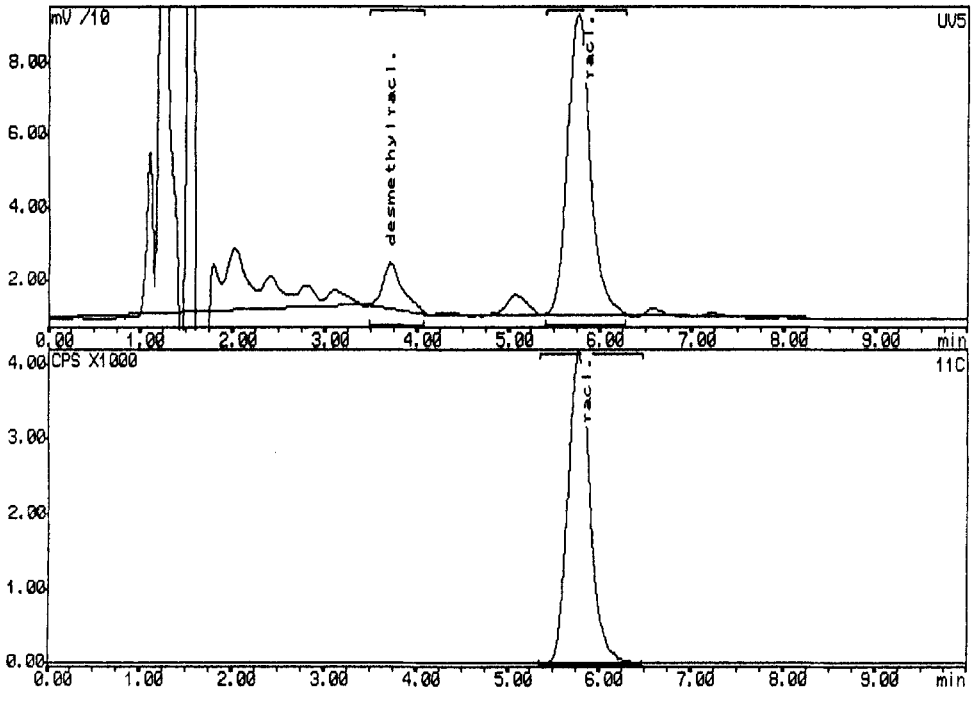


Figure 2: HPLC analysis of a [^{11}C] raclopride sample (conditions see text).
On top: UV detector. Underneath: Radioactivity detector

Synthesis and Quality Control of ^{123}I-Lisuride with High Specific Activities.

BIER, D.; DUTSCHKA, K.; PLÖGER, TH.; and KNUST, E.J. Nuklearchemie und Radiopharmazie, Universitätsklinikum, Hufelandstr. 55, D-45122 ESSEN, Germany

Lisuride, a semi-synthetic ergolene-derivative [3-(9,10-didehydro-6-methyl-8α-ergolinyl)-1,1-diethylurea, see figure 1] stereochemically differs from naturally occuring alkaloids by its 8α-configuration of the diethylurea group thus exhibiting a high affinity for dopamine-D2-receptors. Labelling of lisuride with ^{123}I in position 2 of the molecule [Loc'h und Mazière, 1989, 1991] leads to the dopamine-D2 antagonist 2-^{123}I-lisuride which offers the possibility for clinical application by single photon emission tomography (SPECT). With respect to a high accumulation in the striatum and a routine clinical application a labelling method is required by which the iodinated product is reliably formed with very high specific activities. For this purpose labelling is performed with the oxidizing agent IODOGEN (see table 1). Into a small glass vessel, previously coated with a thin layer of IODOGEN, lisuride in aqueous formic acid and ^{123}I-iodide in 0.01-0.02N NaOH are added. After a reaction time of 15 min at room temperature purification is performed by reverse phase high pressure liquid chromatography (see figure 2). The product is obtained with radiochemical yields of 85±5%, a radiochemical purity of >97% and specific activities in the range of 120-200 Ci/μmol (4440-7400 GBq/μmol) corresponding to 50-85% of the theoretical value. For the preparation of the injection solution, 2-^{123}I-lisuride is dissolved in 0.9% physiological NaCl solution containing 1% of Tween 80 and purified by sterile filtration.

Loc'h C., Mazière B. (1989) : NCA Synthesis of radiohalogenated derivatives of lisuride. J. Lab. Comp. Radiopharm. 26: 100-101
Loc'h C., Mazière B. (1991) : An improved radiosynthesis of the D2-antagonist ^{123}I-iodolisuride. J. Lab. Comp. Radiopharm. 30: 377

Table 1 : Reaction parameters

- IODOGEN*- $CHCl_3$ - solution (50 µl, 17 mg/10 ml), evaporation to dryness
- Addition of lisuride (410 µg in 100 µl 1M HCOOH) and ^{123}I-iodide in 0.01 - 0.02 M NaOH
- 15 min reaction time at room temperature
- Purification and identification by HPLC
  Column : KROMASIL RP-18, 5 µm, 250 x 4.6 mm
  Eluens : CH_3CN - H_2O - $(C_2H_5)_2NH$ = 37 - 63 - 0.2 (Vol)
  Flow rate : 1.5 ml/min
  Detection : UV, 254 nm
  k' : 17.5
- Injection solution
  Physiological NaCl solution with 1% TWEEN 80
  Sterile filtration (0.22 µm)

*IODOGEN = 1,3,4,6-Tetrachloro-3a,6a-diphenylglycouril

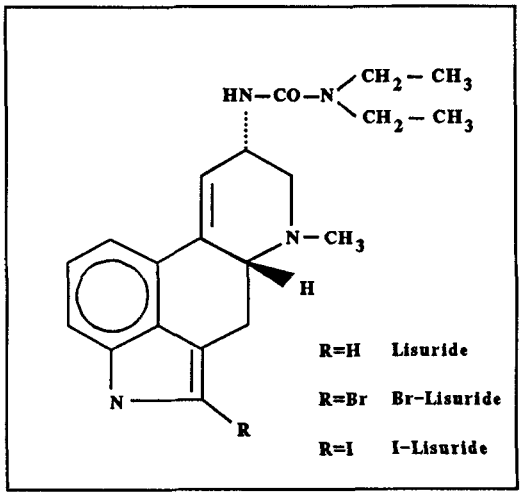


Fig. 1 : Structure of lisuride and derivatives

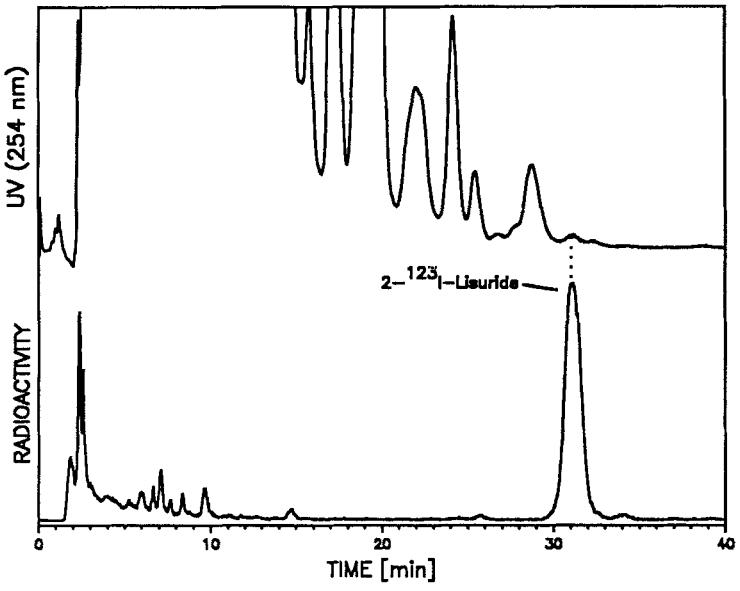


Fig. 2 : HPLC chromatogram for purification of 2-^{123}I-lisuride

A simplified preparation and metabolite analysis of a new D2 SPECT ligand : [123-I]-IBF

G. WESTERA, *H.F. KUNG, A.BUCK, G.K. VON SCHULTHESS. Division of Nuclear Medicine, University Hospital, Zürich, Switzerland, * Department of Radiology, University of Pennsylvania, Philadelphia, USA.

IBF (5-iodo-7-N-[(1-ethyl-2-pyrrolidinyl)methyl]carboxamido-2,3-dihydrobenzofuran) has been characterized as a dopamine D-2 imaging agent (1,2). We are using it to study patients with Parkinson disease ar progressive supranuclear palsy (3).
We have prepared ^{123}I-IBF by the iododestannylation of the corresponding 5-tributylstannyl compound reported previously (1).
The reversed phase HPLC purification used originally (Hamilton PRP-1 eluting with acetonitrile / NH_4HPO_4 (pH=7) = 75 / 25) proved to be very time consuming and not easily reproducible on a routine basis: the retention times were not very reproducible and a large part of the ^{123}I-IBF remained on the column, sometimes as much as 75%. It also takes some time to evaporate the acetonitrile quantitatively.
We have now developed a simplified purification method using a SEP-PAK C-18 cartridge.
The reaction mixture {50 µg 5-tributylstannyl-7-N-[(1-ethyl-2-pyrrolidinyl)-methyl]carboxamido-2,3-dihydrobenzofuran, 50 µl ethanol, 50 µl 1N HCl, ^{123}I-NaI (in NaOH, pH = 11, volume varying from 110-600 µl), 50 µl 3% H_2O_2; followed by 100 µl $Na_2S_2O_5$ (8 mg/ml)} is neutralized with 1 ml of saturated NaHCO3 and the resulting solution transferred to the SEP-PAK cartridge. The SEP-PAK cartridge is washed with
- 1 ml water
- 3 ml water / ethanol = 1 / 1 and the IBF eluted from the column with 9 ml ethanol into a ratavapor vial containing 100 µl ascorbic acid solution (1 mg/ml). After evaporation to almost dryness and redissolution the IBF is > 97% radiochemically pure. The yield is always > 50% of the original activity and is recently usually about 70%.
Quality control (HPLC with acetonitrile / 0.5 mM dimethylglutaric acid = 90 / 10 over PRP-1) only shows one radioactive peak, corresponding to the IBF.
The obvious advantages of this procedure are
- higher and more reproducible radiochemical yield
- faster procedure (the preparation now takes 1.5 h including quality control)
- better reproducibility
- a small amount of alcohol (no HPLC solvent) is the most toxic substance possibly present in the patient dose.

After *i.v.* injection blood was taken during the SPECT scanning period. The blood was centrifuged, plasma and bood cells separated and counted and the plasma analyzed for metabolites.
To plasma was added an equal volume of borate buffer (0.1 M NaOH, 0.1 M H_3BO_3, 0.05 M KCl, pH=11) and this solution was extracted with the same volume of chloroform. Both layers were analyzed by HPLC (same method as for the quality control). As the results were fairly different for various patients, the results of two patients are tabulated in Table 1 a and b.

The plasma IBF concentration decreases rapidly with time suggesting rapid *in vivo* metabolism, but the blood activity initially, unexpectedly, increases with time. This is due to the formation of different amounts of the hydrophilic metabolites.

Table 1a: Metabolite analysis of patient 1 blood after *i.v.* application of IBF

Time min.	Blood act. % *	% BC act. **	% Pl./ H_2O ***	% Pl./ $CHCl_3$ ***	% IBF in $CHCl_3$	% IBF in plasma
5	100	15	56	44	78	34
10	135	18	33	67	48	32
20	145	16	22	78	26	20
60	156	18	63	37	7	3
120	123	14	57	43	5	2
180	107	16	69	31	0	0

Table 1b: Metabolite analysis of patient 2 blood after *i.v.* application of IBF

Time min.	Blood act. % *	% BC act. **	% Pl./ H_2O ***	% Pl./ $CHCl_3$ ***	% IBF in $CHCl_3$	% IBF in plasma
10	100	17	25	75	58	44
20	104	15	29	71	27	19
60	105	26	37	63	11	7
120	81	21	42	58	3	2
180	65	20	43	57	0	0

* The activity / unit volume of the first blood sample was set at 100 %
** The activity in the blood associated with the blood cells
*** Percentage of the plasma activity after extraction with chloroform associated with the water resp. the chloroform phase

References

1. Murphy R.A., Kung H.F., Kung M-P., Billings J. - J. Med. Chem. 33: 171 (1990)
2. Kung M-P., Kung H.F., Billings J., Yang Y., Murphy R.A., Alavi A. J. Nucl. Med. 31: 648 (1990)
3. Buck A., Westera G., Kung H.F., Albani C., Von Schulthess G.K. J. Nucl. Med. 34: 196P (1993)

PREPARATION OF (+)-[PROPYL-^{11}C]7-OH-DPAT, A SELECTIVE DOPAMINE D-3 RECEPTOR AGONIST FOR PET.

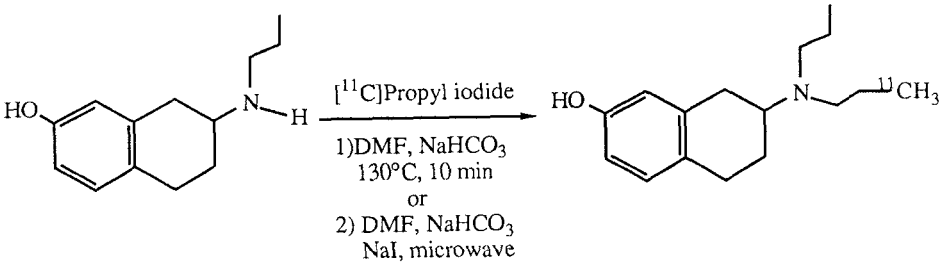
C. Halldin, C.-G. Swahn, T. Suhara, L. Farde, P. Karlsson, P. Sokoloff and G. Sedvall. Department of Psychiatry and Psychology, Karolinska Institute, Stockholm, Sweden and Unité de Neurobiologie et Pharmacologie de Institut National de la Santé et de la Recherche Médicale, Paris, France.

The dopamine D-3 receptor was recently identified. Most antipsychotic drugs used today have affinity for this suptype. [^{11}C]Pergolide has previously been developed as a potential dopamine D-3 ligand for PET. However, the accumulation of radioactivity in the monkey brain was not displaceble (1). [^{3}H]7-OH-DPAT ([^{3}H]7-hydroxy-N,N-di-n-propyl-2-aminotetralin) has been identified as a selective probe for the recently cloned dopamine D-3 receptor and been used to assess the distribution and characteristics of this receptor subtype and establish its distribution and properties in rat brain (2). It binds to dopamine D-3 receptors with a K_D of 0.78 nM, whereas its affinity is approximately 100-, 1000-, and 10000-fold lower for the D-2, D-4 and D-1 receptors, respectively.

The active (+)-enantiomer of [propyl-^{11}C]7-OH-DPAT was prepared by N-alkylation of the free base of the secondary amine with [^{11}C]propyl iodide (3) using either thermal heating or microwave heating conditions (Scheme 1). During our reaction conditions, we found that reaction in DMF with $NaHCO_3$ at 130°C for 10 min resulted in 21% radiochemical incorporation of [^{11}C]propyl iodide, whereas microwave heating (4x2 min, 600 Watt) in DMF with addition of both $NaHCO_3$ and NaI resulted in about 8% radiochemical incorporation. Subsequent reversed-phase semi-preparative HPLC purification resulted in a total radiochemical yield of 15% (from EOB and decay-corrected) with a total synthesis time of 40-45 min and a radiochemical purity >99 %.

(+)-[Propyl-^{11}C]7-OH-DPAT was injected i.v. into a Cynomolgus monkey. PET-examination showed a marked uptake of radioactivity in brain regions such as the striatum. The striatum to cerebellum ratio was 2.2 after 10 minutes. In a displacement experiment, radioactivity in the striatum but not in the cerebellum was markedly reduced after injection of 5 mg raclopride, which has affinity both for dopamine D-2 and D-3 receptors, thus demonstrating the reversibility of (+)-[propyl-^{11}C]7-OH-DPAT binding. Further displacement experiments must be performed to examine the specificity of (+)-[propyl-^{11}C]7-OH-DPAT binding to dopamine D-3 receptors.

1. Halldin C., Swahn C.-G., Hall H. and Farde L. - J, Nucl. Med. 32: 1039 (1991).
2. Lévesque D., Diaz J., Pilon C., Martres M.-P., Giros B., Souil E., Schott D., Morgat J.-L., Schwartz J.-C. and Sokoloff P. - Proc. Natl. Acad. Sci. 89: 8155 (1992).
3. Långström B., Antoni G., Gullberg P., Halldin C., Någren K., Rimland A. and Svärd H. - Appl. Radiat. Isot. 40: 557 (1986).



(+)-[propyl-^{11}C]7-OH-DPAT

Scheme 1.

(R)*trans*-7-OH-PIPAT: A Potential D3 Dopamine Receptor Imaging Agent for SPECT. CHUMPRADIT, S.; KUNG, M.P.; FOULON, C. and KUNG, H.F.* Departments of Radiology and Pharmacology*, University of Pennsylvania, Philadelphia, PA 19104.

The CNS dopaminergic system in mammalian brain is very important for normal brain function, and it is also the apparent action site for various neuroleptic drugs in the treatment of schizophrenia and other mental disorders. Cloning of dopamine receptors has yielded at least six different subtypes[1]; D1, $D2_L$, $D2_S$, D3, D4 and D5, a diversity far beyond the traditional classification of the two subtypes, D1 and D2, proposed by Kebabian in 1979. The D3 receptor differs from D1 and D2 receptors in several ways: amino acid sequence, pharmacological profiles *(in vivo* and *in vitro),* tissue distribution and most likely, the receptor-effector coupling mechanism. These findings presented new challenges in developing PET or SPECT imaging agents specifically for localization of this important dopamine receptor subtype. Recently, [^{3}H]7-OH-DPAT (7-hydroxy-N,N-(di-n-propyl)-2-aminotetralin) was identified as a selective ligand for the D3 receptor expressed in CHO cells, K_d=0.67 nM.[2] Based on 7-OH-DPAT, we have prepared an iodinated derivative, (R,S)-*trans*-7-hydroxy-2-[N-n-propyl-N-(3'-iodo-2'-propenyl]aminotetralin (*trans*-7-OH-PIPAT **5**; scheme 1) by placing the iodine atom on the N-propenyl side chain[3]. This unique feature has led to a stable iodinated derivative with highly desirable properties: higher specific activity (theoretical specific activity for I-125 is 2,200Ci/mmol), more potent binding affinity and lower non-specific binding. The binding characteristics of the racemic (R,S)*trans*-7-OH-PIPAT, **5**, were evaluated with D3 dopamine receptors expressed in *Spodoptera frugiperda* (Sf9) cells[4] (K_d = 0.13 nM in the absence of NaCl). The racemic form of [I-125](R,S)*trans*-7-OH-PIPAT, (R,S)**5**, was resolved into isomer A and isomer B by high pressure liquid chromatography using a chiral column (chiracel OD). However, only the isomer A, which corresponding to R-**5**, showed high D3 dopamine receptor binding. [I-125](R)-trans-7-OH-PIPAT, R-**5**, displayed a high specific and saturable binding in rat striatal membrane homogenates; K_d = 0.48 nM and B_{max} = 240 fmol/mg of protein. Competition binding data exhibited the pharmacological profile of D3 dopamine receptors (Table 1). Further binding studies of R-**5** in the same membrane homogenates indicated that B_{max} values were 180 and 140 fmol/mg of protein with and without the presence of GTP (300 μM), respectively, with no change in K_d. The data suggested that there were GTP sensitive binding sites for this ligand.

In order to elucidate and clearly identify the optical isomer responsible for the specific binding of D3 dopamine receptors, we have employed asymmetric separation method to confirm the geometry of this series of tetralin compounds (scheme 2). Optically active R(-)-α-methoxyphenylacetyl chloride was reacted with **1** to give the diastereomeric amides. (R,R;S,R)**8** . After column chromatography of the diastereoisomers: R,R(+)**8** and R,S(-)**8**[5,6], the corresponding R(+)**1** and S(-)**1** (isolated as hydrochloride salts) were obtained by sequential treatment of potassium t-butoxide and hydrochloric acid solution. Optical rotation measurements of R(+)**1** and S(-)**1** as hydrochloride salts were +71° and -70°, respectively. The values are consistent with those reported in the literature[6]. The resolved optical isomers R(+)**1** and S(-)**1** were suitable starting material for carrying out subsequent reactions. N-alkyation with propynyl chloride, demethylation with BBr_3 and addition of tributyltin hydride and reaction of I_2 in chloroform; as described in scheme 1, gave the desired final products R(+)**5** and S(-)**5**. Radiolabeling with R-**7** via an iododestannylation reaction provides the desired I-125 and I-123 labeled R-**5** in excellent yield and purity. Initial biodistribution studies (Table 2) suggested that [I-123](R)-**5**, penetrated intact blood-brain barrier and localized in brain; however, no selective regional uptake in brain was observed. Further imaging studies are needed to characterize the *in vivo* localization of this new ligand in brain.

Table 1. Inhibition constant of compounds on the [^{125}I](R)*trans*-7-OH-PIPAT, **5** binding in rat striatal homogenates (in presence of 5 μM DTG)

Compound	Ki (nM)	n	Compound	Ki (nM)	n
(±)7-OH-DPAT	1.11±0.10	0.87	(±)Butaclamol	2.76±0.11	1.00
Spiperone	0.012	0.66	(-)Butaclamol	>20,000	
Haloperidol	0.006	0.48	FIDA2	4.01±0.5	1.07
Dopamine	6.57±1.1	0.98	IBZM	2.68±0.4	0.70
Quinpirole	8.13±1.5	0.91	Raclopride	99.7±4.0	0.88

Assay was carried out at 37°C for 30 min. Synapsomes (P-2 fraction) were used, preincubation at 37°C for 20 min to inactivate endogenous DA. Buffer: 50 mM Tris-HCl, pH 7.4 with 2 mM $MgCl_2$, 1μM (±) 7-OH-DPAT was used to define nonspecific binding.

