Paper K1

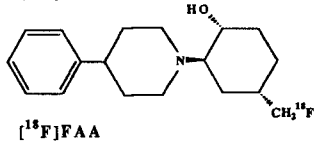
[^{18}F]VESAMICOL DERIVATIVES FOR *IN VIVO* EVALUATION AS TRACERS FOR CHOLINERGIC SYNAPTIC VESICLES

G. Rogers[1], S. Stone-Elander[2,3], L. Eriksson[3], M. Ingvar[3], S. Parsons[1], L. Widén[3]

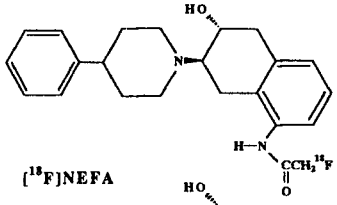
[1]Chemistry & Neurosci Res, Univ of Calif, Santa Barbara, CA, 93106 USA
[2]Karolinska Pharmacy, Box 60024, S-10401 Stockholm, Sweden
[3]Clin Neurophysiology, Karolinska Hospital/Institute, Box 60500, S-10401 Stockholm, Sweden

Vesamicol ((-)trans-2-(4-phenylpiperidino)cyclohexanol) has been shown (1) to bind to a membrane component of acetylcholine containing synaptic vesicles at a site distinct from the transporter active site and inhibits the presynaptic storage of acetylcholine. Through extensive structure-activity studies (2) vesamicol derivatives have been synthesized with varying selectivity for the vesamicol receptor and vesamicol binding protein, as well as varying rates of association and dissociation. Vesamicol derivatives have been recently labelled and evaluated *ex vivo* as SPECT and PET tracers (3 - 6). We present three vesamicol derivatives labelled with no-carrier-added [^{18}F]F$^-$, a kinetic analysis of their whole brain distribution in rats and their regional distribution in monkeys.

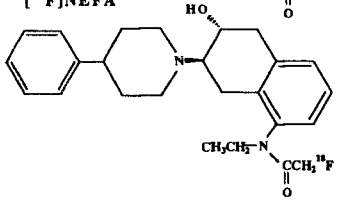
[^{18}F]FMV



[^{18}F]FAA



[^{18}F]NEFA



The tosylate precursors were synthesized from the corresponding alcohols and reacted with the azeotropically dried Kryptofix 2.2.2/K$^+$ complex of [^{18}F]F$^-$ in DMSO at 100 - 130°C for 10 min. After work-up and isolation by reversed-phase HPLC, ^{18}F-labelled FMV, FAA and NEFA were obtained in 25, 5 and 30% yields, radiochemically pure and of high specific activity.

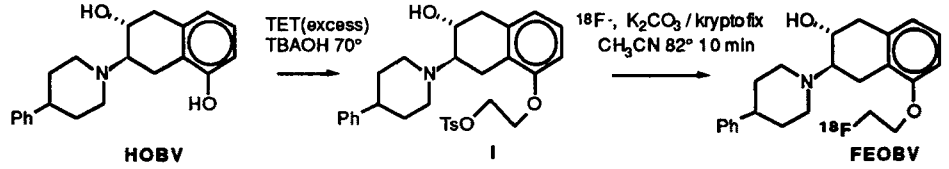
In rats and monkeys FMV was highly extracted into brain and demonstrated an irreversible binding blockable by preinjection of (-)vesamicol (7). FAA was rapidly metabolized within 10 min after i.v. injection. NEFA was extracted to a less degree than FMV, it's regional distribution was less flow-dependent and corresponded well with known patterns of cholinergic innervation. It's binding could be protected by injections of (-)vesamicol. Further characterization of the *in vivo* characteristics of NEFA is therefore underway.

(1) Bahr and Parsons, J. Neurochem. 46: 1214 (1986)
(2) Rogers et al., J. Med. Chem. 32:1217 (1989)
(3) Jung et al., J. Med. Chem. 33: 2068 (1990)
(4) Kilbourn et al., Life Sci. 47: 1955 (1990)
(5) Mulholland et al., J. Cerebr. Blood Flow Metab., 11: S622 (1991)
(6) Efange et al., J. Med. Chem. 34: 2638 (1991)
(7) Widén et al., Neurosci. Lett. in press (1991)

EFFICIENT ONE-STEP SYNTHESIS OF (-)-[^{18}F]FLUOROETHOXY-BENZOVESAMICOL (FEOBV). A NEW TRACER FOR MAPPING CHOLINERGIC NEURONS IN VIVO.

GK Mulholland, Y-W Jung, PS Sherman, TL Pisani, DE Kuhl, DM Wieland and MR Kilbourn.
Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan School of Medicine, Ann Arbor, MI 48109.

Radiolabeled benzovesamicol analogs with high *in vivo* uptake and specific localization in cholinergic tissues are of potential value for early detection of diseases involving alterations in cholinergic function such as Alzheimers. Previously we have examined analogs labeled with radioiodine (1), ^{11}C (2) and ^{18}F (3). We describe here a promising new ^{18}F labeled benzovesamicol which can be made in one step in high yield from tosylate precursor I.



HOBV I FEOBV

Tosylate precursor I was prepared by reaction of 5-hydroxybenzovesamicol (HOBV, made by diazotization of 5-aminobenzovesamicol (4)) with fivefold excess of tosyloxyethyltosylate (TET) and 1 eq. of base in hot CH_3CN, and purified by flash chromatography on silica with pentane: Et_2O solvent. The yields in two preps were 40 and 75%. The (R,R) isomer of I is a crystalline solid (mp 157-159°) which is stable under ambient storage conditions for at least 3 wk.