Table 2. In vivo biodistribution studies with [^{123}I](R)*trans*-7-OH-PIPAT, **5**, in rats i.v. injection (% dose/organ, average of 3 rats ± SD)

Organ	2 min	30 min	60 min	120 min
Blood	2.73±0.18	1.05±0.19	0.96±0.12	0.99±0.04
Heart	1.59±0.27	0.17±0.03	0.10±0.02	0.06±0.01
Muscle	16.41±8.18	14.38±2.01	6.89±1.58	4.64±0.84
Lung	4.07±0.86	0.62±0.11	0.35±0.04	0.22±0.04
Kidney	5.08±0.14	2.15±0.58	1.27±0.56	0.96±0.12
Spleen	0.41±0.14	0.20±0.02	0.13±0.05	0.10±0.03
Liver	11.28±0.89	3.82±0.78	3.32±0.27	3.56±0.96
Skin	4.39±0.49	6.44±0.65	7.01±0.73	4.83±0.39
Thyroid	0.11±0.008	0.07±0.003	0.12±0.06	0.18±0.13
Brain	2.09±0.07	0.46±0.08	0.22±0.05	0.13±0.01

Regional distribution (%dose/gm)

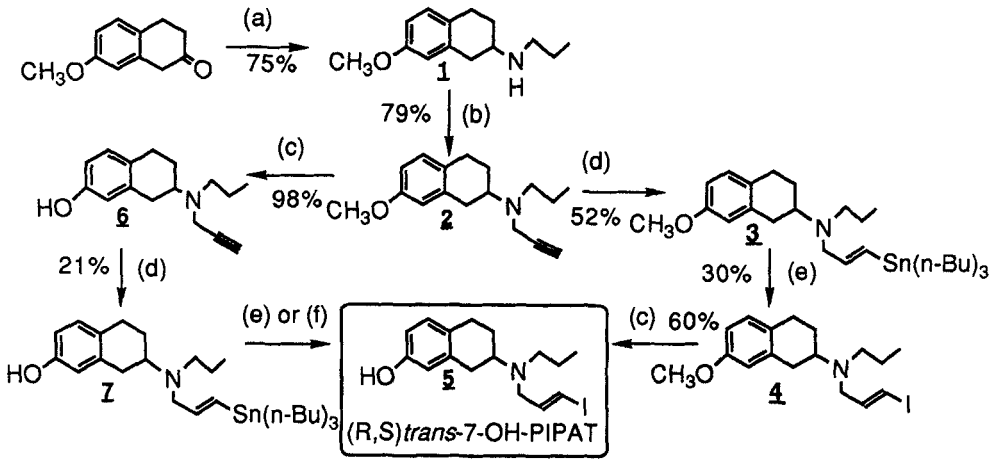
Region	2 min	30 min	60 min	120 min
cerebellum	1.64±0.15	0.32±0.05	0.16±0.04	0.09±0.01
hypothalamus	1.14±0.40	0.37±0.01	0.19±0.05	0.089±0.011
striatum	1.59±0.14	0.42±0.08	0.19±0.05	0.101±0.01
hoppocampus	1.51±0.13	0.38±0.08	0.19±0.04	0.095±0.006
cortex	2.02±0.13	0.35±0.03	0.17±0.02	0.119±0.026

References:

1. Sibley, D. R.; Monsna, F. J. J. *TiPS Rev.* **1992,** 13, 61-69.
2. Levesque, D.; Diaz, J.; Pilon, C.; Martres, M.-P.; Giros, B.; Souil, E.; Schott, D.; Morgat, J.-L.; Schwartz, J.-C.; Sokoloff, P. *Proc. Natl. Acad. Sci. USA.* **1992,** 89, 8155-8159.
3. Foulon, C.; Kung, M.-P.; Kung, H. *J. Med. Chem.* **1993,** in Press.
4. Boundy, V. A.; Luedtke, R. R.; Gallitano, A. L.; Smith, J. E.; Filtz, T. M.; Kallen, R. G.; Molinoff, P. B. *J. Pharm. Exp. Ther.* **1993,** 264,1002.
5. Wikstrom, H.; Sanchez, D.; Lindberg, P.; Hackesell, U.; Arvidsson, L-E.; Johansson, A.M.; Thorberg, S-O.; Nilsson, J.L.G.; Svenssen, K.; Hjorth, S.; Clark, D.; Carlsson, A. *J . Med. Chem.* **1984,** 27, 1030.
6. Wikstrom, H.; Sanchez, D.; Lindberg, P.; Arvidsson, L-E.; Hackesell, U.; Johansson, A.M.; Anderssen, B.; Nilsson, J.L.G.; Svenssen, K.; Hjorth, S.; Carlsson, A. *J. Med. Chem.* **1985,** 28, 215.

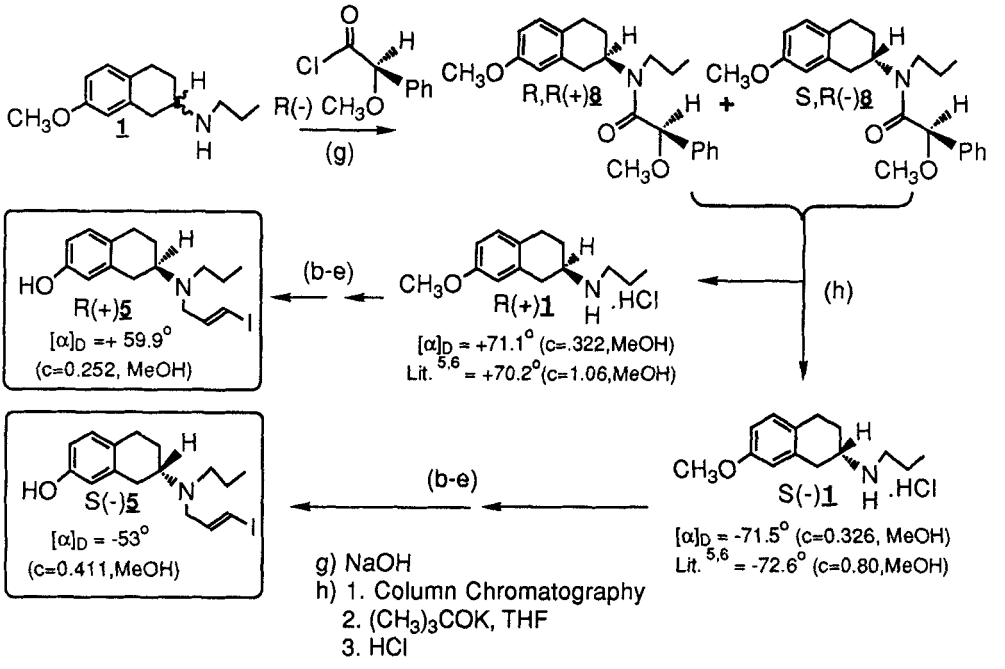
Acknowledgements: This work is supported by a grant awarded by NIH (NS-24538).

Scheme 1. Synthesis of (R,S)*trans*-7-OH-PIPAT, **5**



a). n-propylamine/$NaBH_3CN$ d). $(n\text{-}Bu)_3SnH$, AIBN, toluene
b).2- propynyl chloride/K_2CO_3 e). $I_2/CHCl_3$
c). BBr_3, CH_2Cl_2 f). H_2O_2/ $Na^{125}I$

Scheme 2. Resolution of (R,S)trans-7-OH-PIPAT, **5**



g) NaOH
h) 1. Column Chromatography
2. $(CH_3)_3COK$, THF
3. HCl

Electrophilic synthesis of 6-[^{18}F]fluoro-L-dopa, starting from aqueous [^{18}F]-fluoride

BERGMAN, J.*; HAAPARANTA, M.; LEHIKOINEN, P. and SOLIN, O.*
Medical Cyclotron /PET Center, Radiopharmaceutical Chemistry Laboratory, Turku University and Accelerator Laboratory*, Åbo Akademi University, Porthaninkatu 3, SF-20500 Turku, Finland.

Recent development in the production of electrophilic [^{18}F]F_2 from aqueous [^{18}F]-fluoride (1) and the introduction of a stannylated precursor for 6-[^{18}F]fluoro-L-dopa (**1**) (2) has made possible an easy routine synthesis for PET studies. With this method the specific radioactivity of the radioactive precursor is relatively high (30 mCi/μmol), which gives the advantage that the amount of organometallic starting material needed is small. This makes the synthesis and the purification of the final product easier.

Electrophilic ^{18}F was produced from aqueous [^{18}F]-fluoride according to (1). The amount of carrier F_2 used was 2-4 μmol. A protected 6-trimethylstannyl dopa derivative (N-formyl-3,4-di-t-butoxycarbonyloxy-6-(trimethylstannyl)-L-phenylalanine ethyl ester) (**2**) was prepared as previously described (2,3,4) and used as a precursor. [^{18}F]F_2 in neon was bubbled through a solution of $CFCl_3$ (Freon 11, 1 ml) and precursor **2** (4 μmol, 2.4 mg) at room temperature until the solvent had evaporated (7 min). 300 μL of HBr (48 %) was added and the mixture was hydrolyzed for 10 min at 130 °C. After hydrolysis the reaction mixture was diluted with the HPLC eluent (500 μL) and neutralized with 10.8 M NaOH (170 μL). This solution was injected onto a semipreparative HPLC column (μBondapak C18 (19x150 mm), eluent: 0.9 % NaCl + 0.01% acetic acid at a flow rate of 8 mL/min). The outflow was monitored with U.V. (280 nm) and radioactivity detectors. The final product **1** was collected at 12 min in a volume of 9 mL. (Fig. 1). The radiochemical and chemical purity was analyzed by ion-pair HPLC with electrochemical (coulometric, ESA Coulochem II) and radioactivity detectors. The enantiomeric purity was analyzed by chiral HPLC and the amount of tin contamination by plasma spectrometry.

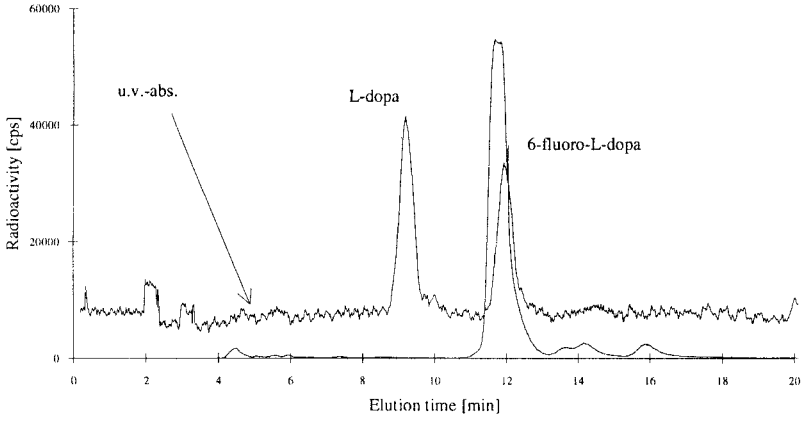


Fig 1. Semipreparative purification of crude reaction mixture.

The decay-corrected radiochemical yield of **1** was 7.8±2.1 % (n= 10) as calculated from the amount of [^{18}F]fluoride at EOB, i.e. starting from 165-270 mCi of [^{18}F]-fluoride we had 5.2-16.5 mCi of **1** at EOS. Total synthesis time was 55 min from EOB. The radiochemical purity of **1** exceeded 98 %

in all cases. The enantiomeric purity was > 99 %. The specific radioactivity was 27±13 mCi/μmol (EOS). The amount of tin contamination was checked from five preparations, and was found to vary between 5 - 12 ppb.

This method makes it possible to produce **1** with high purity, relatively high specific activity, acceptable yield and, more important to utilize [^{18}F]-fluoride as the radioactive precursor in the electrophilic synthesis of 6-[^{18}F]fluoro-L-dopa.

1. Bergman J., Källman K-M., Solin O. et al. - J. Nucl. Med. 34: 69P(1993)
2. Namavari M., Bishop A., Satyamurthy N. et al. - Appl. Radiat. Isot. 43 : 989(1992)
3. Luxen A., Perlmutter M., Bida G. T. et al. - Appl. Radiat. Isot. 41: 275(1990)
4. Schrecker A.W. and Hartwell J.L.-J. Am. Chem. Soc. 79: 3827(1957)

PHARMACOKINETICS OF NO-CARRIER-ADDED (NCA) AND CARRIER-ADDED (CA) 6-[^{18}F]FLUORO-L-DOPA : A COMPARISON.

REDDY,G.N.; HAEBERLI, M.; BEER, H.F.; and SCHUBIGER, P.A.
Division of Radiopharmacy and Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland.

INTRODUCTION:

6-[^{18}F]Fluoro-L-dopa (6-FDOPA) is a viable tracer to define the integrity of central dopaminergic system in patients with Parkinson's disease. Until recently it was produced with ^{18}F-labelled flourine gas generated by irradiation of low energy deutrons on neon and subsequent exchange with carrier flourine gas. However at PET (positron emission tomography) centers that have only low energy accelerated protons, production of ^{18}F-labelled flourine gas is a problem and hence of 6-FDOPA also. Alternatively, if 6-FDOPA could be produced with ^{18}F-labelled flouride which is easily available from proton induced nuclear reaction on ^{18}O-enriched water, then it would be accesible at most centers. Also this mode of production results in no-carrier-added (NCA) form with much higher specific activity. We have been successful in implementing the routine productions of NCA 6-FDOPA.[1] The advantages and disadvantages of NCA form are described here.

METHODS AND MATERIALS:

No-carrier-added (NCA) 6-FDOPA was produced with remote operation as per our publication.[1] Rigorous quality assurance and quality control methods were also developed for clinical applications in Positron Emission Tomographic (PET) investigations of Parkinson's patients.[2] The specific activity of NCA 6-FDOPA ranged from 700 to 1100 Ci / mmol (*vide infra*). Alternatively, carrier-added 6-FDOPA was produced at our Institute by the classical method.[3] The necessary ^{18}F-labelled flourine gas was generated by the nuclear reaction ^{20}Ne (p, x) ^{18}F with medium energy protons (40 - 25 MeV). A remotely operable "black box" was also built for routine productions. The specific activity of the carrier-added 6-FDOPA was calculated to range from 1-2 Ci / mmol. However, the *in vivo* data used for the comparison was taken from the literature.[4]

RESULTS AND DISCUSSION:

In vivo behavior of any tracer with either low or high specific activity would be expected to be similar, other wise the concept of "trace amount" to trace a biochemical pathway non-invasively would not be viable. If the mass of the tracer (with low specific activity) disturbs or gives different information of metabolic pathway under study, then its role as a tracer must be critically evaluated. In PET research next to FDG, 6-FDOPA is the second most extensively used ^{18}F-labelled tracer. Having succeedded in producing NCA 6-FDOPA, we compared its *in vivo* behavior with that of carrier-added 6-FDOPA reported in the literature.[4] Interestingly, the NCA (high specific activity) form exhibits a faster pharmacokinetics in comparison to the carrier-added form. A comparison of pharmacokinetics of these forms in plasma is given in Table I. The disappearance of 6-FDOPA and appearance of 3-OMeFDOPA is faster. However the formation of other metabolites which are sum of peripheral sulfoconjugation and central metabolites clearance is not much different. Curiously, the amount of NCA 6-FDOPA activity lost to red blood cells is significantly much more. Melega et al (private communication) report a loss of 3% activity to erythrocytes. However, our observation shows a loss of 30 to 40% with NCA form. Also, the time activity curves in plasma and blood do not parallel. Thus, there is a significant differential permeability of NCA form between blood and plasma, possibly because catechol-O-methyl transferase (COMT) enzyme, which is found bound to erythrocyte membranes, may behave differentially for the time frame of the PET study and the mass of 6-FDOPA . This finding may have a significant impact in estimating the true arterial input function. Also, an accurate determination of specific activity of NCA form was difficult because of technical limitation of conventional detectors. It varied from one production to other and therefore a reliable method of determining accurate specific activity

of each batch of production must be found. Otherwise, uptake of 6-FDOPA in striatum would be under- or over- estimated which would result in different k3 values. This finding reemphasizes once again the necessity of "trace" amount in non-invasive *in vivo* investigations as well as the effect mass of compounds with pharmacologically significant behavior.

CONCLUSION:

The NCA 6-FDOPA exhibits slightly different metabolic profile compared to the carrier-added form. This means if 6-FDOPA is used to measure the turnover of endogenous dopamine, several other considerations such as the transport, role of different regulatory enzymes, initial rate of appearance etc must be taken into account. If, on the other hand, it is used to evaluate the efficacy of L-dopa therapy or brain graftings, the mass effect must be taken into account.

Acknowledgment: Dr I. Guenther, Ms M. Psylla, Dr A. Antonini and Dr K.L.Leenders are thanked for their help in the evaluation of the tracer.

REFERENCES:

1. Reddy G.N.; Haeberli M.; Beer H.-F. and Schubiger P.A. Appl. Radiat. Isot. 44 : 645 (1993).
2. Reddy G.N.; Haeberli M.; Beer H.-F.; Hasler P. and Schubiger P.A. Appl. Radiat. Isot., in Press, (1993).
3. Luxen A., Perlmutter M., Bida G.T., Van Moffaert G., Cook J.S., Satyamurthy N., Phelps M.E.and Barrio J.R. Appl. Radiat. Isot., 41 : 275 (1990).
4. Melega, W.P., Hoffman J.M., Luxen A., Nissenson C.H.K., Phelps M.E., Barrio J.R. Life Sciences, 47 : 149 (1990).

EXPT	TIME	FDOPA	3-OMeFDOPA	OTHER METABOLITES
FDOPA	30	15%(30%)	43%(28%)	42%(42%)
	60	4%(18%)	55%(38%)	41%(44%)
	120	3%(8%)	67%(52%)	30%(10%)
FDOPA +CARBI	30	30%(42%)	63%(48%)	8%(10%)
	60	15%(25%)	79%(65%)	6%(10%)
	120	3.7 %(10%)	93.2%(78%)	3%(12%)

TABLE 1: COMPARISON OF PHARMACOKINETICS OF NCA (CA) FORMS OF 6-FLUORODOPA

Comparison of Bioavailability of (NCA) 6-[18F]Fluoro-L-dopa with Different Enzyme Inhibitors

GÜNTHER*, I.; PSYLLA*, M.; REDDY+, G.N.; ANTONINI*, A.; HÄBERLI+, M.; BEER+, H.-F. and LEENDERS*, K.L. Paul Scherrer Institute, *PET Program and +Div. of Radiopharmacy, CH-5232 Villigen PSI, Switzerland

Introduction

The tracer 6-[^{18}F]fluoro-L-dopa (6-FD) is used as an analog of L-DOPA to assess the functional integrity of the central dopaminergic system *in vivo* with positron emission tomography (PET). Unlike endogenous, but like exogenously administered DOPA, 6-FD is metabolized peripherally by three major enzymes: catechol-O-methyltransferase (COMT), aromatic amino acid decarboxylase (AAAD) and monoamine oxidase (MAO). An improvement in the bioavailability of 6-FD could be achieved by manipulating the activities of the above enzymes with different inhibitors. CGP 28014 (CGP), Ro 40-7592 (Ro) and OR 611 (OR) are COMT inhibitors with peripheral activity. We studied their effect on the metabolic profiles of (NCA) 6-FD in a Rhesus monkey in combination with or without the AAAD inhibitor Carbidopa. The aim of these studies was to obtain an ideal combination of different enzyme inhibitors so as to improve 6-FD (hence of L-DOPA in therapy) bioavailability to brain.

Materials and Methods

Studies were performed in a female Rhesus monkey weighing 6.5 kg. Anaesthesia was induced with intramuscular injections of ketamine, diazepam and atropine and maintained with intravenous injections of pentobarbital. Carbidopa (5 mg/kg) was given i.v. 60 minutes and the COMT inhibitors (30 mg /kg for CGP, OR and Ro i.v. or p.o. and 10 mg /kg p.o. for Ro) 1-2 hours before the tracer application (1.5-5 mCi i.v.). NCA 6-FD was produced according to a previously published procedure.(1) Over a 2 hour period arterial plasma samples were collected and assayed by HPLC using a sensitive γ coincidence detector. The tracer derived activity was normalized for administered dose and body weight.

Results and Discussion

The effectiveness of inhibition was monitored by the formation of 3-O-methylfluorodopa (3OMFD). Blocking of AAAD action with carbidopa resulted in a slower disappearance of 6-FD and in a higher formation of 3OMFD compared to baseline study.(2) Adequate inhibition of AAAD was obtained when carbidopa was given 60 minutes before 6-FD application. This condition was used in all COMT inhibitor studies. COMT inhibition was dependent on time, dose and mode of administration. CGP more effectively slowed the appearance of 3OMFD when given orally than i.v. (Fig. 1). OR was more effective given orally 90 minutes than 40 minutes before tracer application (Fig. 2). Ro inhibited very potently the formation of 3OMFD either given intravenously or orally (Fig. 3a).(3) Ro, when used orally without carbidopa, resulted in less formation of 3OMFD, because of reduced bioavailability of 6-FD (Fig. 3b). However, this led to a slightly higher formation of fluorodopamine sulfate (data not shown). Of the three tested substances Ro gave the best COMT inhibition (Fig. 4a,b).

Conclusion

Our study indicates that bioavailability of 6-FD (and thus by implication L-DOPA) is considerably improved with effective COMT inhibition in conjunction with carbidopa.

References

1. Reddy G.N., Häberli M., Beer H.-F. and Schubiger A.P.- Appl. Radiat. Isot. 44: 645(1993)
2. Melega W.P., Hoffman J.M., Luxen A., Nissenson C.H.K., Phelps M.E. and Barrio J.R. - Life Science 47: 149(1990)
3. Zürcher G., Colzi A. and Da Prada M. - J. Neural. Transm. [Suppl.] 32: 375(1990)

Fig. 1 Influence of Carbidopa and CGP on 3OMFD Formation

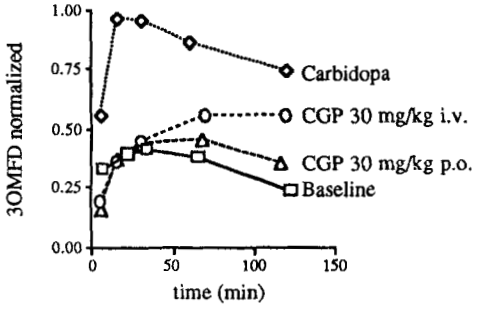


Fig. 2 Influence of Carbidopa and OR (30 mg/kg p.o.) on 3OMFD Formation

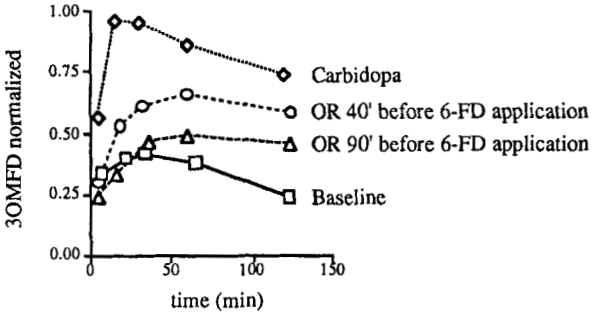


Fig. 3a Influence of Ro with or without Carbidopa on 3OMFD Formation

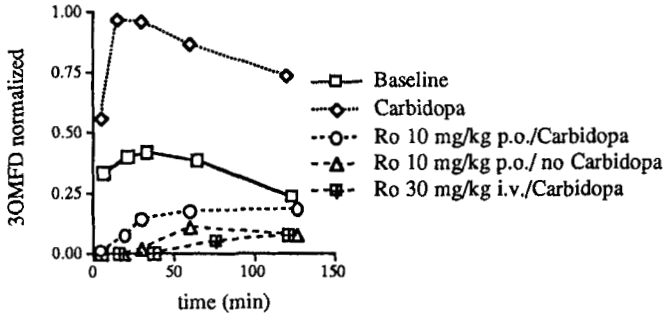


Fig. 3b Influence of Ro with and without Carbidopa on 6-FD Bioavailability

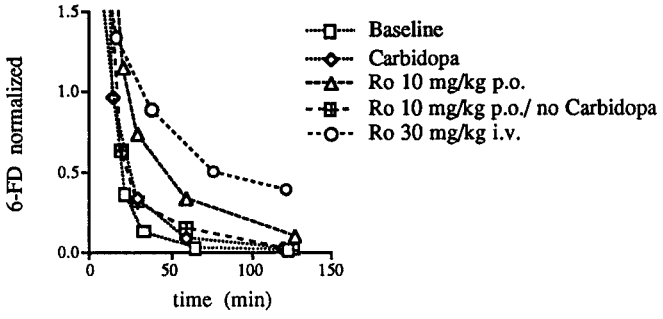


Fig. 4a Comparison of 3OMFD Formation with CGP, Ro and OR

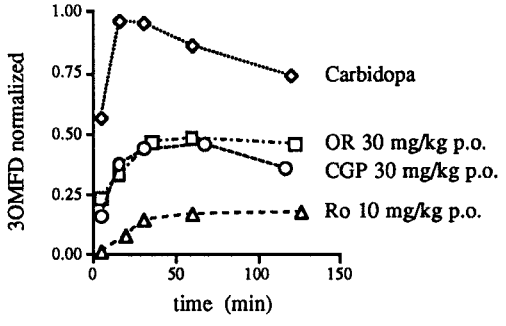
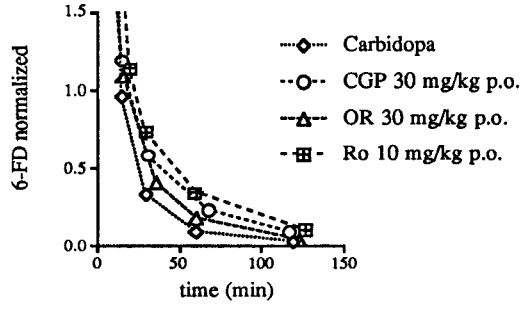


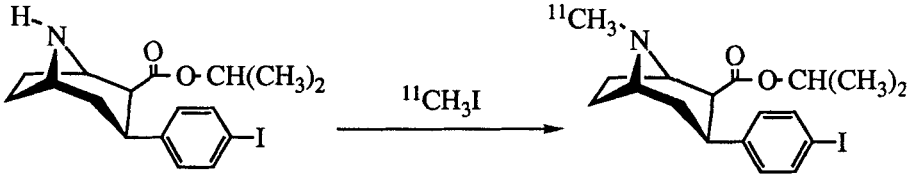
Fig. 4b Comparison of Bioavailability of 6-FD with CGP, Ro and OR



[^{11}C]RTI-121 - A Potential Radioligand for PET Studies of the Dopamine Transporter

BROWN, D.J.; LUTHRA*, S.K.; BROWN, G.D.; CARROLL[1], F.I.; KUHAR[2], M.J.; OSMAN, S.; WATERS, S.L. and BRADY, F. MRC Cyclotron Unit*, Hammersmith Hospital, Ducane Road London W12 OHS ; [1]Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, NC 27709; [2]Neuroscience Branch, Addiction Research Center, National Institute of Drug Abuse, Baltimore MD 21224.

Recently, a series of 3β-substituted phenyltropane analogs having high affinity and selectivity for the dopamine transporter have been identified[1,2]. Two of these analogs, 3β-(4-iodophenyl)tropan-2-carboxylic acid methyl ester (RTI-55 or β-CIT)[3] and 3β-(4-fluorophenyl)tropan-2-carboxylic acid methyl ester (CFT)[4] have been labelled with carbon-11. RTI-55 has also been labelled with iodine-123[5-7] and iodine-125[8]. Although RTI-55 has high affinity compared to CFT, it is not selective for the dopamine transporter in non-human primates[7]. Based on *in vitro* studies[2] and SPECT studies in baboons with ^{123}I-labelled compounds[9], 3β-(4-iodophenyl)tropan-2β-carboxylic acid isopropyl ester (RTI-121) has been suggested as a more selective compound for studying the dopamine transporter. Consequently, we have chosen [^{11}C]RTI-121 (II) as a potential radioligand for studying the dopamine transporter by PET. Labelling has been achieved by *N*-methylation of the desmethyl precursor (I) using [^{11}C]iodomethane.



(I) (II)

[^{11}C]iodomethane was prepared from [^{11}C]carbon dioxide using a remotely controlled apparatus. [^{11}C]Iodomethane was distilled into a vial containing the desmethyl precursor (0.5 mg) (I) in DMF (200 µl). The reaction mixture was heated at 90 ^{0}C for 5 min and then diluted with water (5.0 mL) and injected onto a sample enrichment C_{18}-column (SEC). The SEC column was eluted with water for 3.0 min at a flow rate of 2.2 mLmin^{-1} and then switched in line with the HPLC column (µ-Bondapak C_{18}, 300 x 7.8 mm i.d.). The HPLC column was eluted at 3.0 mLmin^{-1} with a mixture of MeCN and 0.07M KH_2PO_4 [55:45] adjusted to pH 7.0 with triethylamine. [^{11}C]RTI-121 eluted between 12-14 min and the desmethyl precursor eluted between 18-24 min. The fraction containing [^{11}C]RTI-121 was collected in a flask containing ascorbic acid (100 µL, 5.7 µmol). The solvent was removed by rotary evaporation and the residue dissolved in isotonic saline (9.5 mL) containing ethanol (0.5 mL) and millipore filtered. The pH of the formulated product was 6-6.5. The radiochemical yield was ca.95% from [^{11}C]iodomethane. Typical activities achieved at the end of synthesis were in the range 70 to 90 mCi at 40 min after EOB.

The formulated product was analysed using a reverse phase HPLC column (Phenomonex Bondeclone, 10 µm, 250mm x 3.9mm i.d.). The analytical column was eluted at 1.5 mLmin^{-1} with a mixture of MeOH and 0.07M KH_2PO_4 adjusted to pH 7.0 with triethylamine [70 : 30]. The mobile phase was monitored for radioactivity and UV absorbance (232 nm). [^{11}C]RTI-121 eluted at 6.5 min. The radiochemical and chemical purity was > 97%. Typical specific activity was in the range of 20 - 38 GBqµmol^{-1} corresponding to ca. 30 µg of stable RTI-121 in the formulated sample. A sample of the formulated radioactive product examined by mass

spectrometry (CI +ve mode) gave m/z = 414 $[M + H]^+$, corresponding to RTI-121.

For validation of the radiosynthesis, a sample of [$^{11/13}$C]RTI-121 was prepared using [^{11}C]iodomethane and [^{13}C]iodomethane (90 atom %, 1 μL) and the product analysed after decay by mass spectrometry (CI +ve mode) and ^{13}C-NMR . The mass spectrum showed m/z = 415 $[M + H]^+$, corresponding to [^{13}C]RTI-121. Proton decoupled ^{13}C-NMR (CD_3OD) showed a single peak at 41.1 ppm corresponding to N-Me position in authentic RTI-121.

[^{11}C]RTI-121 was found to be unstable (ca. 20% impurity at 40 min), possibly as a result of hydrolysis of the ester group, when formulated in saline alone (pH 6.0). Formulation as the hydrochloride failed to overcome this instability, however stability was conferred by adding ascorbic acid. [^{11}C]RTI-55 similarly labelled in the *N*-methyl position with [^{11}C]iodomethane exhibited similar problems of stability.

Biological evaluation of [^{11}C]RTI-121 and comparison with [^{11}C]RTI-55 is currently being carried out.

References

1. Carroll F.I., Abraham P., Lewin A.H., Parham K.A., Boja J.W. and Kuhar M.J. - J. Med. Chem. 35: 2497(1992).
2. Boja J.W., McNeill R.M., Lewin A.H., Abraham P., Carroll F.I. and Kuhar M.J. - Neuroreport 3: 984(1992).
2. Muller L., Halldin C., Farde L., Karlson P., Hall H., Swahn C.-G., Neumeyer J., Gao Y. and Milius R. - Nucl. Med. Biol. 20: 249(1993)
3. Hantraye P., Brownell A.-L., Elmaleh D., Spealman R.D., Wullner U., Brownell G.L., Madras B.K. and Isacson O. - Neuroreport 3: 265(1992).
4. Carroll F.I., Rahman M.A., Abraham P., Parham K., Lewin A.H., Dannals R.F., Shaye E., Scheffel U., Wong D.F., Boja J.W. and Kuhar M.J. - Med. Chem. Res. 1: 289(1991).
5. Neumeyer J.L., Wong S., Milius R.A., Baldwin R.M., Zea-Ponce Y., Hoffer P.B. Sybirska E., Al-Tikriti M., Charney D.S., Malison R.T., Larielle M. and Innis R.B. - J. Med. Chem. 34, 3144(1991).
6. Laruelle M., Baldwin R.M., Malison R.T., Zea-Ponce Y., Zoghbi S., Al-Tikriti M.S., Sybirska E.H., Zimmermann R.C., Wisniewski G., Neumeyer J.L., Milius R.A., Wang S., Smith E.O., Roth R.H., Charney D.S., Hoffer P.B and Innis R.B. - Synapse 13: 295(1993).
7. Boja J.W., Mitchell W.M., Patel A., Kopajtic T.A., Carroll F.I., Lewin A.H., Abraham P. and Kuhar M.J. - Synapse 12: 27(1992).
8. Scheffel U., Dannals R.F., Wong D.F., Yokoi F., Carroll F.I. and Kuhar M.J. - Neuroreport 3: 969(1992).

Synthesis of [^{11}C]*dl-threo*-Methylphenidate: Drug Pharmacokinetics and Binding to the Presynaptic Dopaminergic Neuron

Y.-S. Ding, J. S. Fowler, N. D. Volkow, S. J. Gatley, S. Dewey, T. Martin, D. Jenkins, D. Alexoff, C. Salata and A. P. Wolf, Brookhaven National Laboratory, Upton, New York 11973.

Methylphenidate (Ritalin) is a mild central nervous system stimulant used primarily in the treatment of attention-deficit hyperactivity disorder (ADHD) [1]. Its greater specificity for dopaminergic neuronal systems makes it a preferred psychostimulant over amphetamine, which affects noradrenergic and serotonergic neurons as well [2]. The psychostimulant and therapeutic properties of methylphenidate are thought to be mediated by its binding to a site on the dopamine transporter, resulting in inhibition of dopamine reuptake and enhanced levels of synaptic dopamine. Unlike amphetamine, but like cocaine, its behavioral and dopamine enhancing effects are sensitive to reserpine and calcium influx suggesting that increases in extracellular dopamine are vesicular in origin [3, 4, 5].

Methylphenidate (MP) has two chiral centers and is marketed as the *dl-threo* form. However, it is known that the pharmacological specificity for the dopamine transporter and its pharmacological activity resides almost entirely in the *d*-enantiomer [6]. The potency (IC_{50}) of *dl-threo*-MP in displacing [^{3}H]*dl-threo*-MP from striatal synaptosomal membrane binding sites is 0.21 μM as compared to 0.088 for the *d* -enantiomer and 1.2 for the *l*-enantiomer [7]. The primary fate of methylphenidate in vivo is metabolism to ritalinic acid.

We report here the synthesis of [^{11}C]*dl-threo*-methylphenidate ([^{11}C]MP) along with PET studies in baboons. This study was undertaken (1) to examine the pharmacokinetics of this widely used therapeutic drug; (2) to provide the pharmacokinetic framework for our recent studies using MP challenge and PET to assess the functional activity of the presynaptic dopaminergic neuron [8] and (3) to evaluate the suitability of [^{11}C]MP as a radiotracer for assessing the presynaptic dopaminergic transporter and its sensitivity to changes in synaptic dopamine in comparison with [^{11}C]cocaine and other labeled compounds binding to the dopamine transporter.

Our first approach to [^{11}C]*d,l-threo*-MP involved direct alkylation of ritalinic acid (obtained from Ciba Geigy Corp.) with [^{11}C]H_3I. However, the potential for both N and O alkylation of the ritalinic acid precursor required that a suitable N-protected derivative be developed. The o-nitrophenylsulfenyl group (NPS) was chosen as the protective group because N-NPS derivatives can easily be prepared from either free amino acids or alkyl esters of amino acids and can be removed rapidly under very mild conditions without affecting the ester functional group [9] (Scheme 1). Thus, [^{11}C]MP was prepared in two steps: O-methylation of the N-protected ritalinic acid derivative with [^{11}C]H_3I followed by hydrolysis (Scheme 2). The subsequent C18 sep-pak and reverse-phase HPLC purification resulted in ca. 40% radiochemical yield (from EOB) with a total synthesis time of 40 minutes and a specific activity of 800-1500 Ci/mmole.

Positron emission tomographic (PET) studies with [^{11}C]MP in baboon demonstrated high regional uptake in striatum. Peak uptake (0.04%/cc) occurred at 5-15 minutes. The half time for clearance from peak uptake for [^{11}C]MP was 60 minutes

and the ratio between the radioactivity in the striatum and that in the cerebellum (ST/CB) was 2.2-2.6 at 40 minutes. Though the regional distribution and peak uptake for [^{11}C]MP and [^{11}C]cocaine are similar, [^{11}C]cocaine peaks and clears from striatum and cerebellum more rapidly in comparative studies carried out in baboon. For example, the clearance halftime from peak for labeled cocaine is about 25 minutes [10]. Time-activity curves for [^{11}C]MP and [^{11}C]cocaine in basal ganglia and cerebellum are shown in Fig. 1 and 2.

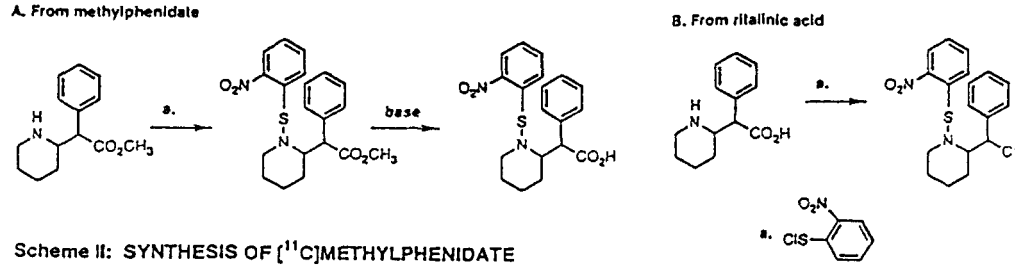
In serial studies on the same baboon, pretreatment with unlabeled methylphenidate (0.5 mg/kg) or GBR12909 (1.5 mg/kg) 30 min prior to [^{11}C]MP injection markedly reduced the striatal but not the cerebellar uptake of [^{11}C]MP, demonstrating the specific binding of [^{11}C]MP to the dopamine transporter in the brain. In both cases, the ratio index (ST/CB) after pretreatment was reduced to 1. The results from the experiment after GBR 12909 pretreatment are shown in Fig.3 and 4.

In summary, we have carried out the first PET studies of methylphenidate, a drug which is widely used in the treatment of hyperactive children. The availability of this labeled drug makes it possible to examine drug pharmacokinetics in the human brain and to optimize drug dosing and timing in the therapeutic application of methylphenidate and in research protocols involving methylphenidate challenge. In addition, the observation of specific binding to dopamine transporter supports further study of *dl-threo*-[^{11}C]methylphenidate and of its labeled active and inactive enantiomers as radiotracers for the presynaptic dopaminergic neuron. Studies are underway to synthesize and evaluate the labeled *d* and *l*-enantiomers of methylphenidate, to determine the selectivity of [^{11}C]methylphenidate binding to the dopamine transporter and its sensitivity to changes in synaptic dopamine and neuronal loss.

Supported by DOE/OHER, NINDS and NIDA.

1. Barkley R.—*J. Child Psychol. Psychiatry Allied Discip.* **18:**137 (1977).
2. Weiner J.--Pharmacologyof CNS stimulants in drug abuse: Proceedings of International Conference, Zarafonetis, C.J. ,ed., Lea and Febiger, Philadelphia, pp 243-251 (1972).
3. Hurd Y. L. Ungerstedt, U.—*synapse* **3:**48 (1989).
4. Braestrup C.—*J. Pharm. Pharmac.* **29:**463 (1977).
5. Chiueh C. C. Moore K.E.—*J. Pharm. Exp. Ther.* **192:**642 (1975).
6. Patrick K. S. Caldwell, R.W., Ferris, R.M., Breese, G.R.—*J. Pharm.Exp. Ther.* **241:**152 (1987).
7. Schweri M. M. Skolnick, P., Rafferty, M.F., Rice, K.C., Janowsky, A.J., Paul, S.M.—*J. Neurochem.* **45:**1062 (1985).
8. Volkow N. D. Fowler, J.S., Wang, G.-J., Schlyer, D.—*J. Nucl. Med.* **34:**67 (1993).
9. Zervas L. Borovas, D., Gazis, E.—*J. Am. Chem. Soc.* **85:**3660 (1963).
10.Fowler J. S., Volkow N. D., Wolf A. P., Dewey S. L., Schlyer D. J. and MacGregor R. R.—*Synapse* **4:**371 (1989).