Labeling conditions similar to those used to make [^{18}F]fluoroethyl tosylate (5) worked well in synthesis of [^{18}F]FEOBV. Purified [^{18}F]fluoride ion (6), kryptofix (10 μmol), K_2CO_3 (3 μmol), and tosylate I (3 μmol) were heated together in 250 μL of CH_3CN. HPLC monitoring of the reaction (C-18, 3:1:1 CH_3CN: MeOH: 20mM pH 6.7 $KHPO_4^-$, 1.5 mL/min) found that maximum incorporation occurs within 10 min at reflux temperature to form a single radioactive peak (7.2 min) coincident with authentic FEOBV, and well separated from I (10 min). The final product [^{18}F]FEOBV was isolated in a high state of purity 15 min after injection of the reaction mixture onto a semi-prep RP-HPLC. Overall yields of formulated [^{18}F]FEOBV (specific activity >2000 Ci/mmol) were in the range of 60% (corrected) in a synthesis time <1hr.

Biodistribution studies of [^{18}F]FEOBV show a localization pattern in rodents that correlates well with cholinergic density in the brain and heart. It is highly extracted by the brain, with > 2% of the injected dose present in the mouse brain at 45 min. Labeled metabolites in the brain could not be detected, and metabolic defluorination, measured as bone activity, was low at 3 hr.

In summary, we report a new ^{18}F labeled benzovesamicol which is made in high yield from a stable precursor. The favorable tissue uptake, localization, and lack of metabolism of [^{18}F]FEOBV seen in preliminary rodent studies give strong support to the use of this new tracer in mapping cholinergic neurons with PET.

Work supported by NIH NS24896, NS22908, NS 15655 and DE-FG2-87ER60561.

(1.) Jung et al., J Med Chem. 33:2065, 1990. **(2.)** Kilbourn et al., Life Sci. 47:1955, 1990. **(3.)** Mulholland et al., J Nucl Med. 32:994, 1991. **(4.)** Rogers et al., J Med Chem 32:1217, 1989. **(5.)** Block et al., J Lab Compd 24:1029, 1986. **(6.)** Mulholland, ARI. 42:1003, 1991.

Paper K3

DEVELOPMENT OF A RADIOTRACER FOR MAPPING ACETYLCHOLINESTERASE IN THE BRAIN IN VIVO

T. Irie, K. Fukushi, Y. Akimoto, H. Tamagami and T. Nozaki
Division of Clinical Research, National Institute of Radiological Sciences, Chiba 260 Japan and Faculty of Hygienic Sciences, Kitasato University, Kanagawa 228 Japan

Selective and intensive decrease in cholinergic enzymes have been reported in the brain of patients with Alzheimer's disease by postmortem biochemical examination. It would be helpful for diagnosis of this disease to develop an in vivo radiotracer for evaluating changes of acetylcholinesterase (AchE). We designed and prepared 7 derivatives of N-[^{14}C]methyl-piperidyl esters (Fig.1) as candidates for mapping AchE. They are expected to be lipophilic for readily crossing the blood-brain barrier into the brain, where they are partly converted into polar radioactive metabolites (aminoalcohol) by enzymes(mainly AchE) and trapped, and partly washed out again from the brain. When the conversion rate of esters by AchE in the brain is not so much faster than the washing out rate of esters, the metabolites converted in the brain would be affected by the enzyme activities. We report the enzymatic characters and biodistributions in mouse brain.

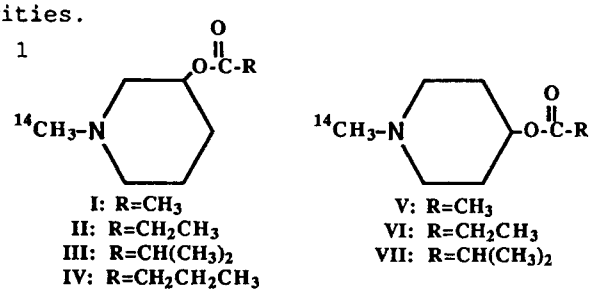
N-[^{14}C]methyl-3-piperidyl esters (I-IV,racemate) and N-[^{14}C]methyl-4-piperidyl esters (V-VII) were synthesized by one step methylation of the corresponding desmethyl amines, 3- and 4-piperidyl esters, with [^{14}C]methyl iodide in acetone at 50-55°C for 30-40 min. The yields were almost 80 % based on [^{14}C]methyl iodide. The hydrolyzed form of esters, N-[^{14}C]methyl-3-piperidinol (MP3OH) and N-[^{14}C]methyl-4-piperidinol (MP4OH), were also synthesized in the same manner.

Selectivity and reactivity of I-VII for enzymatic hydrolysis by AchE were examined in vitro using mouse brain homogenate and a specific AchE inhibitor, BW2854c51. The results (Table 1) showed that esters were relatively high selectivity except butyrate (IV) and that an increase of the C-atom number in acyl group decreased the selectivity and also reactivity.