Scheme I: SYNTHESIS OF PRECURSOR TO [^{11}C]METHYLPHENIDATE



Scheme II: SYNTHESIS OF [^{11}C]METHYLPHENIDATE

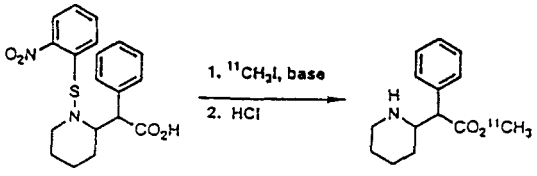


Fig. 1: [^{11}C]METHYLPHENIDATE IN BABOON

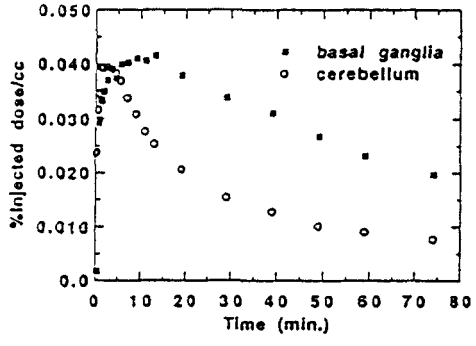


Fig. 2: [^{11}C]COCAINE IN BABOON

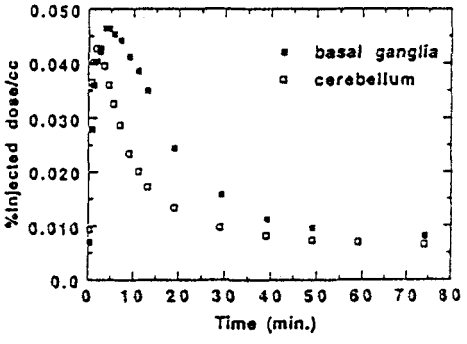


FIG. 3: [^{11}C]METHYLPHENIDATE IN STRIATUM AFTER GBR12909 PRETREATMENT

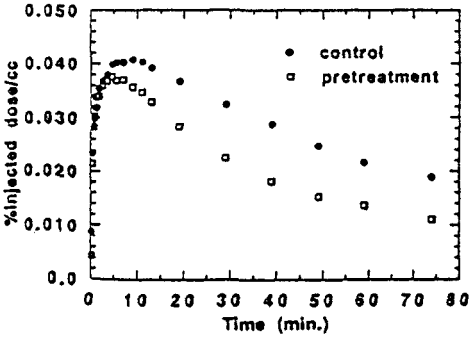
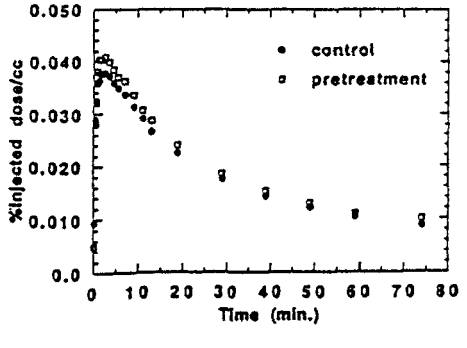


FIG. 4: [^{11}C]METHYLPHENIDATE IN CEREBELI AFTER GBR12909 PRETREATMENT



Synthesis of N-3-[^{18}F]Fluoropropyl-2ß-Carbomethoxy-3ß-(4-Chlorophenyl)Tropane: A High Affinity Neuroligand to Map Dopamine Reuptake Sites by PET

GOODMAN, M.M.; KABALKA, G.W.; KUNG, M.P.; KUNG, H.F.; and MEYER, M.A.
Biomedical Imaging Center, University of Tennessee at Knoxville, Knoxville, TN 37920 USA

Cocaine is one of the most powerful positive reinforcing psychoactive drugs. The binding properties of (-)cocaine and a variety of analogs have been extensively evaluated to delineate the neurochemical mechanism responsible for the reinforcing properties of this drug (1). Cocaine has been hypothesized to bind to the presynaptic dopamine transporter which results in the inhibition of the reuptake of dopamine. The dopamine transporter plays a pivotal role in regulating dopamine transmission. For this reason, the development of radioligands labeled with gamma emitting isotopes which exhibit pronounced brain uptake, very high selectivity and affinity for the transporter would be excellent probes to study the relationship between alterations in presynaptic dopamine binding and neurodegenerative diseases such as Parkinson's disease.

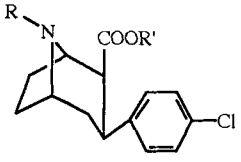
We have developed a new radioligand iodine-123 labeled 2ß-carbomethoxy-3ß-(4-chlorophenyl)-8-(3E-iodopropen-2-yl)nortropane which exhibits high uptake and retention of radioactivity in striatum of rats (0.6% dose/g at 120 min.) and primates with high striatum to cerebellum ratios, 16.5:1 and 5:1 respectively. These results clearly demonstrate that bulk tolerance exists at the 8-aza position of this molecule (2). Because of the higher resolution (6-8 mm) currently available with PET in comparison with SPECT (15-18 mm) imaging we performed competitive binding studies on a series of 8-substituted fluoroalkyl 2ß-carbomethoxy-3ß-(4-chlorophenyl)nortropanes to determine whether an analog was suitable for labeling with fluorine-18 for PET studies. The competitive binding data using [^{125}I]RTI-55 are shown in Table 1. The results from the binding studies demonstrate that 2ß-carbomethoxy-3ß-(4-chlorophenyl)-8-(3-fluoropropyl)nortropane is a potent inhibitor of [^{125}I]RTI-55 and prompted us to synthesize fluorine-18 labeled 2ß-carbomethoxy-3ß-(4-chlorophenyl)-8-(3-fluoropropyl)nortropane for evaluation as a potential PET presynaptic dopamine reuptake site imaging agent.

2ß-Carbomethoxy-3ß-(4-chlorophenyl)-8-(3-fluoropropyl)nortropane (Figure 1) was synthesized by treatment of 2ß-carbomethoxy-3ß-(4-chlorophenyl)nortropane with 1-bromo-3-fluoropropane in acetonitrile at 90°C. Fluorine-18 labeled 2ß-carbomethoxy-3ß-(4-chlorophenyl)-8-(3-fluoropropyl)nortropane was prepared by treatment of 1-bromo-3-trifluoromethanesulfonylpropane with NCA K^{18}F/K222 in acetonitrile at 90°C for 5 min. followed by filtration through a silica seppak and subsequent treatment with the nor substrate in acetonitrile at 90°C for 60 min. The fluorine-18 labeled 2ß-carbomethoxy-3ß-(4-chlorophenyl)-8-(3-fluoropropyl)nortropane was obtained in 25% radiochemical yield (EOB).

References

1. Carroll, F.I., Lewin, A.H., Boja, J.W., Kuhar, M.J. - J.Med.Chem. 35: 969-981 (1992)
2. Goodman, M.M., Kabalka, G.W., Longford, C.P.D., et al. - J.Nucl.Med. 33: 890 (1992)
3. Goodman, M.M., Kabalka, G.W., Kung, M.P., Kung, H.F., et al. - J.Med.Chem. (in press)

Table 1. Inhibition of 8-Substituted Fluoroalkyl 2ß-Carboalkoxy-3ß-(4-chlorophenyl)Nortropanes on the Binding of [^{125}I]RTI-55 to Rat Striatal Homogenates



R	R'	Ki (nM)
$CH_2CH{=}CH(E)I$	CH_3	3.57
CH_3	i-C_3H_7	7.75
CH_2CH_2F	CH_3	32.4
$CH_2CH_2CH_2F$	CH_3	8.2
CH_2CH_2F	i-C_3H_7	132
$CH_2CH_2CH_2F$	i-C_3H_7	42.9

0.2-0.5 nM of [^{125}I]RTI-55 was incubated with rat striatal homogenates and 7-11 concentrations of compounds. Values are from 2-3 independent determination in duplicate.

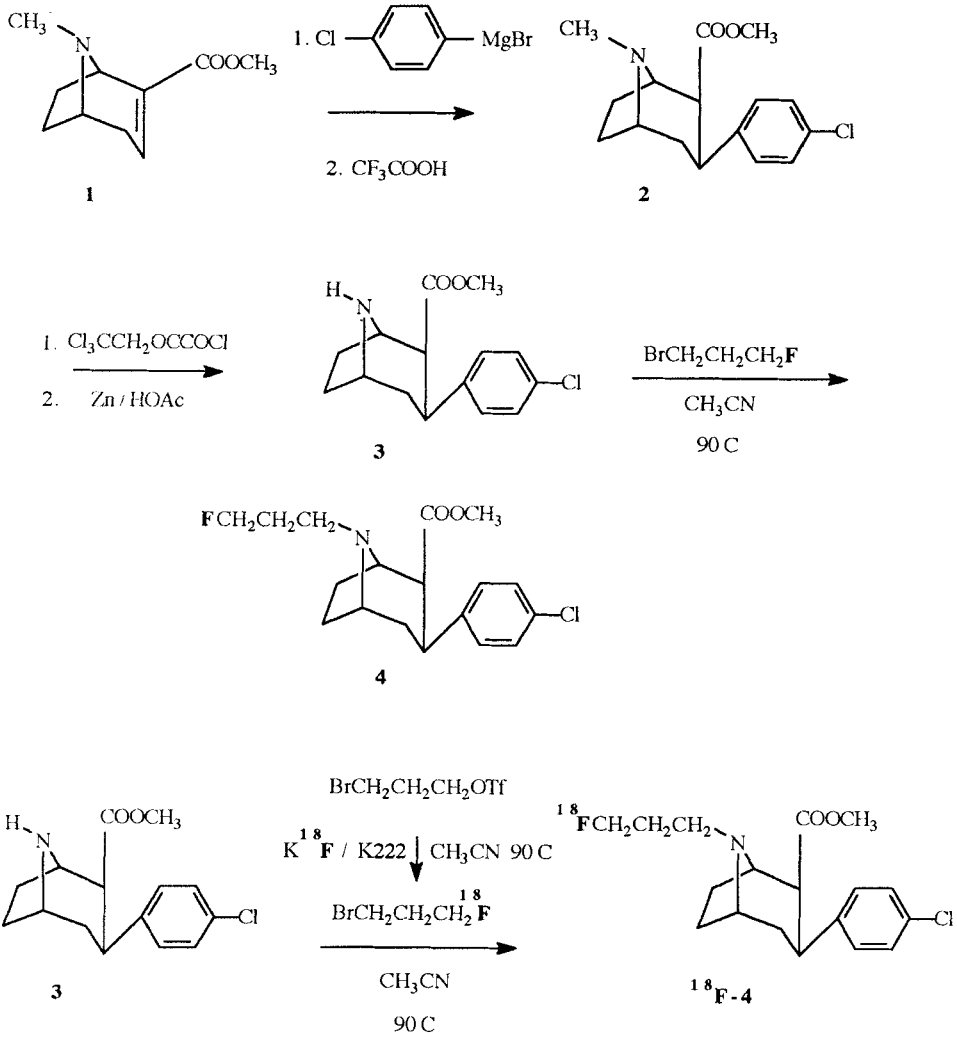


Figure 1 . Synthesis of N-3-[^{18}F]fluoropropyl-2ß-carbomethoxy-3ß-(4-chlorophenyl)tropane

Radiosynthesis and Evaluation of E and Z N-Iodoallyl-2b-Carbomethoxy-3b-(4-fluoropheny (I) Tropane (IACFT)

ELMALEH, D.R., BYON, C., FISCHMAN, A.J., HANSON R.N., MELTZER, P. AND MADRAS, B.K. Department of Radiology, Massachusetts General Hospital, Boston, MA, Northeastern University, Boston, MA and Harvard Medical School, Boston, MA

2B-carbomethoxy-3B-(4-fluorophenyl)tropane (CFT) is a cocaine analog that was found to have high affinity for and selectivity to the dopamine transporter sites. CFT inhibits the uptake of dopamine and other monoamines. It has been shown that the binding specificity of ^{3}H-CFT is similar to that of the dopamine transporter in human and nonhuman primate caudate and putamen. Our work with ^{11}C-labeled CFT has demonstrated the excellent properties of this cocaine analog for measuring dopamine terminals with PET.

Following the development of the tritiated and ^{11}C-labeled CFT, 2-carbomethoxy-3-(4-iodophenyl)tropane (RTI-55) was synthesized and radiolabeled. This compound showed a tenfold higher affinity (IC_{50} = 1.5 nmol) than CFT (IC_{50} = 17 nmol) for the cocaine sites in rodent striatum. RTI-55 autoradiography studies in monkey brain revealed high concentrations in dopamine-and serotonin-rich regions (1). The in-vitro binding studies and in-vivo imaging studies indicated that both CFT and RTI-55 are suitable as imaging probes for the dopamine nerve terminals. A recent study by Kaufman and Madras (1) comparing autoradiography with tritiated CFT and RTI-55 showed that both compounds concentrate in the dopamine-rich brain regions; however, RTI-55 accumulates in serotonin-rich brain regions as well as the cortex and thalamus to a greater extent than tritiated CFT. Both probes and possibly other congeners may be useful for studying the cocaine recognition sites in dopamine nerve terminals, striatum and other brain regions. Since RTI-55 shows a higher accumulation in serotonin-rich regions, and 2-carbomethoxy-3-(4-chlorophenyl)tropane CCT behaves similarly, we decided not to significantly alter the aromatic ring in CFT but to derivatize the molecule on the nitrogen (less sensitive to selectivity and does not alter affinity significantly) (1-5). We developed the synthesis for the E isomer of N-iodoallyl-2β-carbomethoxy-3β-(4-fluorophenyl)tropane (IACFT) as a radioiodinated version for SPECT (scheme 1). Our studies indicated that IACFT has higher affinity to the dopamine transporter sites (IC_{50} = 5.7 nmol) than CFT and better selectivity than RTI-55 (no uptake was observed in cortex or thalamus).

Goodman et al. have reported on two iodoallyl CCT isomers. The CCT isomers were chosen because we reported that CCT has higher affinity than CFT (6). The nonspecific binding to the presynaptic serotonin sites that we obtained in our more recent studies in which ^{11}C-CCT showed high accumulation in serotonin-rich regions such as the cortex, thalamus and caudate-putamen was not anticipated (Table 1). We observed that a symptomatic parkinsonian MPTP-treated monkey with no caudate-putaman activity (due to >95% decrease in dopamine fiber) when imaged with ^{11}C-CFT had high accumulation in serotonin-rich regions such as the cortex, thalamus and caudate-putamen.

The uptake could not be associated with the dopamine regions. It is our conclusion that the higher affinity of ligands like GBR, RTI-55 and CCT does not necessarily increase selectivity. On the contrary, in these cases the nonspecific binding to other sites (serotonin) increased and therfore made the compound less useful for this specific task.

Therefore, the optimal structure for this series is CFT, with fluorine in the para position, as the basic structure for radioiodination. Our results show that this approach gives a very potent and selective compound.

1. Kaufman M.J. and Madras B.K. Synapse, 12: 99-111(1992).

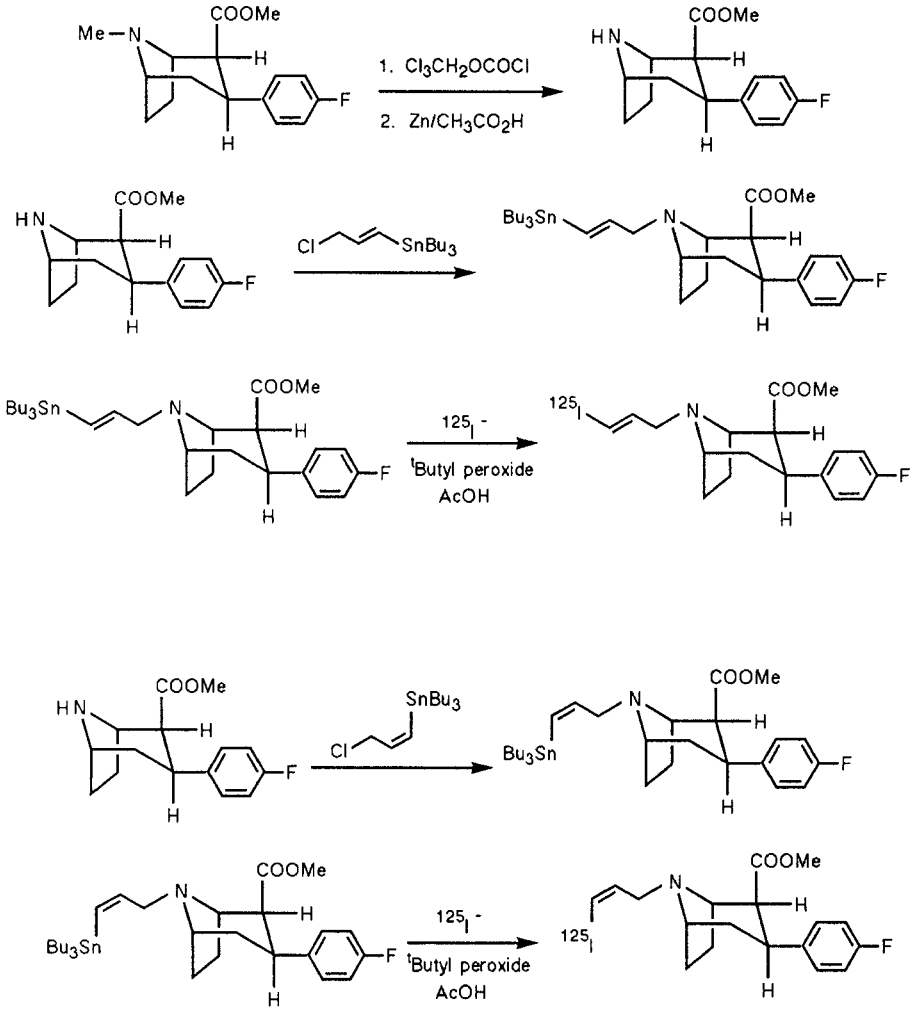
2. Mitchell WM, Boja JW, Cline EJ, Lew R, Carroll FI, Lewin A and Kuhar MJ. . Soc. Neuroscien, Abstrat, 17: 155(1991).
3. Carroll FI, Rahman MA, Abraham P, Parham K, Lewin AH, Dannals RF, Shaya E, Scheffel U, Wong DF, Boja JW, and Kuhar NJ. Med Chem Res, 289-294(1991).
4. Laruella RM, Baldwin Y, Zea-Ponce Y, Zoghbi SS, Al-Tikriti MS, Sybirska EH, Nalison RT, Neumeyer JL, Milius R, Wong S, Smith EO, Wisniewski G, Charney DS, Hoffer, PB, Innis RB. J Nucl Med, 33: 945(1992).
5. Boja JW, Patel A, Carroll FI, Rahman MA, Philip A, Lewin AH, Kopajtic TA, Kuhar MJ. Eur J Pharmacol, 194: 133-134(1991).
6. Goodman MM, Kabalka, GW, Collier TL and Longford CPD. J Nucl Med, 33: 890(1992).

Table 1

Potencies of Cocaine Analogs for Inhibiting Binding of ^{3}H-CFT to primate caudate-putaman

Compound	R_1	R_2	R_3	IC_{50} (nM)
1 (CFT, Win 35,428)	H	F	CH_3	11.0 ± 1
2 (CCT)	H	C	CH_3	1.40 ± 0.035
3 (Win 35,065-2)	H	H	CH_3	65 ± 12
4 (RTI-55)	H	I	CH_3	1.08 ± 0.06
5 (IACFT)	H	F		5.70 ± 1.00
6 ((-) Cocaine)				95.6 ± 14.4

Synthesis and Radiolabeling of [I-125]-N-Iodoallyl-2β-carbomethoxy-3β-phenyltropane



PREPARATION OF [PROPYL-^{11}C]OSU 191, A HIGHLY POTENT AND SELECTIVE 5-HT$_{1A}$ AGONIST FOR PET.

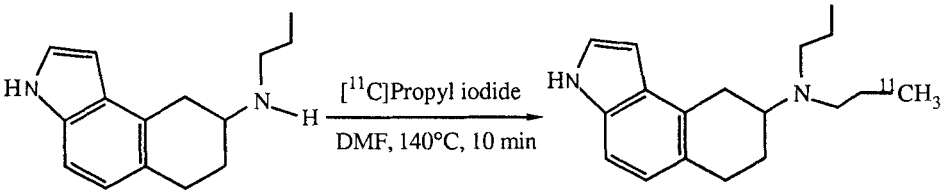
C. Halldin, H. Wikström, C.-G. Swahn, G. Sedvall P., Stjernlöf and L. Farde. Department of Psychiatry and Psychology, Karolinska Institute, Stockholm, Sweden, University Centre for Pharmacy, University of Groningen, Groningen, The Netherlands and Department of Pharmacology, Göteborg University, Göteborg, Sweden.

In the search for orally active bio-mimetics of the widely used 5-HT$_{1A}$ agonist 8-OH-DPAT, different heterocycles have been studied. One of them, OSU 191 (1-formyl-6,7,8,9-tetrahydro-benz[e]indol-8-(N,N-dipropyl)amine) is a highly potent and selective 5-HT$_{1A}$ agonist (IC$_{50}$ (nM) = 1.2 (5-HT$_{1A}$), >1800 (D-2)) (1). As OSU 191 contains two N-propyl groups we used [^{11}C]propyl iodide (2) as the labelling precursor to prepare [propyl-^{11}C]OSU 191.

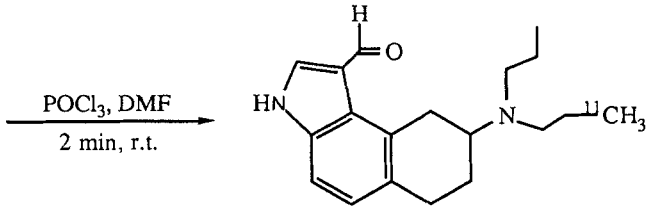
The first approach examined was a two-step synthesis starting from [^{11}C]propyl iodide (Scheme 1). The free base of the secondary amine of the despropyl "indolic" aminotetralin (OSU 1032) was labelled by N-alkylation with [^{11}C]propyl iodide using thermal heating. Reaction in DMF at 140°C for 10 min resulted in 17% radiochemical incorporation. Then, the 1-formylated analog ([propyl-^{11}C]OSU 191) was accomplished by a Wilsmeyer formulation using $POCl_3$ in DMF at r.t. for 2 min in 60-90% yield. Subsequent reversed-phase semi-preparative HPLC purification resulted in a total radiochemical yield of about 10% (from EOB and decay-corrected) with a total synthesis time of 50-60 min and a radiochemical purity >99%.

When the appropriate precursor (OSU 1120) was available a second labelling approach was tested which consists of a one-step synthesis starting from [^{11}C]propyl iodide (Scheme 2). The despropyl 1-formylated aminotetralin (OSU 1120) was labelled directly by N-alkylation of the free base of the secondary amine with [^{11}C]propyl iodide using either thermal heating or microwave heating conditions. By varying the reaction conditions we found that reaction in DMF with $NaHCO_3$ at 140°C for 10 min resulted in 12% radiochemical incorporation, whereas microwave heating (4x2 min, 600 Watt) in DMF with addition of both $NaHCO_3$ and NaI resulted in 20% radiochemical incorporation. Reversed-phase semi-preparative HPLC purification resulted in a total radiochemical yield of 12-15% (from EOB and decay-corrected) with a total synthesis time of 40-50 min and a radiochemical purity >99%.

1. Stjernlöf P., Gullme M., Elebring T., Andersson B., Wikström H., Lagerquist S., Svensson K., Ekman A., Carlsson A. and Sundell S. - J. Med. Chem. (in press) (1993).
2. Långström B., Antoni G., Gullberg P., Halldin C., Någren K., Rimland A. and Svärd H. - Appl. Radiat. Isot. 40: 557 (1986).