The brain biodistributions of I-VII were examined in mice at 1, 5, 15, 30 and 60 min. after i.v. injection. All of esters showed high brain uptakes (approximate 10 %dose/g) at 1 min. and the radioactivities were gradually decreased (Fig.2, a typical example) Brain biodistribution were also examined in mice administered with MP3OH and MP4OH, hydrolyzed form of esters. The maximal brain uptakes were 1.09 and 0.51 %dose/g, respectively. These facts indicates that esters freely enter into the brain, where they are hydrolyzed enzymatically and trapped, though the trapped metabolites are slowly eliminated. We compared the regional brain uptakes of tracers with AchE activities and [^{123}I]isopropyl-p-iodoamphetamine(IMP) uptakes as a relative blood-flow indicator. The results summarized in Table 2 showed that in all esters examined the rank order of regional uptakes at 30 min. was striatum >cortex >cerebellum, which was agree with that of regional AchE activities rather than that of regional uptakes of IMP(cortex >striatum >cerebellum). The regional uptakes of esters in three regions were explained as a hyperbolic relation between the uptakes

and AchE activities corrected with blood flows by compartment model analysis. Therefore the tracers uptakes would perceive AchE activities.

Fig. 1



I: R=CH_3
II: R=CH_2CH_3
III: R=$CH(CH_3)_2$
IV: R=$CH_2CH_2CH_3$

V: R=CH_3
VI: R=CH_2CH_3
VII: R=$CH(CH_3)_2$

Fig. 2 Time sequential curves of ^{14}C in the brain of mice administered with I(MP3A)

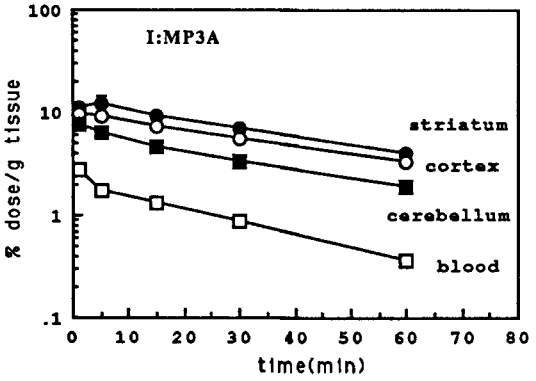


Table 1 First order rate constants of enzymatic hydrolysis in mouse brain homogenate

esters	total rate constant*	% rate constant of AchE to total (selectivity for AchE)
I:MP3A	3.81	99 %
II:MP3P	2.33	96 %
III:MP3IB	1.02	89 %
IV:MP3B	0.24	45 %
V:MP4A	4.43	99 %
VI:MP4P	0.97	97 %
VII:MP4IB	0.10	82 %

* fraction/min for brain tissue concentration of 1g/1ml
rate constants of I-IV were determined as a racemate

Table 2 Comparison between uptakes of esters, and IMP uptakes and AchE activities

	uptakes at 30 min (% dose/g) (N=3)		
	striatum	cortex	cerebellum
I:MP3A	6.97	5.61	3.29
II:MP3P	7.20	4.91	2.51
III:MP3IB	6.84	5.43	3.08
IV:MP3B	2.06	1.73	1.53
V:MP4A	6.44	5.37	2.74
VI:MP4P	7.35	3.95	1.93
VII:MP4IB	3.33	1.60	1.18
IMP uptake* (N = 5)	8.38 ± 0.93	8.72 ± 1.09	7.59 ± 0.95
AchE activity (nmole/min/mg)	56.2 ± 5.4	10.5 ± 1.3	2.3 ± 0.3

* uptakes at 2min postinjection

Iodo-tomoxetine derivatives: Selective ligands for serotonin reuptake sites

Chumpradit, S.; Kung, M.P.; Brooks, B.P.; Szabo, S.A.; Panyachotipun, C.; Prapansiri, V. and Kung, H.F. Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104.

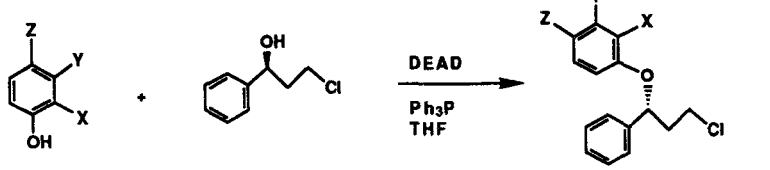
Pharmacological actions of many antidepressants are based on blockage of presynaptic reuptake sites for serotonin (5-HT) or norepinephrine (NE). In order to develop selective radioactive ligands for studying the 5-HT reuptake sites, a series of iodinated derivatives of tomoxetine (TMT) was synthesized and the structure activity relationship was investigated. Using Mitsunobu condition, iodinated cresols or 3-iodophenol were condensed with (R-) or (S-)-3-chloro-1-phenyl-1-propanol.[1] As expected, the reaction resulted in an inversion of the configuration of the chiral center (Scheme 1). The optical purities of these compounds (**1R**, **1S**, **9R**, **9S**) were measured by HPLC (chiral column). In all cases the optical purities were > 99%. To determine the absolute configuration, **9R** and **9S** were debrominated to produced (R-) and (S-)TMT, respectively. Both showed the same configuration (by HPLC) and optical rotation values as previously reported.[1] In vitro competition binding study indicated that **1R**, **2R** and **2S**, which have iodine at the 4-position of the phenyl ring, displayed higher binding affinities to the 5-HT reuptake sites (Table 1). Stereoselectivity was observed in **1R** vs. **1S**, containing a 2-methyl group. Changing the position of the methyl group (**2R**, **2S**, **3R**, **3S**) resulted in a dramatic loss of stereoselectivity. The substitution of the N-methyl group with N-ethyl, N-propyl and N-cyclopropyl resulted in derivatives with much lower binding affinity for 5-HT uptake sites. Due to its high affinity and better selectivity for 5-HT reuptake sites, compound **1R** was chosen for radioiodination and further characterization. The [^{125}I]-labeled **1R** ([^{125}I]**1R**) was prepared from the corresponding tributyl-tin derivative via a hydrogen peroxide-catalyzed iododestannylation reaction (Scheme II). In vitro binding studies of [^{125}I]**1R** displayed high affinity and saturable binding to rat cortical membrane preparations (Kd=0.03nM)(Figure 1). The competition data with various compounds (Table 2) demonstrated that [^{125}I]**1R** is a very selective ligand with the pharmacological profile corresponding to a serotonin reuptake blocker. These data suggest that these iodinated ligands may be useful as potential tools for mapping serotonin neuron projections in vivo and in vitro.

Reference:

1. Srebnik M., Ramachandran P.V. and Brown H.C.- J.Org.Chem. 53:2916(1988).

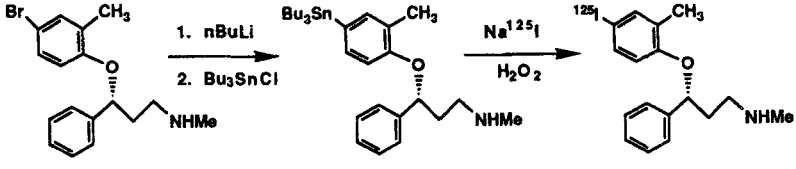
Acknowledgments: This work is supported by grants awarded by NIH (NS-24538 and MH-48125.

SCHEME I



Compounds	X	Y	Z	R
1R, 1S	CH_3	H	I	CH_3
2R, 2S	H	CH_3	I	CH_3
3R, 3S	I	H	CH_3	CH_3
4R, 4S	H	I	H	CH_3
5R, 5S	CH_3	H	I	C_2H_5
6R, 6S	CH_3	H	I	C_3H_7
7R, 7S	CH_3	H	I	
8R, 8S	CH_3	H	I	H
9R, 9S	CH_3	H	Br	CH_3
(R, S) Tomoxetine (TMT)	CH_3	H	H	CH_3

SCHEME II



9R

[^{125}I]1R

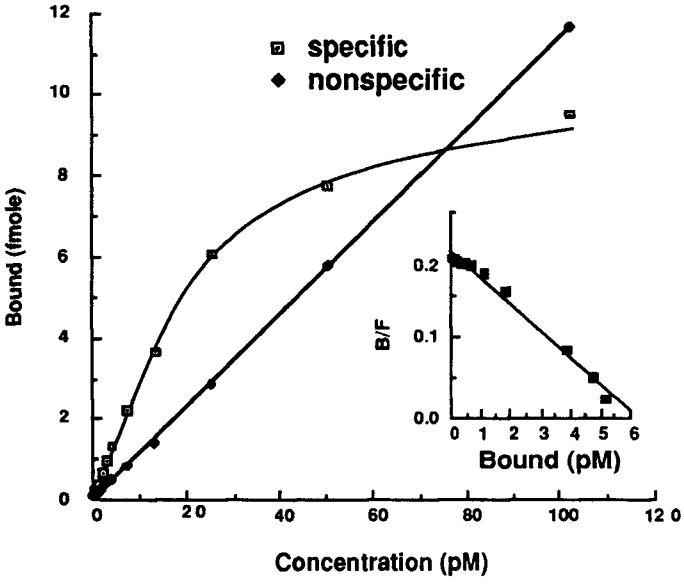
Table 1. Inhibition of [^{3}H]citalopram binding to serotonin uptake sites in rat cerebral cortical membranes

Compound	Ki (nM, mean±SEM)	Compound	Ki (nM, mean±SEM)
Paroxetine	0.08 ± 0.01		
R-TMT	25.1 ± 1.0		
1R	0.65 ± 0.04	**3R**	5.18 ± 0.51
1S	13.9 ± 1.0	**3S**	5.73 ± 1.14
2S	0.30 ± 0.06	**4R**	14.4 ± 3.0
2R	0.69 ± 0.13	**4S**	4.0 ± 0.5

Table 2. Inhibition of [^{125}I]1R binding to rat cortical membrane preparations

Drug	Ki (nM, mean±SEM)	Drug	Ki (nM, mean±SEM)
Paroxetine	0.06 ± 0.003	Serotonin	129 ± 20
1R	0.16 ± 0.01	(±)Propranolol	812 ± 105
1S	2.33 ± 0.25	Atropine	>5000
Chloroimipramine	0.10 ± 0.01	Clonidine	>10000
R-Nisoxetine	70 ± 14	Spiperone	>2000
R-Tomoxetine	9.1 ± 1.0	Mianserin	>2000
Zimelidine	22.3 ± 1.8	Naloxone	>5000

Figure 1. In vitro binding of [^{125}I]1R in rat cortex membrane (K_d =0.03nM)



Paper K5

CARBON-11 LABELED ALPHA-2 ADRENERGIC RECEPTOR ANTAGONIST: SYNTHESIS of [^{11}C]WY26703 AND ITS BIODISTRIBUTION IN RODENTS.