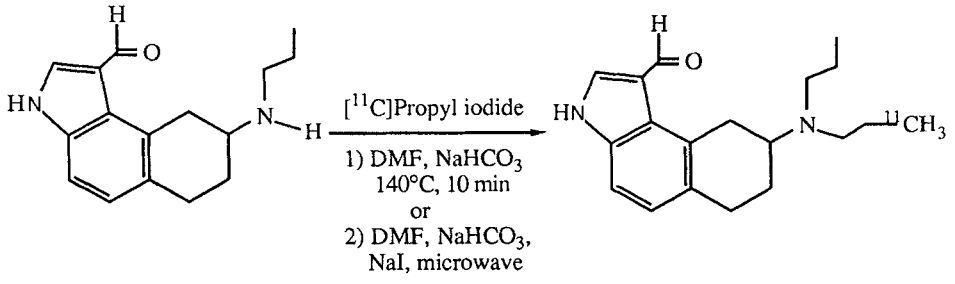


OSU 1032 **[Propyl-^{11}C]OSU 190**



[Propyl-^{11}C]OSU 191

Scheme 1.



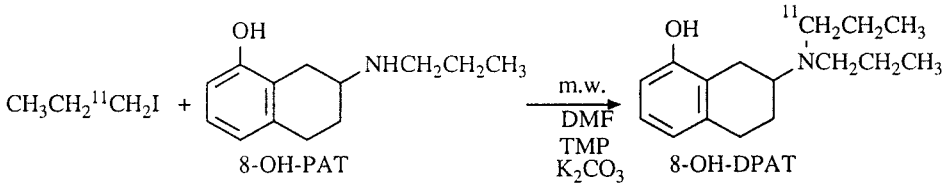
OSU 1120 **[Propyl-^{11}C]OSU 191**

Scheme 2.

Microwave-Induced Synthesis of [N-Propyl-^{11}C]-8-hydroxy-2-(di-N-propylamino)tetralin

THORELL, J-O.[a,b], STONE-ELANDER, S.[a,b] and INGVAR, M.[b] [a]Karolinska Pharmacy, Box 60024, and [b]Clinical Neurophysiology, Karolinska Hospital and Institute, S-104 01 Stockholm, Sweden

The serotonin agonist, 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT) (1), binds with high affinity to the 5-HT_{1A} sites (2) in rat cortical membranes (IC_{50}=~5 nM). To investigate the potential of this ligand as an *in vivo* probe for this receptor subtype, a method for labelling with [^{11}C]propyl iodide was developed, using the corresponding 8-hydroxy-2-(N-propylamino)tetralin (8-OH-PAT) as starting material, as shown below.



[^{11}C]Propyl iodide was synthesized by the reaction of [^{11}C]CO_2 with ethylmagnesium chloride in THF by a standard one-pot method (3) and was trapped in DMF. In preliminary experiments, very little [^{11}C]-8-OH-DPAT was obtained under any of the thermal conditions investigated. It has previously been shown that microwave treatment can be used to speed up N-alkylations with [^{18}F]alkyl halides which could not be performed under thermal conditions (4). Using a prototype wave-guide microwave cavity (5), alkylations were successfully performed using the HBr salt of both racemic and R-(+)-8-OH-PAT and the combined bases 2,2,6,6-tetramethylpiperidine (TMP) and K_2CO_3 in DMF. Treatment with microwaves at 100 W for 40 sec gave 50-60% conversion of the [^{11}C]propyl iodide according to analytical radio HPLC. Conversions were not appreciably increased by additional intermittent microwave treatments of ~1 min each up to a total of 3 min, which indicated that the microwave treatment generated a suitably intense field in the sample for good conversions in <1 min. A radiolabelled by-product (probably due to O-alkylation with [^{11}C]propyl iodide) was observed when stronger bases were used in the alkylation, but was produced in very small amounts (<5%) under the conditions described above. Isolation of [^{11}C]-8-OH-DPAT was achieved by semi-preparative HPLC (μ-Bondapak C-18, mobile phase = CH_3OH:H_2O:Et_3N). This radioligand is now available for *in vivo* characterization with PET, which is currently being pursued.

The gift of 8-OH-PAT and 8-OH-DPAT by Lars-Erik Arvidsson, BioProcess Technology AB, Uppsala and Uli Hacksell, Astra CNS R&D, Södertälje, is gratefully acknowledged.

References

1 Arvidsson L-E., Hacksell U., Nilsson J.L.G., Hjorth S., Carlsson A., Lindberg P., Sanchez D. and Wikström H. -J. Med. Chem. 24: 921 (1981).

2 Björk L., Höök B., Nelson D., Andén N.-E. and Hacksell U. -J. Med. Chem. 32: 779 (1989).

3 Långström B., Antoni G., Gullberg P., Halldin C., Någren K., Rimland A. and Svärd H. -Appl. Radiat. Isot. 37: 1141 (1986)

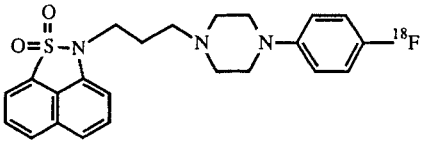
4 Zijlstra S., de Groot T.J., Kok L.P., Visser G.M. and Vaalburg W. -J. Org. Chem. 58 1643 (1993)

5 Stone-Elander S. and Elander N. -J. Label. Cmpds. Radiopharm. 32: 154 (1993)

[^{18}F]RP 62203 : A High Affinity and Selective Radioligand as a Potential PET Tracer for Serotonin 5HT2 Receptor. Das M.K. and Mukherjee, J. Department of Radiology, University of Chicago, Chicago, Il 60637

Introduction

Several positron emitting radioligands have been reported for the serotonin $5HT_2$ receptor and used in *in vivo* studies (for example: Crouzel et al., 1988; Lever et al., 1989; Blin et al., 1990; Sadzot et al., 1991). However, the selectivity of the available tracers is not entirely suitable to evaluate only the $5HT_2$ receptor specifically in *in vivo* studies. In PET experiments, ^{18}F-setoperone has been shown to bind significantly to the dopamine D_2 receptor sites in caudate-putamen (Blin et al., 1990). The naphthosultam, RP 62203 shows an affinity of 0.26 nM and weak affinities for $5HT_{1A}$, $5HT_{1C}$, H_1, D_2, and α_1 receptors (Malleron et al., 1991). Autoradiographic studies of ^{3}H-RP 62203 has demonstrated the relatively better selective nature of this compound (Malgouris et al., 1993). We have therefore considered radiolabelling RP 62203 by replacing its native fluorine with fluorine-18.



^{18}F-RP 62203

Radiosynthesis

Since the fluorine atom in RP 62203 is on the activated phenyl ring, a multi-step radiosynthesis had to be considered. A recent report on the synthesis of fluorine-18 labeled piperazines (Collins et al., 1992) was adapted to the radiosynthesis. This procedure involved the radiosynthesis of ^{18}F-fluoroaniline, which was then subsequently coupled with *bis*-(2-bromoethyl)amine or *bis*-(2-ditosyloxyethyl)amine. Therefore, the ditosylate of the naphthosultam derivative **6** was prepared as shown in Figure-1. Naphthosultam was reacted with dibromopropane to provide the bromopropyl derivative **3**. This was heated with bis-diethanolamine in dimethylformamide at 150 °C to provide the diol **5**, which was subsequently tosylated with tosyl chloride.

^{18}F-Fluoroaniline was prepared from dinitrobenzene by reported methods (Shiue et al., 1984). Dintrobenzene was reacted with ^{18}F-fluoride, potassium carbonate and Kryptofix in DMSO at 130 °C for 30 minutes. The reaction was cooled and the mixture reduced with tin chloride/HCl. The mixture was made basic and the ^{18}F-fluoroaniline extracted with ether. The ^{18}F-fluoroaniline was converted to the hydrochloride and taken to dryness. The ^{18}F-fluoroaniline hydrochloride was mixed with ditosylate **6** and potassium carbonate in DMF. This mixture was heated at 150 °C for one hour. Purification was carried out by reverse-phase HPLC with the product eluting at 13.3 minutes. Radiochemical yield for the conversion of ^{18}F-fluoroaniline to ^{18}F-RP 62203 was 10-15%, decay corrected. Apparent specific activity was in the range of 500-700 Ci/mmol. The product was characterized by coelution of an authentic sample of RP 62203. The authentic sample and the radioactive peak at 13.3 minutes had the same retention time on HPLC and the same R_f (0.70 in 9:1 $CHCl_3$:MeOH). *In vivo* evaluation of this radiotracer is currently in progress.

Figure-1

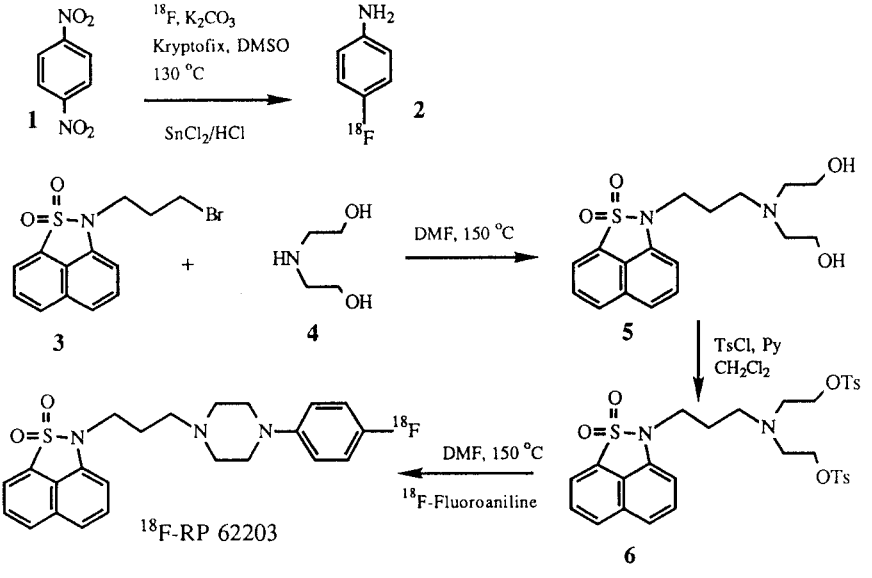
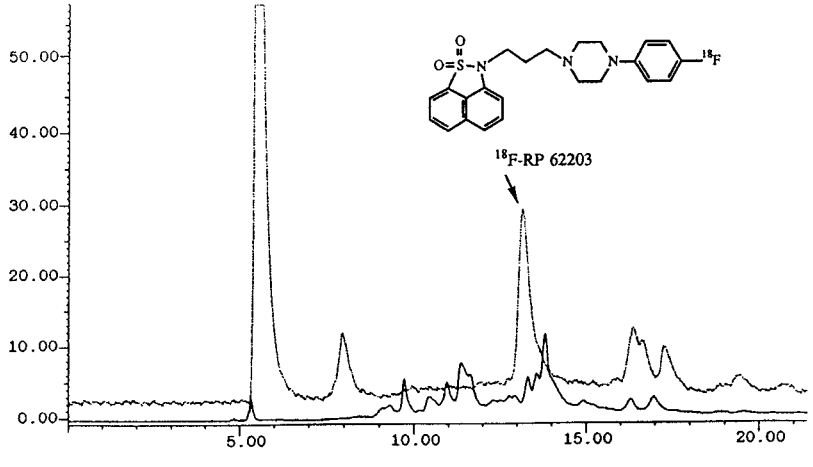


Figure-2: Reverse-phase HPLC purification of ^{18}F-RP 62203 (semi-preparative C-18 column, mobile phase contained 0.01M H_3PO_4 and aceteonitrile. Acetonitrile concentration increased from 0 to 60% during the first 10 min and then remained constant at 60% for the remainder of the time; flow rate was 3 mls/min). The radioactive peak at 5.6 minutes was ^{18}F-fluoroaniline. Radioactive peak at 13.3 minutes was ^{18}F-RP 62203.



References:

Blin J, Sette G, Fiorelli M, Bletry O, Elghozi JL, Crouzel C and Baron JC. J. Neurochem. **54**, 1744-1754 (1990).
Collins M, Lasne MC, Barre L and Baron JC. IXth Int. Symp. Radiopharm. Chem., Paris, 6-10 April, 1992, p-112.
Crouzel C, Venet M, Sanz G and Denis A . **25**, 403 (1988).
Lever JR, Dannals RF, Wilson AA, Ravert HT, Scheffel U, Hoffman BT, Hartig PR, Wong DF and Wagner HN. Nucl. Med. Biol. **16**, 697 (1989)..
Malgouris C, Flamand F and Doble A. Eur. J. Pharm. 233, 29-35 (1993).
Malleron JL, Comte M-T, Gueremy C, Peyronel JF, Truchon A, Blanchard JC, Doble A, Piot O, Zundel JL, Huon C, Martin B, Mouton P, Viroulaud A, Allam D and Betschart J. J. Med. Chem. **34**, 2477-2483 (1991).
Sadzot B, Lemaire C, Cantineau R, Hermanne JP, Plenevaux A, Maquet P, Salmon E, Degueldre C, Guillaume M and Franck G. J. Nucl. Med. **32**, 980 (1991).
Shiue CY, Watanabe M, Wolf AP, Fowler JS and Salvadori F. J. Labeled Compds. Radiopharm. **21**, 533 (1984).

Acknowledgements: We like to thank financial support from Department of Energy (contract No. DE-FG02-86ER60438). Technical assistance of Z.-Y. Yang and P. Nicole is gratefully acknowledged.

Synthesis and Evaluation of 2N-[^{11}C]Methylpinoline: A Serotonin Reuptake Tracer for PET
SHOUP, T.M., ELMALEH, D.R., and FISCHMAN, A.J. Division of Nuclear Medicine, Department of Radiology, Massachusetts General Hospital, Boston, MA 02114

Beta-Carboline alkaloids (THBC) alkaloids are trace endogenous compounds formed in the pineal gland, presumably from cellular condensation of carbonyl compounds with neuroactive indoleamines (1,2). In pharmacological studies, THBC compounds inhibit monoamine oxidase and competitively inhibit the high affinity uptake of 5-HT (serotonin) in brain homogenates. They can also displace citalopram from the 5-HT uptake site on human platelets in nM concentrations (3).
It has been suggested that certain N-methylated beta-carbolines, if accumulated in the brain over time, could have long-term initiator roles in idiopathic parkinsonism (PD). Studies with mice have shown concentration of N-methyl THBC increases with stress and age (4). The link to PD is due in part to changes in the brain similar to that created by MPTP, a compound that selectively lesions the nigrostriatal pathway and causes parkinsonism. N-Methyl-THBC resembles MPTP in reducing 3,4-dihydroxyphenylacetic acid levels in caudate and in altering levels of serotonin and 5-hydroxyindoleacetic in substantia nigra.
In our study, we examined the biodistribution of 2N-[^{11}C]]methylpinoline (CMP) in rats and evaluated its use for PET imaging in monkeys. CMP was labeled by direct methylation with [C-11]methyliodide and purified by HPLC on a C_{18} cartridge (<99% radiochemical purity, specific activity >2000 Ci/mmol). The biodistribution study was done in rats at 5, 30 and 60 min. The highest uptake (%ID/g) at 5 min was in the cortex: 1.47, striatum: 1.31, thalamus: 1.19 and cerebellum: 0.99%. This activity decreased with time. Similar results were observed in the monkey imaging studies. The behavior of ^{11}C-pinoline in rat brain is similar to that of ^{11}C-MPTP, at 5 min the %Dose/g in cortex: 1.14, striatum: 1.34, thalamus: 1.10 and cerebellum: 0.89 (5). However, washout of activity from monkey brain regions was observed with ^{11}C-pinoline but not with ^{11}C-MPTP. The relatively fast brain activity washout can explain the temporary parkinsonian symptoms observed with this compound. The pharmacokinetics of this compound is relatively complex and its usefulness as a PET tracer is not clear as yet.

1. Callaway J.C., Morimoto H. et al.-J. Labelled Comp. Radiopharm. 31: 354(1991).
2. Komulainen H, Tuomisto J. et al.-Acta Pharmacol. et Toxicol. 46: 179(1985).
3. Cascio C.S., Kellar K.J.-Neuropharmacology, 21: 1219(1982).
4. Collins M.A., Neafsey E.J.-Neuroscience Let. 55: 179(1985).
5. Livni E, Elmaleh D.R. et al.-J. Nuc. Med., 27: 1600(1986).

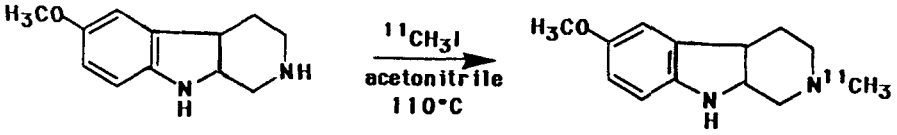


Table 1. C-11 Methylpinoline Biodistribution in Rats at 5, 30 and 60 min

Brain Region	5 min	30 min	60 min
Thalamus	1.19	0.25	0.04
Cerebellum	0.99	0.32	0.06
Cortex	1.47	0.40	0.07
Striatum	1.31	0.42	0.08
Hypothalamus	1.06	0.30	0.15
Pons	0.90	0.28	0.10

% Dose per gram

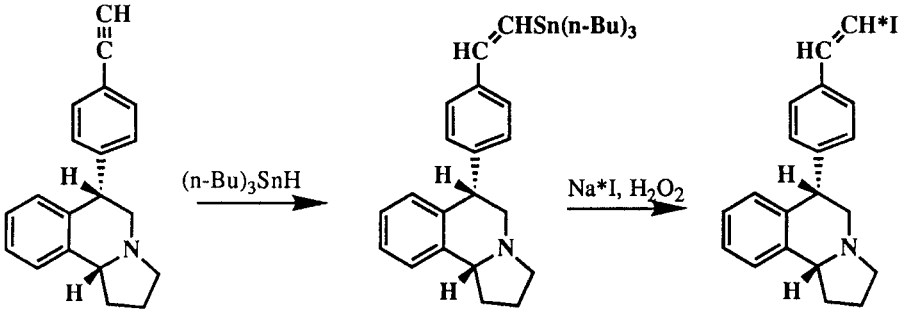
Synthesis of radioiodinated analogs of McN6398 as SPECT radiotracers for 5-HT uptake sites.

M. Suehiro, H. T. Ravert, R. F. Dannals, J. L. Musachio, U. Scheffel, and H. N. Wagner, Jr.
Division of Nuclear Medicine and Radiation Health Sciences, The Johns Hopkins Medical Institutions, Baltimore, MD 21205-2179, U.S.A.

A PET/SPECT radiotracer that selectively labels the presynaptic element of central serotonergic (5-HT) neurons could provide valuable information regarding the functional role of serotonergic neurons in human brain in healthy normal subjects and in patients with a wide variety of disorders. It would also facilitate the clinical assessment of individuals exposed to potentially neurotoxic drugs such as 3,4-methylene-dioxymethamphetamine (MDMA) or fenfluramine.

Recently, we have shown that [^{11}C]McN5652, a highly potent 5-HT uptake blocker with a K_i value of 0.6 nM vs [^{3}H]5-HT (1 - 3), shows promising biological behavior *in vivo* with high target-to-nontarget ratios and high selectivity and specificity in binding to 5-HT uptake sites (4, 5). The properties of McN5652 suggest that a radioiodinated analog of McN5652 may be useful as a SPECT radiotracer.

Among the McN pyrroloisoquinoline antidepressants, trans-1,2,3,5,6,10b-hexahydro-6-(4'-ethynylphenyl)-pyrrolo[2,1-a]isoquinoline, McN6398, has been found to possess high *in vitro* potency in 5-HT uptake inhibition with a K_i value of 1.0 nM (1, 2). We derivatized this compound into 4'-[2(*E*)-, and 2(*Z*)-iodovinyl]- analogs according to the literature procedure (*e.g.* 5, 6), tested *in vitro*, and radioiodinated with ^{125}I to study their *in vivo* behavior.



McN6398

The 2(*E*)-, and 2(*Z*)-iodovinyl derivatives showed IC50 values of 32 and 0.39 nM, respectively, in *in vitro* [^{3}H]paroxetine binding inhibition. The radiosynthesis was performed by iododestannylation of the tributyltin precursor with Na^{125}I. The tributyltin precursor prepared in hexamethylphosphorous triamide at 60°C gave the 4'-[2(*E*)- and 2(*Z*)-^{125}I-iodovinyl]- analogs in a ratio of 1.2:1, while the precursor prepared in toluene at 90°C in the presence of azobis(isobutyronitrile) gave only the *E* isomer. The radiolabeling proceeded fast and effectively. In 5 minutes at room temperature, 80-90 % of ^{125}I was incorporated. The ^{125}I labeled *E* and *Z* isomers were separated by HPLC using a semi-preparative reversed phase column and formulated for injection. The specific activity was 1600 - 2000 mCi/μmol. *In vivo* biodistribution studies are under way.

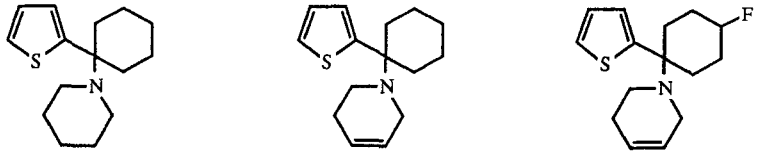
Acknowledgements: The authors would like to thank Dr. Bruce E. Maryanoff of McNeil Pharmaceutical for providing samples of McN6398. They also thank Mr. Stanley Kim for performing *in vitro* binding assays. This work was supported in part by U.S.P.H.S. grant numbers NS-15080, CA-32845, and DA-06309.