R.C. Pleus[1], C.-Y. Shiue[2], G.G. Shiue[2], J.A. Rysavy[2], H. Huang[2], M.P. Frick[2], and D.B. Bylund[1]. 1. Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE; 2. Center for Metabolic Imaging, Creighton University, Omaha, NE, USA.

Norepinephrine and epinephrine are important neurotransmitters in the central nervous system and alpha-2 adrenergic receptors (A2ARs) mediate some of their physiologic effects. In humans A2AR agonists ameliorate the symptoms of opiate withdrawal and depression and exert anesthesia. A2AR density is increased on platelets of depressed patients (1) and these elevated A2AR levels can be reversed to normal values with tricyclic antidepressant treatment (2). A2AR density is decreased on platelets in patients with borderline personality disorder (3). A2ARs can be pharmacologically divided into four subtypes (4,5). WY26703, an antagonist, has selectively high affinity for A2ARs. K_i values derived from inhibition studies using [^{3}H]rauwolscine as the radioligand for A2ARs, ranged from 0.52 to 1.55 nM in tissues that express a single A2AR subtype (i.e., 2A, 2B, and 2C). Therefore, it seemed worthwhile to label this ligand with a positron emitting isotope and to study its distribution in the tissues of rodents.

No-carrier-added [^{11}C]WY26703 (II) was synthesized by methylation of WY27050 (I) with [^{11}C]H_3I followed by purification with HPLC (C_{18}, 10 x 300 mm Spherisorb 5 ODS, Phenomenex; CH_3CN:0.1 M ammonium formate (60:40) as the solvent with a flow rate of 7.5 ml/min) (Scheme 1). The radiochemical yield of [^{11}C]WY26703 was 14% in a synthesis time of 35 minutes from EOB. Radiochemical purity was 98% and specific activity was 1.53 Ci/μmol (EOB).

The anatomical biodistribution of [^{11}C]WY26703 in mice revealed that the compound passes the blood brain barrier and that peak [^{11}C] activity occurs at the 5 minute post-injection interval in the brain, heart, lungs, spleen, and kidneys (Table 1). [^{11}C] values for 30 and 60 minutes post-injection were approximately 50% lower than the 5 minute interval but relatively constant. In rat studies, the A2AR antagonist atipamezole was used to compete for A2ARs *in vivo*. Atipamezole treated rats had less [^{11}C] binding in brain, lungs, and kidneys compared to their controls, however, only brain values reached statistical reliability (Table 2). These data suggest that [^{11}C]WY26703 has nM affinity for the A2ARs, crosses the blood-brain barrier, and its binding can be inhibited by atipamezole. These data suggest that [^{11}C]WY26703 may be useful to image A2ARs by PET.

WY27050 and WY26703 and atipamezole were generous gifts from Wyeth Research Ltd. (Berkshire, UK) and Farmos Pharmaceuticals (Turku, Finland), respectively.

1. Piletz, J.E., Schubert, S.P., Halaris, A., - Life Sci. 39, 1589 (1986).
2. Piletz, J.E., Halaris, A., Saran, A., Marker, M.R., - Arch. Gen. Psychiat. 48, 813 (1991).
3. Southwick, S.M., Yehuda, R., Giller, E.L., Perry, B.D., - Am. J. Psychiat. 147, 1014 (1990).
4. Blaxall, H.S., Murphy, T.J., Baker, J.C., Ray, C., Bylund, D.B., - J. Pharmacol. Exp. Ther. 259, 323 (1991).
5. Simonneaux, V., Ebadi, M., Bylund, D.B., - Mol. Pharmacol. 40, 235 (1991).

Scheme 1. Synthesis of No-Carrier-Added [^{11}C] WY26703

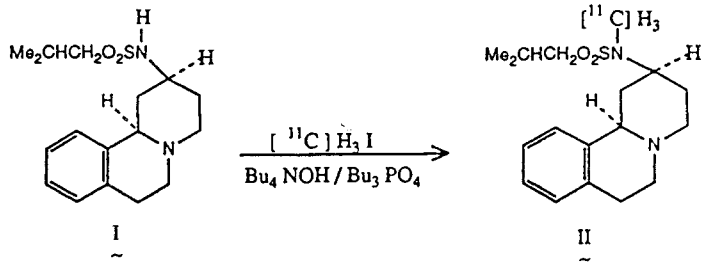


Table 1. TISSUE DISTRIBUTION OF [^{11}C]WY26703 IN MICE
% Dose/g (mean ± SEM) (n = 7-8)

Organ	Time after injection*		
	5 mins	30 mins	60 mins
Brain	4.4 ± 0.4	1.9 ± 0.1	1.5 ± 0.1
Blood	1.0 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
Heart	2.9 ± 0.3	1.2 ± 0.1	1.1 ± 0.1
Lungs	7.9 ± 0.6	3.8 ± 0.2	3.3 ± 0.2
Liver	6.1 ± 0.4	6.5 ± 0.7	5.5 ± 0.7
Spleen	5.1 ± 0.4	3.5 ± 0.6	3.0 ± 0.2
Kidneys	7.0 ± 0.6	3.8 ± 0.3	3.1 ± 0.3
Sm Intestine	5.4 ± 0.5	6.6 ± 0.8	7.5 ± 0.8
Muscle	2.0 ± 0.2	0.8 ± 0.1	0.8 ± 0.1

Table 2. DISTRIBUTION OF [^{11}C]WY26703 IN RAT WITH OR WITHOUT CO-ADMINISTRATION OF 3 mg/kg ATIPAMEZOLE
% Dose/g (mean ± SEM) (n = 4-5)

	Whole Tissue		Tissue Homogenized and Filtered (% injected dose/g tissue)		
Organ	Saline	Atipamezole	Saline	Atipamezole	% Inhibition
Brain	0.61 ± 0.05	0.48 ±0.03†	0.15 ± 0.03	0.07 ± 0.01*	53
Lung	1.21 ± 0.14	0.92 ± 0.12	0.33 ± 0.04	0.20 ± 0.05	40
Kidney	0.96 ± 0.08	0.81 ± 0.04	0.18 ± 0.03	0.12 ± 0.03	33
Heart	0.41 ± 0.04	0.38 ± 0.03	-	-	-

Rats were co-injected with .25 ml of [^{11}C]WY26703 with or without 3 mg/kg atipamezole, i.v., under light anesthesia. Ten minutes later, rats were sacrificed and organs harvested. * $p<.04$ Student's one tailed t-test, † $p<.07$ Student's one tailed t-test as compared to saline.

Paper K6

[^{11}C]RX 821002 as a Potential PET Radioligand for Central α_2 Adrenoceptors

Pike V.W., Hume S.P., Aigbirhio F., Turton D.R and Nutt D.J.[1]

MRC Cyclotron Unit, Hammersmith Hospital, Ducane Road, London W12 OHS, U.K.

[1]Reckitt & Colman Psychopharmacology Unit, School of Medical Sciences, University Walk, Bristol BS8 1TD, U.K.

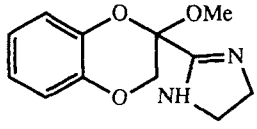
Continuing investigation of the role of central α_2 adrenoceptors is of interest in elucidating the biochemical basis of clinical depression and of other affective disorders.[1] So far it has not been possible to pursue these studies in living man through lack of an effective radioligand for the external detection of α_2 receptors. RX 821002 [2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline] (I) is a potent and highly selective antagonist at central α_2 receptors. Tritiated RX 821002 has been used successfully to identify α_2 receptors *in vitro* in rat brain,[3] in a human cell-line[4] and in human frontal cortex.[5] These favourable *in vitro* characteristics suggest that suitably labelled RX 821002 might also behave as an effective radioligand *in vivo*. We therefore sought to explore this possibility.

The biodistribution of radioactivity in the brains of rats following *i.v.* administration of [^{3}H]RX 821002 was measured by *ex vivo* dissection and scintillation counting. A regional distribution of radioactivity was clearly detectable from 30 min after injection, with α_2 receptor-rich regions showing the highest sustained uptake. Pre-dosing the rats with a potent α_2 receptor antagonist, idazoxan, resulted in the rapid loss of radioactivity from all regions down to plasma levels at 60 min after injection of [^{3}H]RX 821002 (*e.g.* for entorhinal cortex, see Figure 1). By the application of compartmental models to this time-radioactivity data it was possible to calculate regional *binding potentials* (B_{max}/K_D values) for α_2 receptors, ranging from 0.2 for cerebellum to 1.9 for hypothalamus. The regional distribution of binding potential correlated closely with the distribution of specific binding observed[3] *in vitro* (Figure 2). These results show that [^{3}H]RX 821002 can be used experimentally to mark central α_2 receptors *in vivo*. Furthermore, the size and rate of expression of the α_2 receptor-specific signal seen with [^{3}H]RX 821002 justify the development of [^{11}C]RX 821002 for evaluation as a radioligand for PET studies of the central α_2 receptor.

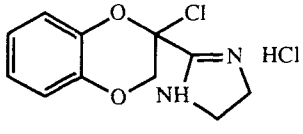
RX 821002 (I) contains an *O*-methyl group on a quaternary carbon bonded to oxygen and an imidazolidino group. These structural considerations suggested the possibility to label RX 821002 (I) by methanolysis of a suitable halo precursor with n.c.a. [^{11}C]methanol, a previously much neglected labelling agent.[6] The chloro precursor (II) was found to convert smoothly into RX 821002 (I) in acetonitrile (500 μL) containing *ca* 1% methanol and trace ammonia solution at 90 - 100 °C. Nonetheless, the use of n.c.a. [^{11}C]methanol under these conditions fails to give any significant labelling. However, the addition of silver oxide to this reaction medium results in the formation of n.c.a. [^{11}C]RX 821002 (III) as the major non-volatile labelled product (Scheme 1) as assessed by TLC with autoradiography on two systems. Work is in progress to optimize the radiochemical yield in order to provide sufficient [^{11}C]RX 821002 for evaluation as a PET radioligand *in vivo*.

Acknowledgement. The authors are grateful to Dr C.B. Chapleo (Reckitt & Colman Products Ltd) for the gift of compounds related to RX 821002.