1. Maryanoff BE, McComsey DF, Gardocki JF, Shank RP, Costanzo MJ, Nortey SO, Schneider CR, Setler PE. Pyrroloisoquinoline antidepressants. 2. In-depth exploration of structure-activity relationships. J Med Chem 30: 1433-1454, 1987
2. Maryanoff BE, Vaught JL, Shank RP, McComsey DF, Costanzo MJ, Nortey SO. Pyrroloisoquinoline antidepressants. 3. A focus on serotonin. J Med Chem 33: 2793-2797, 1990
3. Suehiro M, Scheffel U, Dannals RF, Ravert HT, Ricaurte GA, Wagner HN, Jr. A PET radiotracer for studying serotonin uptake sites: Carbon-11-McN5652Z. J Nucl Med 34: 120-127, 1993
4. Suehiro M, Scheffel U, Ravert HT, Dannals RF, Wagner HN, Jr. [^{11}C](+)McN5652 as a radiotracer for imaging serotonin uptake sites withPET. Life Sciences, in press
5. Ensley HE, Buescher RR, Lee K. Reaction of organotin hydrides with acetylenic alcohols. J Org Chem 47: 404-408, 1982
6. Hoyte RM, Rosner W, Johnson IS, Zielinski J, Hochberg RB. Synthesis and evaluation of potential radioligands for the progesterone receptor. J Med Chem 28: 1695-1699, 1985

[^{18}F]fluorine Labeled TCP-derivatives for the NMDA Receptor-Linked Calcium Ionophore. Mukherjee, J.; Ouyang, X.-H. and Yang, Z.-Y., Department of Radiology, University of Chicago, Chicago, Il 60637

Introduction: We have been interested in the development of radiotracers which will enable non-invasive studies of the NMDA receptor-linked ionophore. TCP and MK-801 have been shown to selectively bind within the ion-channel and have thus attracted attention as potential lead compounds for the development of appropriate tracers. A number of potential positron-tracers, both fluorine-18 and carbon-11 labeled, have been prepared, but have not shown successful *in vivo* use (for example: Mukherjee et al., 1988; Van Dort et al., 1988; Ponchant et al., 1992). Development of radiolabeled TCP or MK-801 derivative has been a challenge in terms of obtaining a tracer that will: i) show good affinity for the NMDA receptor, ii) have optimal lipohilicity, and iii) be amenable to labeling with [F-18]fluoride.

Figure-1

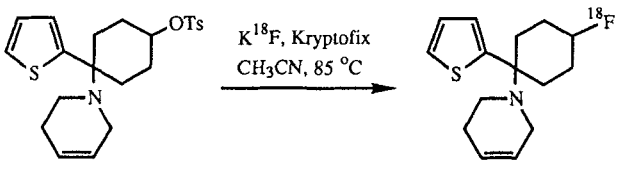


1 **2** **3**

We are currently evaluating structure-activity-lipophilicity profile of TCP analogs that are likely to provide high affinity fluorinated derivatives. Shown in Figure-1 are TCP **1** (Ki = 20 nM; using ^{3}H-TCP) and an unsaturated analog of TCP **2** (Ki = 12 nM; using ^{3}H-TCP, Rice et al. 1989). We have developed a fluorinated unsaturated analog **3** for *in vivo* evaluation.

Radiosyntheses: Direct nucleophilic ^{18}F-fluoride reaction of the tosylate precursor (prepared using procedures outlined in Rice et al., 1989) was carried out in the presence of potassium carbonate and Kryptofix in acetonitrile at 85 °C for 25 minutes. Radiochemical purity was >95% (Figure-3) and yield of the product was 25%, EOB decay-corrected. The mixture was purified by reverse-phase HPLC to provide pure ^{18}F-**3** in apparent specific activities of 800 Ci/mmol.

Figure-2



Rat biodistribution studies: *In vivo* studies were carried out in male Sprague-Dawley rats after intravenous administration of ^{18}F-**3**. Rats were sacrificed at various time-points and the following regions of the brains were isolated: hippocampus, frontal cortex, parietal cortex, striata and cerebellum. ^{18}F-**3** showed good initial brain uptake. The selectivity for the various regions were assessed using cerebellum as the reference region. Cerebellum in the rat brain has been shown to contain approx. 3% of the ^{3}H-TCP binding-sites compared to that in the hippocampus (Maragos et al., 1988). The ratio in the four regions (hippocampus, frontal cortex, striata and parietal cortex) versus cerebellum is shown in Figure-4. The ratio in all the four regions improved

with time. Parietal cortex showed maximal ratios, approximately 3, three hours p.i. Hippocampus showed only moderate ratios. Compared to previously reported ^{18}F-FTCP (Ferrarese et al., 1991; Miletich et al., 1991), ^{18}F-**3** shows some regional selectivity. Further evaluation of this compound is in progress.

Figure-3

Reverse-phase HPLC purification of ^{18}F-**3** (semi-preparative C-18 column, mobile phase contained 0.01M H_3PO_4 and aceteonitrile. Acetonitrile concentration increased from 5 to 95% during the first 20 min and then remained constant at 95% for the remainder of the time; flow rate was 3 mls/min). The radioactive peak at 12.5 minutes was ^{18}F-**3**. UV peak at 13.5 min was the tosylate precursor.

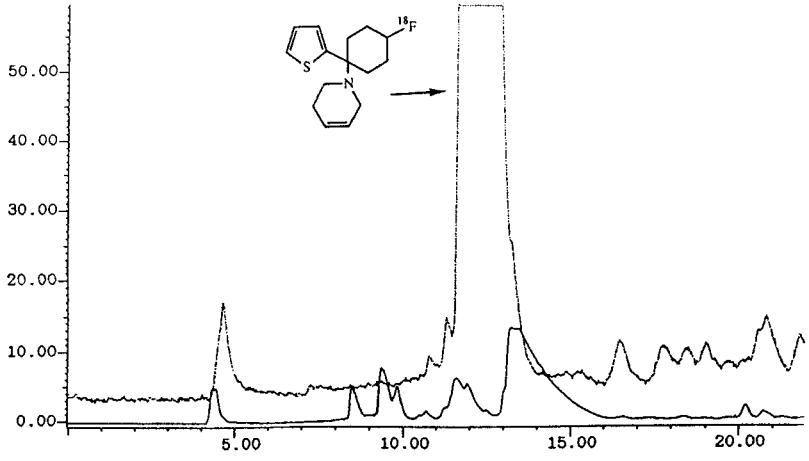
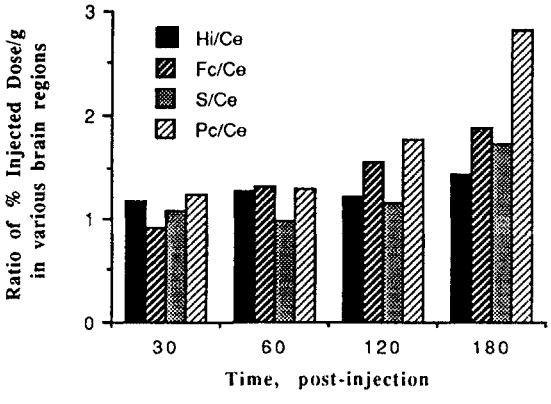


Figure-4: Rat brain distribution of ^{18}F-**3**. Figure shows ratios of brain regions. Data are an average of two rats at each time point.



References:

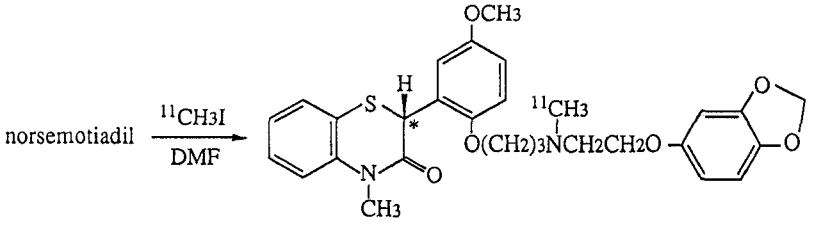
Ferrarese, C., Guidotti, A., Costa, E., Miletich, R.S., Rice, K.C., DeCosta, B.R., Fulham, M.J. and DiChiro, G. Neuropharm. **30**: 899-905 (1991).
Miletich, R.S., Ferrarrese, C., Kiesewetter, D.O., Brooks, R., Carson, R.E., Fulham, M., Linfante, I., Hayashida, K., Rice, K.C., deCosta, B.R., Costa, E., Guidotti, A. and DiChiro, G. J. Nucl. Med. **32**: 1008 (1991).
Maragos, W.F., Penny, J.B. and Young, A.B. J. Neurosci. **8**:493-501 (1988).
Mukherjee, J., Murphy, S.N., Hartwig, W., Miller, R.J. and Cooper, M.D. J. Nucl. Med. **29**:932 (1988).
Ponchant, M., Crouzel, C., Kamenka, J.M. and Pappata, S. IX th Int. Symp. Radiopharm. Chem. Paris, April 6-10, 1992, page-352.
Rice, K.C., Jacobseon, A.E., Thurkauf,A., Mattson, M.V., O'Donohue, T.L., Contreras, P.C. and Gray, N.M. EP 0317953 A2.
Van Dort, M.E., Yang, D.J., Kilbourn, M.R., Gole, D.J., Kalir,A., Domino, E.F., Young, A.B. and Wieland, D,M. J. Nucl. Med. **29**:767 (1988).
Wong, E.H.F., Kemp, J.A., Priestly, T., Knight, A.R., Woodruff, G.N. and Iverson, L.L. Proc. Natl. Acad. Sci. (USA), **83**:7104-7108 (1988).

Acknowledgements: We like to thank financial support from Department of Energy (contract No. DE-FG02-86ER60438). Technical assistance of Dr. M.K. Das and P. Nicole is gratefully acknowledged.

Synthesis and In Vivo characteristics of [^{11}C]Semotiadil, a Calcium Antagonist.

ISHIWATA, K.; SEKI*, H.; ISHII, S.; ISHII, K.; NOZAKI*, T.; and SENDA M. (Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, Tokyo, *Faculty of Hygienic Sciences, Kitasato University, Japan)

(+)-(*R*)-3,4-dihydro-2-[5-methoxy-2-[3-[*N*-methyl-*N*-[2-[(3,4-methylenedioxy)phenoxy]ethyl]-amino]propoxy]phenyl]-4-methyl-3-oxo-2*H*-1,4-benzothiazine (semotiadil) has Ca^{2+} antagonistic activity (1,2). In order to develop a ligand for visualizing calcium channels by PET, ^{11}C-labeled semotiadil was synthesized and its *in vivo* potential were investigated.



^{11}C-semotiadil

Radiosynthesis of [^{11}C]semotiadil was carried out using norsemotiadil as shown in scheme above. [^{11}C]CH_3I prepared as previously described (3) was trapped in a solution of 0.1 μmol norsemotiadil in 0.3 mL DMF. The solution was heated at 75-130 °C for 10 min and applied to HPLC separation using a Megapak SIL C18-10 column (7.2 mm x 250 mm), which was eluted with CH_3OH/50 mM AcONa (9/1, v/v) as a mobile phase with a flow rate of 5 mL/min. The [^{11}C]semotiadil fraction eluted at 6.8 min was collected and evaporated to dryness. The residue was dissolved in physiological saline and passed through a 0.22 μm membrane filter for injection. Radiochemical purity was analyzed by HPLC using Crestpak C18S-10 (4 mm x 150 mm) with CH_3OH/50 mM AcONa (9/1, v/v) with a flow rate of 2.0 mL/min. The retention times of norsemotiadil and semotiadil were 3.8 and 5.3 min, respectively. ^{11}C-Labeled S-enantiomer which has less potency as a Ca^{2+} antagonist was also prepared from a corresponding desmethyl derivative. [^{11}C]semotiadil and its enantiomer were injected i.v. into mice, and tissue distribution was measured as % injected dose per g tissue.

Methylation yields of norsemotiadil with [^{11}C]CH_3I were summarized in Table 1. In HPLC separation, semotiadil was eluted faster than norsemotiadil in the conditions described above using a semipreparative reverse phase column: retention time of semotiadil and norsemotiadil was 6.8 min and 9.0 min, respectively. Therefore, [^{11}C]semotiadil and its enantiomer with radiochemical and chemical purity of >99% was prepared within 40 min from EOB. Specific activity was 11~50 GBq/μmol (n = 6), and optical purity was retained.

Tissue distribution of [^{11}C]semotiadil in mice was summarized in Table 2. Highest uptake was found in the lung, followed by liver, kidney, small intestine and spleen. Radioactivity level in the heart decreased with time, but was twice or three times as high as that in blood upto 30 min. Brain uptake was the lowest, but the maximal uptake was observed at 15 min after injection. Regional brain distribution of the [^{11}C]semotiadil and effect of loading doses on it were summarized in Table 3.

Hippocampus, striatum and hypothalamus showed higher uptake than the other regional tissues in the lowest loading dose, and decreased with loading doses upto 2.1 nmol. These regions are rich in L-type calcium channels (4).The saturable uptake was not observed in the heart nor in the regional distribution of ^{11}C-labeled (S)-enantiomer.

Theses results indicate that [^{11}C]semotiadil has a potential as a ligand for visualizing calcium channels in brain by PET.

Table 1. Radiochemical yields of [^{11}C]semotiadil.

Norsemotiadil		Temp.	Reaction time	Radiochemical yield
free base, 1 μmol/0.3 mL DMF		130 °C	2 min	41.0 %
		130 °C	5 min	45.4 %
		130 °C	10 min	50.0 %
		75 °C	5 min	42.5 %
fumarate, 1μmol/0.3 mL DMF	1.1 μmol TMP*	75 °C	5 min	23.9 %
	11 μmol TMP*	75 °C	5 min	12.5 %
		75 °C	5 min	22.6 %**
		130 °C	5 min	38.5 %

*2,2,6,6-tetramethylpiperidine. **Mean (n = 3).

Table 2. Tissue distribution of radioactivity in mice after injection of [^{11}C]semotiadil.

	% Injected dose/g tissue			
	5 min	15 min	30 min	60 min
Blood	1.23 ± 0.16	0.85 ± 0.12	0.67 ± 0.10	0.51 ± 0.11
Brain	0.64 ± 0.12	0.72 ± 0.10	0.52 ± 0.10	0.45 ± 0.10
Heart	3.66 ± 0.47	1.57 ± 0.22	1.14 ± 0.30	0.68 ± 0.17
Lung	17.75 ± 1.57	12.78 ± 2.52	5.44 ± 1.91	2.71 ± 0.68
Liver	10.66 ± 1.76	8.68 ± 1.53	7.61 ± 1.38	5.84 ± 1.06
Pancreas	7.16 ± 1.40	8.58 ± 1.20	6.28 ± 1.75	5.81 ± 2.63
Spleen	5.93 ± 2.48	3.93 ± 0.65	2.52 ± 0.96	1.30 ± 0.33
Small intestine	9.06 ± 2.05	8.70 ± 2.43	5.34 ± 0.96	3.44 ± 0.88
Kidney	11.37 ± 2.09	6.68 ± 1.50	4.64 ±1.25	2.95 ± 0.61
Muscle	2.22 ± 0.24	1.31 ± 0.13	0.88 ± 0.14	0.61 ± 0.19

Mean ± sd (n = 4)

Table 3. Effects of loading dose on the tissue distribution of radioactivity (% Injected dose/g tissue) in mice at 15 min after injection of [^{11}C]semotiadil.

	Loading dose				
	7.9 pmol	79 pmol	2.1 nmol	20 nmol	200 nmol
Blood	0.56 ± 0.10	0.69 ± 0.11	0.70 ± 0.10	0.73 ± 0.02	0.72 ± 0.08
Heart	1.25 ± 0.16	1.24 ± 0.17	1.35 ± 0.31	1.44 ± 0.29	1.77 ± 0.26
Cortex	0.48 ± 0.07	0.46 ± 0.07	0.45 ± 0.05	0.52 ± 0.05	1.15 ± 0.14
Hippocampus	0.85 ± 0.15	0.41 ± 0.09*	0.38 ± 0.07*	0.58 ± 0.06**	1.03 ± 0.10
Striatum	0.95 ± 0.12	0.62 ± 0.14*	0.51 ± 0.03*	0.64 ± 0.19***	0.98 ± 0.10
Hypothalamus	0.69 ± 0.15	0.44 ± 0.05**	0.48 ± 0.07***	0.52 ± 0.08	0.94 ± 0.04
Midbrain	0.48 ± 0.09	0.40 ± 0.06	0.42 ± 0.05	0.49 ± 0.04	1.01 ± 0.13
Medulla oblongata	0.48 ± 0.17	0.43 ± 0.05	0.43 ± 0.03	0.49 ± 0.04	0.99 ± 0.13
Cerebellum	0.52 ± 0.12	0.45 ± 0.04	0.44 ± 0.05	0.51 ± 0.05	0.98 ± 0.17

Mean ± sd (n = 4~5)

*p<0.01, **p<0.02, ***p<0.05 (Student's t-test, compared to the 7.9 nmol dose)

References

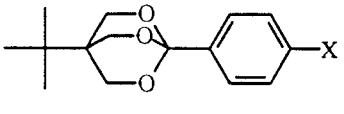
1. Fujita M., Ito S., Ota A., et al.- J.Med.Chem. 33:1898(1990)
2. Nakayama K., Morimoto K., Nozawa Y. and Tanaka Y.- J.Cardiovasc.Pharmacol. 20:380(1992)
3. Ishiwata K., Ishii S. and Senda M. - Appl.Radiat.Isot. (in press)
4. Schoemaker H., Lee H.R., Roeske W.R. and Yamamura H.I. - Eur.J.Pharmcol. 88:275(1983)

Synthesis of [^{18}F]2-(4-Fluorophenyl)-1,3-Dithianes as Potential PET Imaging Agents for the GABA$_A$ Chloride Ion Channel.

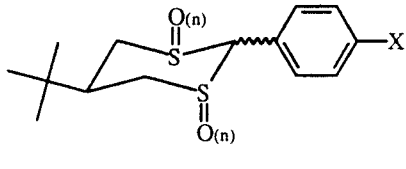
SNYDER, S.E. and KILBOURN, M.R. Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan School of Medicine, Ann Arbor, MI 48109.

γ-Aminobutyric acid (GABA), is an inhibitory neurotransmitter in the central nervous system which upon binding to the GABA$_A$ receptor complex opens neuronal chloride ion channels. This results in hyperpolarization of the neuron due to Cl^- influx. Deficiencies in GABA-mediated neuromodulation has been implicated in a number of neurological abnormalities including epilepsy and anxiety disorders. Several classes of compounds, including benzodiazepines, barbiturates and steroids, have been shown to modulate the effects of GABA by binding to ancillary sites on the GABA$_A$ receptor complex. Two other classes of compounds, the bicycloorthobenzoates and the phenyldithianes (along with their *S*-oxidation products),have been identified as noncompetitive inhibitors of GABAergic neurotransmission.[1] These agents, unlike the benzodiazepines and barbiturates, are thought to act through binding to sites within the ion channel itself. Thus, radiolabeled analogues of these "channel blockers" could provide probes for the GABA-gated ion channel. In particular, PET imaging agents based on these compounds could provide *in vivo* functional markers for the GABA$_A$ receptor complex.

In previous attempts to prepare radiolabeled channel binding agents for the GABA$_A$ receptor-coupled Cl^- channel, the [^{18}F]- and [^{123}I]-labeled derivatives of *t*-butylbicycloorthobenzoate **1** have been successfully synthesized.[2,3] Neither of these agents has shown any potential as *in vivo* imaging agents: in particular, the ^{18}F-labeled derivative exhibited poor stability toward hydrolysis. The phenyldithianes and the corresponding *S*-oxidation products, however, should be much more resistant to hydrolysis. One series of dithianes in particular, the 4'-substituted-4-*t*-butyl-2-phenyl-1,3-dithianes **2** and the corresponding sulfone and sulfoxide derivatives, show low nanomolar affintiy for GABA receptor complexes in bovine and rat brain.[1,4] Thus, we have undertaken the synthesis of positron emitter-labeled analogues of this series of ligands.



1 X = ^{18}F, ^{123}I

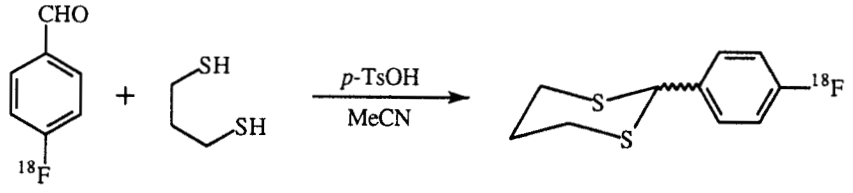


2 X = H, Br, ethynyl

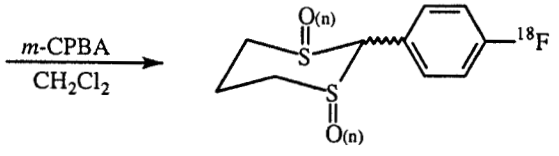
In order to show the feasibility of the formation and oxidation of phenyldithianes within the time frame necessary for work with positron emitting radionuclides, we have synthesized [^{18}F]2-(4-fluorophenyl)-1,3-dithiane, **3**, by a modification of the procedure of Wacher *et al.*[4] Thus, commercially available 1,3-propanedithiol was condensed with [^{18}F]4-fluorobenzaldehyde[5] in the presence of *p*-toluenesulfonic acid. Dithiane formation occured within 15 minutes with complete consumption of the starting aldehyde and formation of the desired product in 92% radiochemical purity. Oxidation of this dithiane *via* treatment with *m*-chloroperoxybenzoic acid (*m*-CPBA) was instantaneous and gave a mixture products which were separable on TLC.