1. Crews F.T. and Smith C.B., *Science*, 1978, **202**, 322.
2. Crews F.T. and Smith C.B., *J. Pharmacol. Exp. Ther.*, 1980, **215**, 143.
3. Hudson A.L., Mallard, N.J., Tyacke R. and Nutt D.J., *Mol. Neuropharmacol.*, 1991. In Press.
4. Langin D., Lafontan M., Stillings M.R. and Paris H., *Eur. J. Pharmacol.*, 1989, **167**. 95.
5. Vauquelin G., de Vos H., de Backer J.-P. and Ebinger G., *Neurochem. Int.*, 1990, **16**, 537.
6. Watkins G.L., Jewett D.M. and Kilbourn M.R., *J. Label. Compd Radiopharm.*, 1989, **26**, 506.

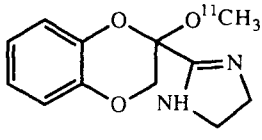


RX 821002 (I)



(II)

n.c.a. [^{11}C]Methanol
MeCN/AgO_2/NH_3
15 min , 100 °C



(III)

Scheme 1: Radiosynthesis of n.c.a. [^{11}C]RX 821002

Figure 1. Time course for radioactivity uptake into entorhinal cortex after i.v. injection of tritiated RX 821002 into non-predosed rats (closed symbols) and rats predosed with idazoxan (open symbols).

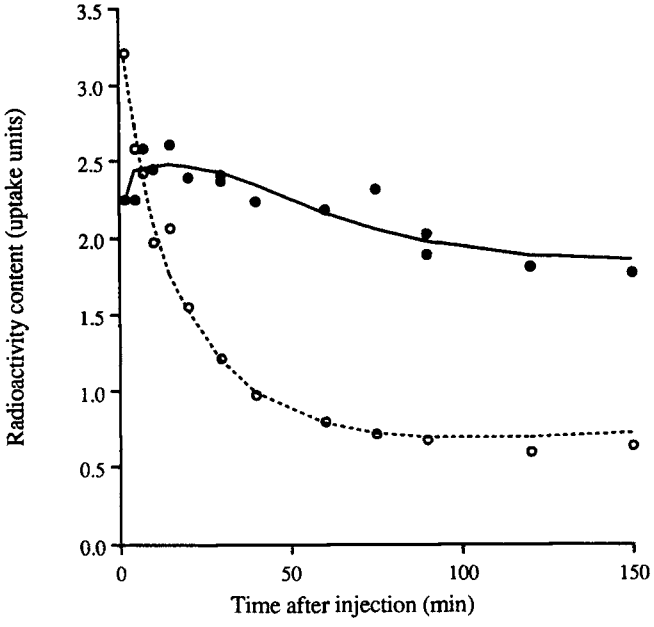
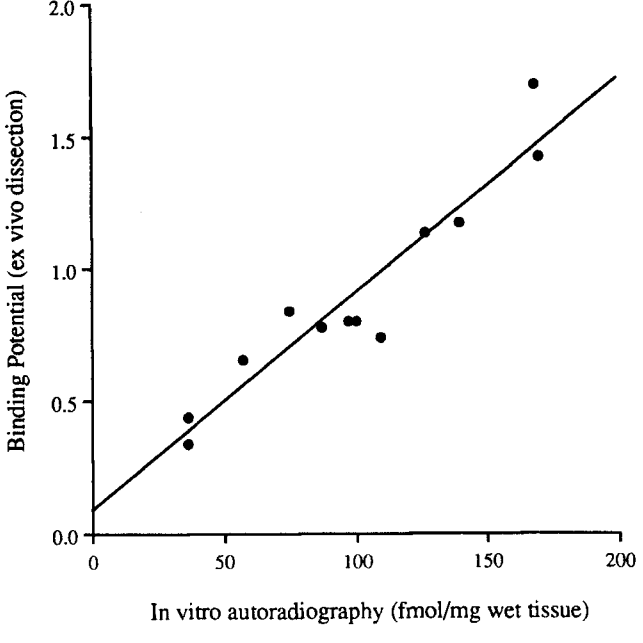


Figure 2: Correlation of regional binding potentials for tritiated RX 821002 in regions of rat brain from this study with in vitro binding distribution from autoradiography (reference 3).



Paper K7

SELECTIVE N.C.A. ^{11}C-LABELLING OF TRAMADOL AND ITS MAJOR METABOLITES FOR PET PHARMACOKINETICS

R. Gail, H.H. Coenen, K. Hamacher, G. Stöcklin
Institut für Nuklearchemie, Forschungszentrum Jülich, 5170 Jülich, Germany

Tramadol is a strong analgetic which acts at least partially at opiate receptors. Its biotransformation proceeds rather rapidly via N- and/or O-desmethylation to the metabolites M2 and/or M1 (1), the O-desmethyl derivative M1 (1-(3-hydroxyphenyl)-2-dimethylaminomethyl-cyclohexan-1-ol) having also analgetic properties (2). For in vivo studies of the regional cerebral pharmacokinetics of Tramadol and its metabolites in mouse and man with PET, the (+) and (-) isomers were prepared labelled in the N- and O-methyl group, respectively (cf. Fig. 1).

Reaction parameters of methylation at various functional groups were studied in detail such as the dependence on educt concentration, reaction time, amount of base added, and of carrier methyl iodide. The phenolate exhibited a much higher reactivity than the secondary amino group while quaternization of tertiary amine was only observed at higher methyl iodide concentration. [O-methyl-^{11}C]Tramadol 1 was obtained in enantiomerically pure form from the O-desmethyl derivate M1 at room temperature with n.c.a. [^{11}C]methyl iodide in dimethylsulfoxide in presence of NaOH. After 5 min reaction time radiochemical yields above 85% were achieved. Similarly, the [N-methyl-^{11}C]Tramadol 2 was synthesized starting from the N-desmethyl derivative M2 with radiochemical yields up to 90% without NaOH addition.