We were thus able to synthesize [^{18}F]-labeled dithianes with good radiochemical yield and in less than 75 minutes from resolubilized ^{18}F. Further, the various oxidation products could be synthesized *via* a single oxidation step, eliminating the need for more time-consuming oxidation

schemes. Work is currently underway to isolate and identify the oxidation products formed and to synthesize the 4-*t*-butyldithiane analogues.



3



Acknowledgments: This work was supported by NIH grants NS15655 and 5-T32-CA09015-17.

(1) Hawkinson, J. E.; Casida, J. E. *Mol. Pharmacol.* **1992**, *42*, 1069-1076.

(2) Mulholland, G. K.; Kilbourn, M. R. *J. Label. Compd. Radiopharm.* **1993**, *32*, 313.

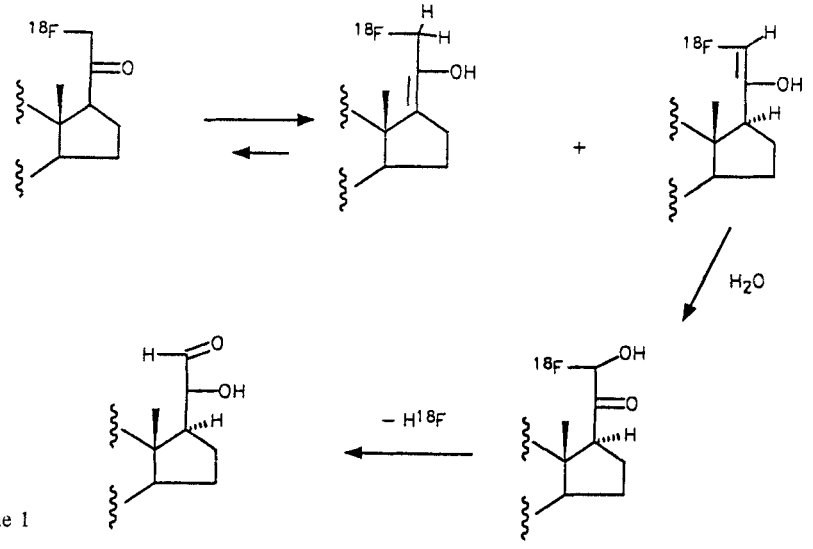
(3) Culbert, P. A.; Chan, S.; Wearring, A. V.; Chamberlain, M. J.; Hunter, D. H. *Nucl. Med. Biol.* **1993**, *20*, 469-475.

(4) Wacher, V. J.; Toia, R. F.; Casida, J. E. *J. Agric. Food Chem.* **1992**, *40*, 497-505.

(5) Chakraborty, P. K.; Kilbourn, M. R. *Appl. Radiat. Isot.* **1991**, *42*, 673-681.

Synthesis and distribution of ^{18}F-Fluoro-Org6141, a high-affinity fluorine-substituted glucocorticoid receptor ligand in the rat brain. *Visser, G.M.[1], Krugers, H.J.[2], Luurtsema, G.[1], Van Waarde, A.[1], Elsinga, P.H.[1], Korf J.[2], and Vaalburg W.[1]*, PET Center and Groningen Center for Catalysis and Synthesis[1], Department of Biological Psychiatry and Center for Behavioral Cognitive and Neural Sciences[2], University of Groningen, P.O.Box 30.001, 9700 RB, Groningen, The Netherlands

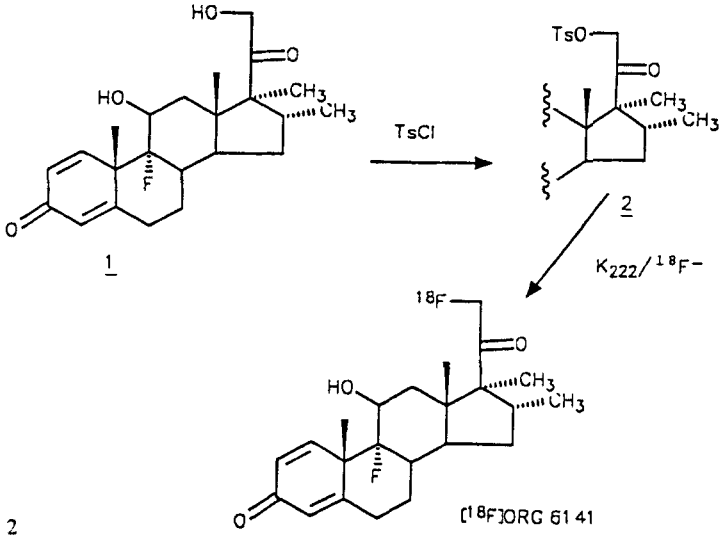
Corticosteroids are released during the circadian rhythm and elevated plasma levels are often found as a response to stress. They bind to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) in the brain. In 1992 Pomper et al. (1) described the synthesis and distribution of fluorine-substituted corticosteroids in the rat using Positron Emission Tomography but failed to demonstrate selective accumulation and retention of several [^{18}F]-substituted MR and GR ligands in the brain. One of the problems with [^{18}F]-labelled steroid analoges reported till now are the lipophilicity and susceptibility of the C-21 position to defluorination (2, 3, 4, 5, 6, 7). Besides significant metabolic reduction of the C-20 carboxyl group, a chemical enolisation as depicted in scheme 1 might be expected.



Scheme 1

Therefore we embarked upon a program to develop a high affinity and lipophilicity GR ligand ^{18}F-Fluoro-Org6141 3, which promised to be a good candidate because of i) enhanced metabolic/chemical stability towards defluorination due to enolisation blockade at 17 position, ii) high affinity (K_d approximately 0.6 nM). The radiochemical synthesis of ^{18}F-Fluoro-Org6141 was analogous to that reported for 21-fluoro-16-α-ethyl-19-norprogesterone (FENP) as described by De Groot et al. (8) and proceeded by ^{18}F-fluoride ion displacement of the C-21 substituted tosylate precursor (Scheme 2). The resolubilized ^{18}F-fluoride/K-222 (kryptofix) potassium carbonate mixture in acetonitrile was transferred to a glass vial containing the appropriate precursor 2 obtained via selective tosylation of derivative 1 (OrgND82, 9, 10) with tosyl chloride. The reaction mixture was heated at 70 °C for 20 minutes, passed through a silica SEP-PAK column. The column was

Radiolabeled material was isolated after purification by normal phase HPLC. Radiochemical yield was approximately 10% (decay corrected) and a specific activity of approximately 1000 Ci/mmol was attained. The biodistribution of the purified product was examined in adrenalectomized (ADX) and sham-adrenalectomized (sADX) rats and 50 μCi portions were injected. Male Wistar rats (230-250 g, locally bred at the Central Animal Laboratory, University of Groningen) were housed individually in a temperature controlled room at a light-dark regime of 12:12 hours. Three animals were bilaterally adrenalectomized under ether anaesthesia, to remove endogenous corticosterone and minimize competition with the radioligand, and remained on 0.9% saline as drinking solution. Three animals were sham-adrenalectomized. Two days after surgery the animals were anaesthesized with pentobarbital and ^{18}F-Fluoro-Org6141 was administered i.v. via a tail vein using a 10% ethanol-saline vehicle. Kinetics and distribution were followed for one hour using the PET-camera. After one hour animals were killed by overdosage pentobarbital and several peripheral organs and brain areas were dissected, weighed and activity was counted. Uptake was calculated as %Injected Dose/g. Data in table 1 indicate little uptake in the brain (tissue levels below plasma levels). Pituitary uptake is higher than uptake in the brain but uptake in several brain areas and pituitary is not statistically significant higher in ADX animals than in sADX animals. Uptake in peripheral organs is neither different in sADX animals than in ADX animals. The considerable uptake in bone and fat indicate respectively in vivo defluorination and lipophilicity. When compared to Pomper et al. (1), which investigated the GR ligand 21-^{18}F-Fluoro-21-deoxytriamcinolone acetonide, ^{18}F-Fluoro-Org6141 shows more uptake in the hippocampus, several other brain areas, and plasma but a reduced uptake in cortex, pituitary and bone. These authors studied another GR ligand [^{18}F]RU28362 which also did not show specific binding in vivo (1). We conclude that the brain does not accumulate [^{18}F]Org6141 over blood levels and that the uptake is probably not receptor mediated since adrenalectomy does not affect uptake in liver and hypothalamus which are rich in GR. Although [^{18}F]Org6141 is theoretically more stable to defluorination and more lipophilic than tracers used thus far it does not meet the criteria for imaging of glucocorticoid receptors in the brain.



Scheme 2

TABLE 1. ^{18}F-ORG6141, Distribution in peripheral organs and the central nervous system, 60 minutes after i.v. injection.

Tissue	sADX			ADX		
%ID/g ± SEM						
Bladder	1.12	±	0.25	1.38	±	0.40
Fat	0.39	±	0.10	0.62	±	0.32
Heart	0.58	±	0.18	0.58	±	0.03
Ileum	0.62	±	0.18	0.54	±	0.04
Kidney	0.64	±	0.16	0.94	±	0.04
Liver	1.03	±	0.08	1.08	±	0.15
Lung	0.44	±	0.15	0.86	±	0.23
Muscle	0.11	±	0.01	0.15	±	0.01
Pancreas	0.54	±	0.08	0.58	±	0.05
Plasma	0.42	±	0.06	0.47	±	0.08
RBC	0.24	±	0.02	0.23	±	0.03
Spleen	0.38	±	0.13	0.27	±	0.13
Stomach	0.24	±	0.07	0.27	±	0.03
Submandibularis	0.30	±	0.04	0.30	±	0.01
Testes	0.15	±	0.01	0.17	±	0.02
Trachea	0.63	±	0.04	0.53	±	0.02
Bone	1.20	±	0.12	1.04	±	0.13
Bulbus Olfactorius	0.17	±	0.05	0.15	±	0.02
Bulbus	0.11	±	0.04	0.16	±	0.03
Septum	0.12	±	0.04	0.13	±	0.02
Striatum	0.10	±	0.05	0.11	±	0.01
Cortex	0.09	±	0.04	0.13	±	0.01
Hypothalamus	0.13	±	0.03	0.13	±	0.02
Thalamus	0.15	±	0.06	0.17	±	0.03
Amygdala	0.10	±	0.03	0.13	±	0.03
Hippocampus	0.08	±	0.02	0.12	±	0.02
Brainstem	0.11	±	0.02	0.17	±	0.03
Cerebellum	0.10	±	0.03	0.16	±	0.02
Pituitary	0.31	±	0.07	0.35	±	0.02

References

1) Pomper, M.G., Kochanny, M.J., Thieme, A.M., Carlson, K.E., VanBrocklin, H.F., Mathias, C.J., Welch, M.J. and Katzenellenbogen, J.A., Nucl.Med.Biol., 19, 481-489, 1992.

2) Verhagen, A., PHD Thesis 1993, Groningen.

3) DaSilva, J.N., and Crouzel, C., J.label.Compd.Radiopharm., 30, pp 240, 1991.

4) DaSilva, J.N., Crouzel, C., Stulzaft, O., Khalili-Varasteh, M., Hantraye, P., Nucl.Med.Biol., 19, 167-173, 1992.

5) Kochanny, M.J., Pomper, M.G., Carlson, K.E., Mathias, C.J., VanBrocklin, H.F., Welch, M.J., Katzenellenbogen, J.label.Compd. Radiopharm., 30, pp 241, 1991.

6) Kochanny, M.J., VanBrocklin, H.F., Kym, P.R., Carlson, K.E., O'Neil, J.P., Bonasera, T.A., Welch, M.J., Katzenellenbogen, J.A., J.Med.Chem., 36, 1120-1127, 1993.

7) Kym, P.R., Carlson, K.E., Katzenellenbogen, J.A., J.Med.Chem., 36, 1111-1119, 1993.

8) De Groot, T., Verhagen, A., Elsinga, P.H., Vaalburg, W., Appl.Radiat Isot., 42, 471, 1991.

9) Hewett, C.L., Woods, G.F., and Logan, R.T., U.S.P., 3, 520, 908, 1970.

10) Schaub, R.E., and Weiss, M.J., J.Med.Chem., 10, 789-795, 1969.

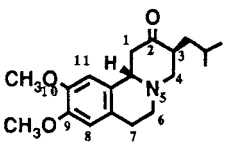
The authors would like to thank Prof.Dr.E.R.DeKloet, Prof.Dr.B.Bohus, Prof.Dr.K.G.Go, and Dr.M.B.Groen for useful discussions.

Amino/Amido-Tetrabenazine Analogs as Potential Monoamine Transporter Ligands

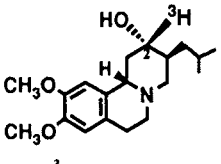
CANNEY, D. J.; KUNG, M-P.; GUO, Y-Z.; KUNG, H. F., Department of Radiology, University of Pennsylvania, 3700 Market St., Suite 305, Philadelphia, PA 19104

Synaptic neurotransmission in the central nervous system (CNS) is closely coupled to the active transport of monoamines into storage vesicles in presynaptic nerve terminals. Radiolabeled derivatives of tetrabenazine (TBZ; **1**) have been shown to be useful markers for in vitro binding and autoradiography studies of the vesicular monoamine reuptake site. For example, using α-[^{3}H]dihydrotetrabenazine ([^{3}H]DH-TBZ; **2**), postmortem binding studies demonstrated a significant reduction of [^{3}H]DH-TBZ binding in the putamen and caudate nucleus of the brains of Parkinsonian patients as compared to controls (1). Recently, in vivo PET studies in human subjects showed promising results for ^{11}C-labeled TBZ as a potential radiotracer for the transporter (2). Our attempts to develop a radioiodinated TBZ analog for in vivo SPECT studies of the transporter have been disappointing thus far. The high lipophilicity and consequent high nonspecific binding of a 2-iodovinyl TBZ (I-TBZ; **3**) analog has precluded the use of this radiotracer in in vivo SPECT imaging studies (3,4).

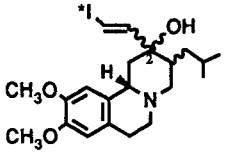
In an effort to reduce the lipophilicity of the iodovinyl ligand, while maintaining high specificity and affinity for the transporter, amino- and amido-TBZ analogs have been synthesized as potential precursors in the development of SPECT imaging agents for the vesicular monoamine transporter. As shown in Scheme I, the synthesis of amines, **4a**, **4b**, and **4c**, was achieved by reductive amination reactions involving TBZ and ammonium acetate, propargyl amine and N-methyl propargyl amine, respectively, in the presence of $NaCNBH_3$. Acetamide **5a** was prepared by reacting amine **4a** with acetyl chloride in the presence of triethylamine. Reaction of amine **4a** with 4-iodobenzoyl chloride in the presence of triethylamine afforded benzamide **5b**.



TBZ (1)



[^{3}H]DH-TBZ (2)



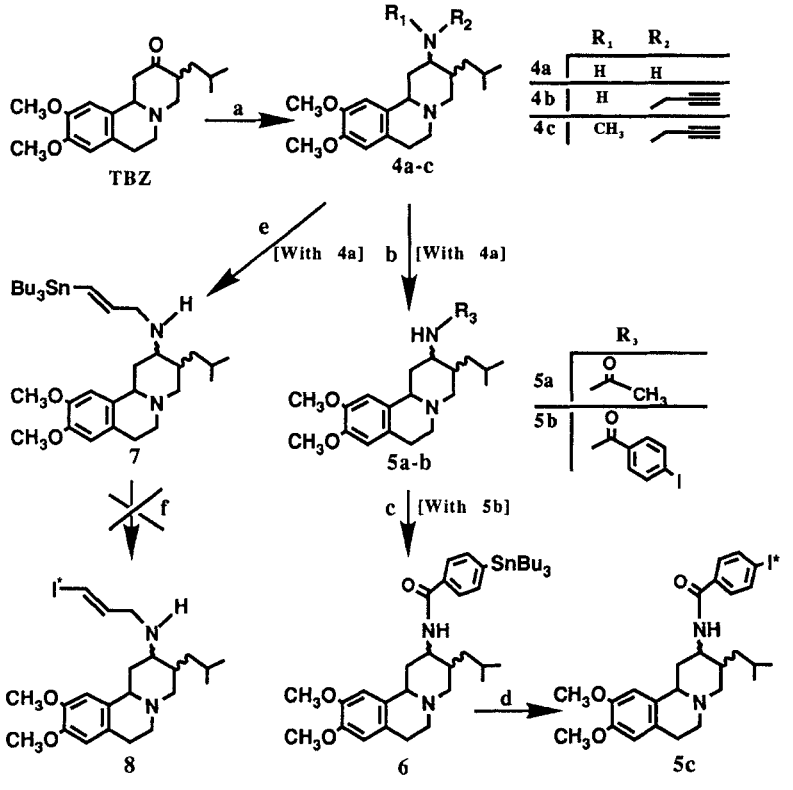
I-TBZ (3a = I-TBZ)
(3b = ^{125}I-TBZ)

Binding experiments performed using [^{125}I]I-TBZ (**3b**-Fraction I) as the radioligand for the monoamine transporter demonstrated that secondary amine **4b** had good affinity for the transporter (Ki = 7.6nM), while tertiary amine **4c** had only moderate affinity (Ki = 72 nM; Table 1). The primary amine **4a** and TBZ were found to have Ki values (32 nM and 6.7 nM, respectively) consistent with those reported by Scherman and coworkers (5). Amide derivatives **5a** and **5b** were poor inhibitors (Ki = 730 nM and >10,000 nM, respectively) of specifically bound [^{125}I]I-TBZ, **3b**. Reaction of compounds **4b** and **5b** with tributytin hydride (AIBN) and hexabutylditin [$(Ph_3P)_4Pd$], respectively, afforded the corresponding tributyltin derivatives **7** and **6**. Attempts to prepare the iodinated amino-TBZ analog **8** were unsuccessful, due, presumably, to the instability of the iodo-allyl functionality. The radioiodinated benzamide analog **5c** was prepared from the tributyltin precursor in good yield, but was found to be very lipophilic (P.C. > 3000) and to have low affinity (Ki > 10,000 nM) for the transporter. Of the compounds evaluated here, the secondary propargylamine analog **4b** exhibits the most promising characteristics (good affinity, presumably lower lipophilicity than **3b**) as a potential ligand for the monoamine transporter. However, we have been unable to successfully prepare the radiolabeled form of the compound using standard iododestannylation techniques. We are currently exploring alternative approaches to decreasing the lipophilicity of this series of TBZ analogs while maintaining the desired high binding affinity.

Acknowledgements: This work is supported by a grant from Nihon Medi-Physics Inc.

Refs: 1. Pierot L., Desnos C., Blin J., Raisman R., Scherman D. et al. - J. Neurol. Sci. 86: 291 (1988).
2. Kilbourn M.R., Frey K.A., Koeppe R.A., DaSilva J.N. et al. - J. Soc. Nucl. Med. 34: 25P, Abstr 92 (1993).
3. Canney D. J., Guo Y-Z., Kung M-P., Kung, H. F. - J. Labeled. Compds. Radiopharm. 33: 355 (1993).
4. Kung M-P., Canney D.J., Guo Y-Z., Billings J.J., Kung H.F. - Soc. Neurosc. (Abstr 202.13), 18: 475 (1992)
5. Scherman, D.; Gasnier, B.; Jaudon, P.; Henry, J-P. - Mol. Pharmacol. 33: 72 (1988).

Scheme I. Synthesis of Amino/Amido-Tetrabenazine (TBZ) Derivatives



a) $CH_3CO_2NH_4$ (1a), propargylamine (1b), or N-methylpropargylamine (1c), $NaBH_3CN$, HCl/CH_3OH; b) acetylchloride (2a) or 4-iodobenzoyl chloride (2b), CH_2Cl_2,triethylamine; c) $(SnBu_3)_2$, $(Ph_3P)_4Pd$, toluene, 100 °C; d) $Na^{125}I$, 0.1 N HCl/EtOH, H_2O_2; e) HSnBu3, AIBN, toluene; f) $Na^{125}I$, 0.1 N HCl/EtOH, H_2O_2, or I_2, $CHCl_3$, r.t.

Table 1. Inhibition Constants of Compounds on the Binding of ^{125}I-TBZ (3b)-Fraction I to Rat Striatal Homogenates[a]

Compound	Ki (nM)	Compound	Ki (nM)[b]
TBZ	6.7±1.2	Haloperidol	424±120
DH-TBZ[b]	8.6±1.8	Reserpine	5373±698
I-TBZ (3a)	25.8±2.9	Atropine	>30,000
4a	32.0±6.4	Paroxetine	>18,000
4b	7.6±1.5	Spiperone	>17,000
4c	72.2±12	Dopamine	>50,000
5a	730±138	NE	>100,000
5b	>10,000	5-HT	>80,000

[a] Kd for [^{125}I]I-TBZ = 0.22 nM. ^{125}I-TBZ (0.2nM) incubated in the presence of 7-11 concs of indicated compds and of membrane prep from rat striata. Each value represents the mean ±SEM of 3-5 determinations. [b] Values taken from ref 4.

[^{18}F]Labeled 1,2-Diacylglycerols ; A New Tracer for the Imaging of Second Messenger System.

TAKAHASHI, T.; IDO, T.; ASHINO, H.; OOTAKE, A.; IWATA, R.; and YANAI, K. Cyclotron and Radioisotope Center, Tohoku University, Sendai, 980 Japan.

The past several years have witnessed increasing interest in the study of the phosphatidyl-inositol (PI) turnover, newly defined as a second messenger system. The system may play a central role in cellular signals in the central nervous system (1,2). From this viewpoint, second messenger imaging in vivo studies using PET has been suggested to be effective for the observation of neuronal functions manifested by the synaptic transmission process. 1,2-Diacylglycerol (1,2-DAG) is one component in the PI turnover. We report here the development of [^{18}F]labeled 1,2-diacylglycerols (1,2-[^{18}F]FDAGs) as a potential second messenger system imaging tracer by PET.

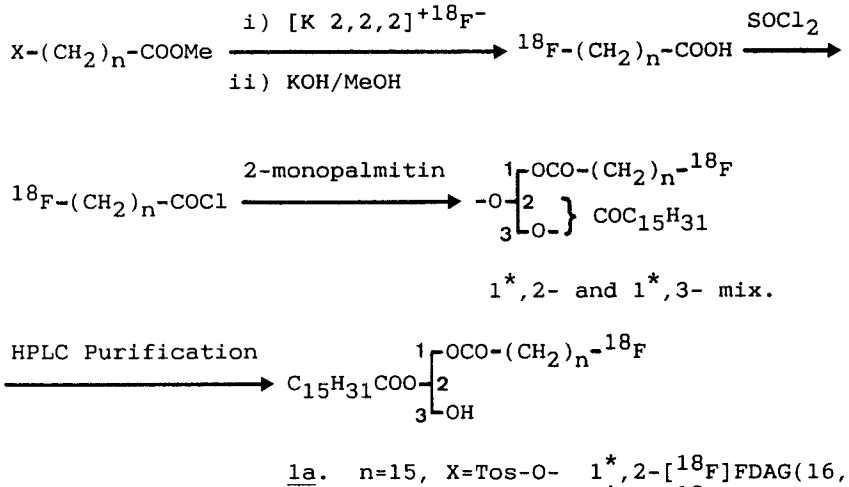
[^{18}F]Labeled 1,2-DAGs at 1-position (1*,2-[^{18}F]FDAG) and 2-position (1,2*-[^{18}F]FDAG) were prepared. ^{18}F was introduced by the substitution reaction according to the method already reported (3,4). The synthetic schemes of 1,2-[^{18}F]FDAGs were shown in Fig. 1 and Fig. 2. In the synthesis of 1,2*-[^{18}F]FDAG (2), the reaction of w-[^{18}F]fluorofatty acid chloride with 1-monopalmitin (Method A) was disadvantageous because 1*,3-[^{18}F]FDAG was a main product. Therefore, we chose the another synthetic route as shown in Fig. 2 (Method B). Compound 3 was synthesized according to a literature procedure (5). While it has been known that acyl group at 2 position in 1,2-diacylglycerol readily undergoes rearrangement to 3-position (6). In both syntheses (Method A and Method B), 1,3-[^{18}F]FDAGs were formed after reaction and the further HPLC purification was needed for removing 1,3-isomers. 1,2- and 1,3-[^{18}F]FDAG could be easily separated by HPLC. The HPLC system is as follows ; Column : YMC-023-5-06 S-5 60A Sil (10 mm x 250 mm), Solvent : Hexane/Ether/isoPrOH=400/80/1.5(V/V), Flow : 7 mL/min. The experimental data for 1,2-[^{18}F]FDAG synthesis are summarized in Table 1.

For the characterization of these 1,2-[^{18}F]FDAGs as ligands for PET measurement, tissue distribution studies and metabolic studies were performed. In tissue distribution studies, two 1,2-[^{18}F]FDAG(16,16) compounds (1a and 2a) showed the similar biodistribution patterns in the blood, liver and brain. On the other hand, 1*,2-[^{18}F]FDAG(8,16) (1b) showed the different biodistribution pattern, lower uptake in the liver and higher uptake in the brain, compared with those of two 1,2-[^{18}F]FDAG(16,16) compounds (1a and 2a). In metabolic studies, the extraction of metabolites from the excised tissue samples was carried out according to Folch's method (7). The extract was analyzed by analytical radio-TLC system and the radioactivity of each spot was estimated with an Imaging Plate and BAS 3000 System (Fuji Photo Film Co., Ltd). The TLC system is as follows ; Plate : Silica gel (Kieselgel 60 F_{254} (MERCK)), Solvent : $CHCl_3$/MeOH/AcOH/H_2O=340/50/50/1.2(V/V). The results are summarized in Table 2. In the brain, 1*,2-[^{18}F]FDAG(16,16) (1a) was mainly incorporated into PI turnover and 1*,2-[^{18}F]FDAG(8,16) (1b) was into phosphatidylcholine (PC) synthesis.

According to these properties, it has been suggested that total chain length is an important determinant of brain uptake and incorporation into PI turnover of 1,2-[^{18}F]FDAGs. Among three radiopharmaceuticals, 1*,2-[^{18}F]FDAG(16,16) (1a) might be a suitable tracer for the imaging of second messenger system by PET.

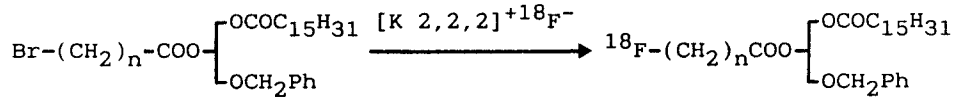
REFERENCES
1. Berridge M. J. – Biochem. J. 220, 345 (1984)
2. Nishizuka Y. – Science 233, 305 (1986)
3. Coenen H. H., Klatte B., Knöchel A. et al. – J. Labelled Compd. Radiopharm. 23, 455 (1986)
4. Takahashi T., Ido T. and Iwata R. – Appl. Radiat. Isot. 42, 801 (1991)
5. Eibl von H., Arnold D., Weltzien H. U. et al. – Liebigs Ann. Chem. 709, 226 (1967)
6. Fischer E. – Ber. 53, 1621 (1920)
7. Folch J., Lees M. and Stanley G. H. S. – J. Biol. Chem. 226, 497 (1957)

Fig. 1 [^{18}F]Labeling of 1,2-DAG at 1 position (Method A)

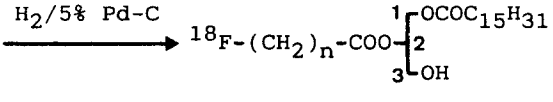


1a. n=15, X=Tos-O- 1*,2-[^{18}F]FDAG(16,16)
1b. n= 7, X=Br- 1*,2-[^{18}F]FDAG(8,16)

Fig. 2 [^{18}F]Labeling of 1,2-DAG at 2-position (Method B)



3.



2a. n=15 1,2*-[^{18}F]FDAG(16,16)

Table 1. Experimental data for 1,2-[^{18}F]FDAG synthesis

	1a	1b	2a
Radiochemical yield (%) [a]	13 - 26	13 - 35	10 - 15
Radiochemical purity (%) [b]	> 97	> 97	> 97
Synthesis time (min) [c]	104 - 135	110 - 120	145 - 160

a) based on $^{18}F^-$ b) determined by analytical HPLC (Conditions - Column : Zorbax Sil (4.6 mm x 250 mm), Solvent : Hexane/Ether/isoPrOH =400/80/1.5(V/V), Flow : 3 mL/min) c) including the time required for HPLC purification

Table 2 Distribution of ^{18}F radioactivity [a]

	% of total activity [b]					
	TAG	DAG	PA	PC	PI	PIP&PIP$_2$ [c]
1*,2-[^{18}F]FDAG(16,16) (1a)						
Brain	13.8	7.7	N.D.	16.0	54.8	5.5
Liver	17.6	1.2	0.4	18.4	58.4	4.0
Blood	84.6	6.1	N.D.	0.3	7.6	1.5
1*,2-[^{18}F]FDAG(8,16) (1b)						
Brain	N.D.	12.0	N.D.	52.6	18.5	13.7
Liver	N.D.	3.1	7.2	73.8	7.6	8.3
Blood	N.D.	3.6	N.D.	60.3	24.0	12.3
1,2*-[^{18}F]FDAG(16,16) (2a)						
Brain	11.6	9.1	N.D.	35.2	36.7	8.4
Liver	55.9	0.7	N.D.	9.4	30.9	3.1
Blood	72.2	1.6	N.D.	12.2	10.4	3.6

a) at 30 min intervals after injection b) based on extracted radioactivity c) TAG : Triacylglycerol, DAG : Diacylglycerol, PA : Phosphatidic Acid, PC : Phosphatidylcholine, PI : Phosphatidylinositol, PIP : Phosphatidylinositol-4-phosphate, PIP$_2$: Phosphatidylinositol-4,5-bisphosphate.

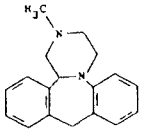
Labeling of Histamine H_1, H_2, and H_3 Receptor Antagonists with Carbon-11 Using On-Line Methylation System: Potential Radiopharmaceuticals for PET Studies.

YANAI, K, RYU, J.H.; WATANABE, T.; HIGUCHI*, M.; FUJIWARA*, T.; ITOH, M*.; IWATA*, R.; TAKAHASI*, T.; IDO*, T. Department of Pharmacology I, School of Medicine and *Cyclotron and Radioisotope Center, Tohoku University, Sendai 980, Japan.

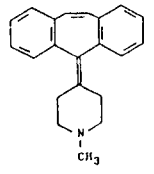
Histamine is now widely recognized as a neurotransmitter acting through the three specific receptors denoted as H_1, H_2, and H_3. Positron emission tomography (PET) is a useful technique for identifying receptors in humans *in vivo*. We have successfully labeled [^{11}C]pyrilamine (an antihistaminics) and [^{11}C]doxepin (a tricyclic antidepressant with high affinity toward H_1 receptors) [1], and validated *in vivo* imaging methods to measure histamine H_1 receptors in normal volunteers using PET [2,3]. We are now applying the measurement of histamine H_1 receptors using PET to various neurological disorders [4,5]. The present article describes the preparation of the following histamine H_1, H_2, and H_3 antagonists with carbon-11 methyliodide as potential radiotracers for PET studies: a) Histamine H-1 antagonists (Non-selective histamine H_1 receptor antagonists such as mianserin and cyproheptadine), b) Histamine H-2 antagonist (^{11}C-nizatidine), c) Histamine H-3 antagonist (S-[^{11}C]Methylthioperamide as a carbon-11 labeled H_3 antagonist).

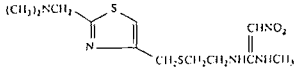
Several histamine antagonists listed below were labeled with carbon-11 by N-alkylation of their desmethylated precursors with [^{11}C]methyliodide, and purified by preparative high performance liquid chromatography. The chemically and radiochemically pure labeled histamine antagonists were obtained with specific radioactivity of approximately 1000-1500 mCi/μmol at end-of-synthesis. The apparatus used in these preparations is the on-line methylation system previously described [6]. The advantages of the synthesis system are simple operation, rapid injection of the reaction mixtures without loss, and reduction of radiation dose to chemists. The preparations were completed in approximately 25 min after end-of-bombardment with radiochemical yields of 40-90 % (based on [^{11}C]methyliodide). *In vivo* distribution studies in mice indicate that carbon-11 labeled H_1 antagonists can easily penetrate the blood-brain barrier and accumulate into the brain. In contrast the H_2 and H_3 antagonists accumulate into brain relatively to a lesser extent. Carbon-11 labeled nizatidine and S-methylthioperamide should be used for imaging H_2 and H_3 receptors, respectively, in peripheral tissues, for example, heart and adrenal gland. The [^{3}H]labeled histamine antagonists were also synthesized with [^{3}H]methyliodide (Amersham) and were used for further characterization of binding *in vivo* and *in vitro*. These ^{11}C-labeled radiopharmaceuticals could be used to image the three distinct histamine receptors with PET.

Mianserin

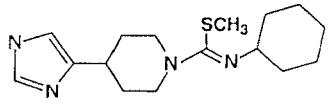


Cyproheptadine





Nizatidine



S-Methylthioperamide

References

1. K. Yanai, R.F. Dannals, A.A. Wilson, H.T. Ravert, *et al.* J. Nucl. Med. Biol. 15: 605-610 (1988)
2. K. Yanai, T. Watanabe, H. Yokoyama, J. Hatazawa, *et al.* J. Neurochem. 59: 128-136 (1992)
3. K. Yanai, T. Watanabe, H. Yokoyama, K. Meguro, *et al.* Neurosci. Lett. 137: 145-148 (1988)
4. K. Yanai, T. Watanabe, K. Meguro, H. Yokoyama, *et al.* NeuroReport 3: 433-436 (1992)
5. K. Iinuma, H. Yokoyama, T. Otsuki, K. Yanai, *et al.* Lancet 341: 238 (1992)
6. R. Iwata, C. Pascali, M. Yuasa, K. Yanai, *et al.* Appl. Radiat. Isot. 43: 1083-1088 (1992)

A New Radioiodinated Ligand for SPECT Studies of Peripheral Benzodiazepine Binding Sites: 4'-Iodo-PK11195.

IIDA Y.; SAJI H.; NAKATSUKA I.*; KATAOKA M.*; MAGATA Y.; YOSHITAKE A.* and YOKOYAMA A. Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto. *Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd., Osaka, Japan.

An increase in the peripheral-type benzodiazepine binding sites has recently been reported in various cerebral disorders, such as ischemia, glioma and glioblastoma. Visualization of peripheral benzodiazepine binding sites has thus been of great interest (1). In this study, taking into account of the superior radiation properties of I-123 and its good spatial resolution of recent SPECT, the development of a radioiodinated ligand with high affinity and selectivity for peripheral benzodiazepine binding sites was attempted. Based on studies on the structure-activity relationship of the 1-phenyl-3-isoquinoline carboxamides, the position 4' in the 1-phenyl ring of PK11195 [1-(2-chlorophenyl)-N-methyl-N-(1-methyl-propyl)-3-isoquinoline carboxamide] was selected as the exploitable site for iodination.

An analog of PK11195 iodinated at position 4' (4'-I-PK11195) was synthesized from p-bromobenzoyl chloride by condensation with 1-methyl-2-hydroxy-2-phenylethylamine, cyclization and followed by oxidation. The 3-isoquinoline carboxlic acid derivative was then condensed with 3-methyl-N-methyl-propylamine to yield 4'-brominated analog of PK11195 (4'-Br-PK11195). The 4'-I-PK11195 was synthesized by a halogen-exchange reaction with 4'-Br-PK11195. The structure of the compound was confirmed by infrared, ^{1}H-NMR and mass spectrometric analyses.

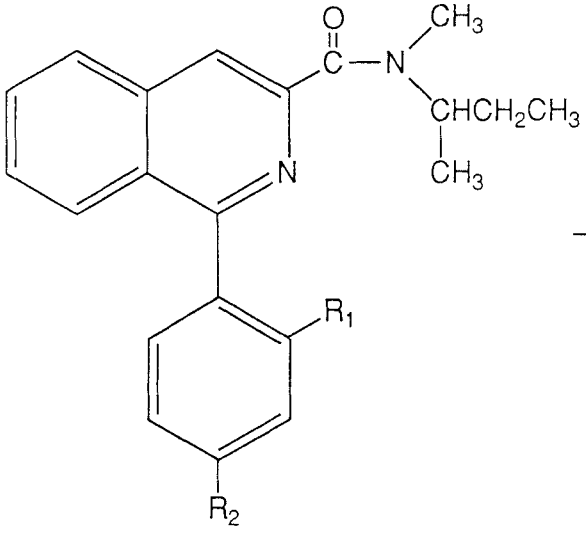
The affinity of 4'-I-PK11195 for peripheral benzodiazepine binding sites was measured from its ability to inhibit specific ^{3}H-PK11195 bindings to rat kidney membranes. The 4'-I-PK11195 showed high affinity for the benzodiazepine binding sites, which were similar as those of PK11195 and its 2'-iodinated analog.

With regard to in vivo biodistribution studies, no-carrier added 4'-^{125}I-PK11195 was obtained by the bromine-radioiodine exchange reaction of 4'-Br-PK11195 under a solid-state condition in 50 - 60 % radiochemical yield of > 98 % purity.

Distribution studies of 4'-^{125}I-PK11195 in mice showed a high uptake by the lungs, adrenals, heart, and kidneys. Although a moderate accumulation of radioactivity in the brain was observed during the early period, the counts in the blood and stomach were low. This biodistribution profile was substantially similar to that reported for PK11195. Thus, this newly designed 4'-I-PK11195 seemed to hold great potential for the SPECT study of peripheral benzodiazepine binding sites. Further in vivo studies including the effect of various drugs and metabolism are now under progress.

1). V.W.Pike, et al., Nucl. Med. Biol., 20: 503-525 (1993).

Fig. 1. Chemical structure of 4'-I-PK11195 and its related compounds.



	R_1	R_2
PK11195	Cl	H
2'-I-PK11195	I	H
4'-I-PK11195	H	I

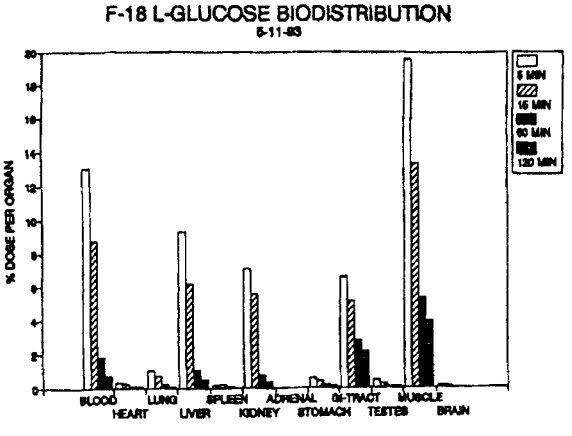
2-[F-18]Fluoro-2-Deoxy-L-Glucose: A New Tracer for Measuring Changes in Intact Brain Transport.

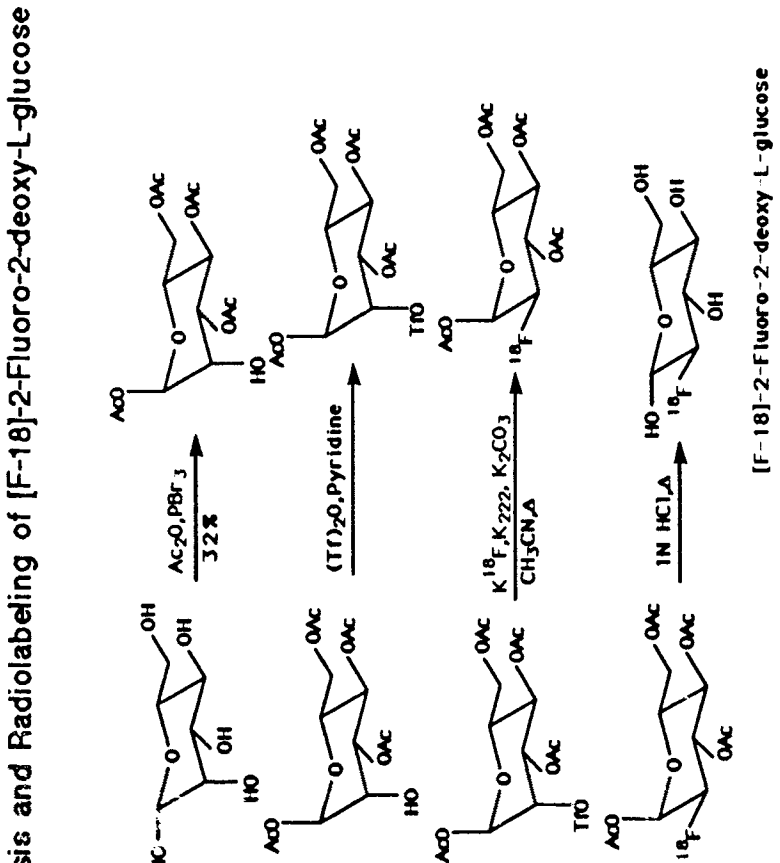
MENG, X-J., ELMALEH, D.R., BROWNELL, A-L. and FISCHMAN, A.J. Department of Radiology, Massachusetts General Hospital, Boston, MA 02114

[F-18] (2-[F-18]fluoro-2-deoxy-D-glucose) is an established PET tracer for measuring glucose metabolism in brain and other tissues. Its optical isomer, L-[F-18]FDG (2-[F-18]fluoro-2-deoxy-L-glucose), is not a substrate for transport in the brain. However, this isomer may be useful for accounting for specific permeability effects in quantitative studies of glucose transport in brain tumors, ischemic injury or other disorders associated with brain transport disruption. A higher contrast in tracer activity between normal and injured brain might be achieved with the use of L-[F-18]FDG than [F-18]FDG because of the lack of uptake of the former in normal brain and its presumptive transport in injured brain tissue.

We synthesized and radiolabeled 2-fluoro-2-deoxy-L-glucose according to the following procedure (Scheme 1). L-mannose was treated with acetic anhydride in the presence of trace amounts of perchloric acid, followed by treatment with phosphorous tribromide and then sodium acetate in aqueous solution, to give pure 1,3,4,6-tetra-o-acetyl-L-mannose, mp 155°C, $[\alpha]_D^{25}$ = + 22.5 in 32% yield (1). This compound was treated with trifluoromethanesulfonic anhydride and pyridine/methylene chloride at O°C to give 1,3,4,6-tetra-o-acetyl-2-o-triflyl-L-mannose (76% yield). The precursor was then reacted with K[F-18]F, Kryptofix K_{222}/K_2CO_3 in acetonitrile at 120°C to give L-[F-18]FDG (22% yield). The biodistribution in rats is presented in Figure 1. As expected, imaging of the monkey brain after IV administration of L-[F-18]FDG did not show brain uptake. In conclusion, this glucose analog could be labeled in the same manner as [F-18]FDG and be used to complement or enhance the information obtained with [F-18]FDG.

1. Deferrari J.O, Gros E.G, Mastronardi I.O.-Carbohyd. Res. 4: 432-434(1967).




Synthesis and Radiolabeling of [F-18]-2-Fluoro-2-deoxy-L-glucose
Ac2O, PBr3
32%
(Tf)2O, Pyridine
K18F, K222, K2CO3
CH3CN, Δ
1N HCl, Δ
[F-18]-2-Fluoro-2-deoxy-L-glucose