The n.c.a. methylation of the bis-desmethyl compound (1-(3-hydroxyphenyl)-methylaminomethyl-cyclohexan-1-ol (3) was of special interest in view of the possible intramolecular competition reactions (cf. Fig. 2). In the absence of base the N-^{11}C-methylated M1(4) is exclusively formed (> 95% RCY), while the O-^{11}C-methylated M2(5) is only obtained together with ^{11}C-M1 even at high concentration of NaOH (Fig. 3). An optimal radiochemical yield of 5 is reached at a molar ratio of 3.5/1 of base to 3 with 70% RCY.

In agreement with animal studies, pharmacokinetics in man with PET showed no enantiomeric selectivity for ^{11}C-Tramadol. The metabolite ^{11}C-M1 exhibited a tenfold smaller brain uptake, indicating a small - if any - contribution to the CNS analgetic effect.

1. **Frankus E., Friderichs E., Kim S.M., Osterloh G. - Arzneim.-Forsch./Drug Res. 28: 114 (1978)**
2. **Lintz W., Eerlacin S., Frankus E., Uragg H. - Arzneim.-Forsch./Drug Res. 31: 1932 (1981)**

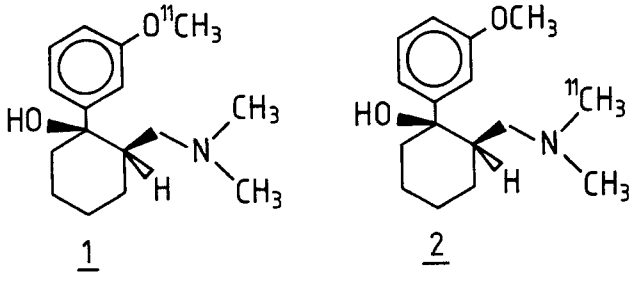


Fig. 1 Structure and labelling position of ^{11}C-Tramadol

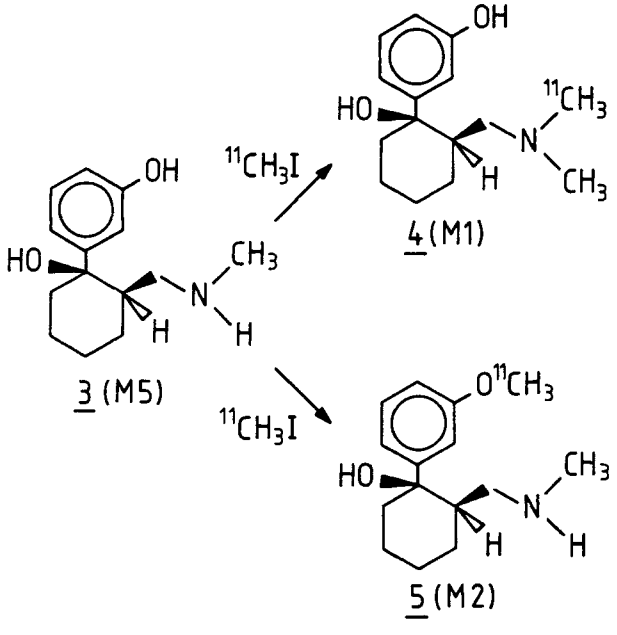


Fig. 2 Competing reactions in ^{11}C-methylation of (+) and (-) 1-(3-hydroxyphenyl)-methylaminomethyl-cyclohexan-1-ol

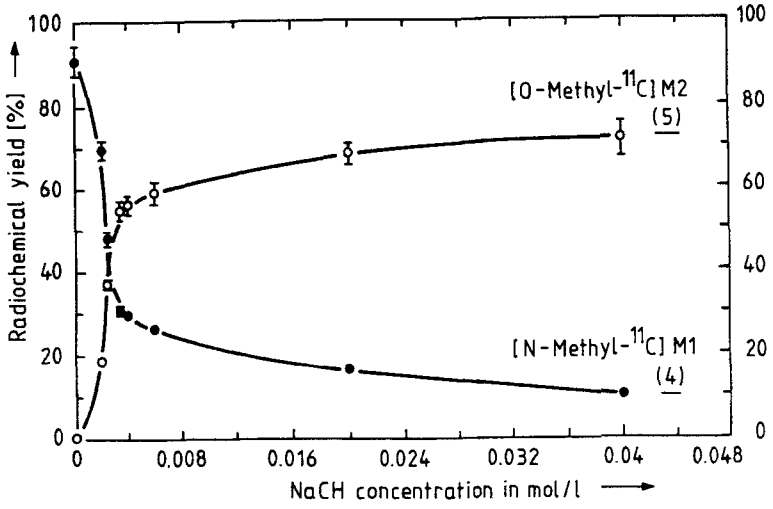


Fig. 3 Influence of base concentration on the ^{11}C-methylation of (+) and (-) 1-(3-hydroxyphenyl)-methylaminomethyl-cyclohexan-1-ol (C(3) = 1.1 10^{-2} mol/l, 80°C, 10 min, 0.5 ml DMSO, nc.a. [^{11}C]CH_3I)

Fluorine-18 Labeled BMY 14802: Synthesis and Anatomical Distribution in Rodents.

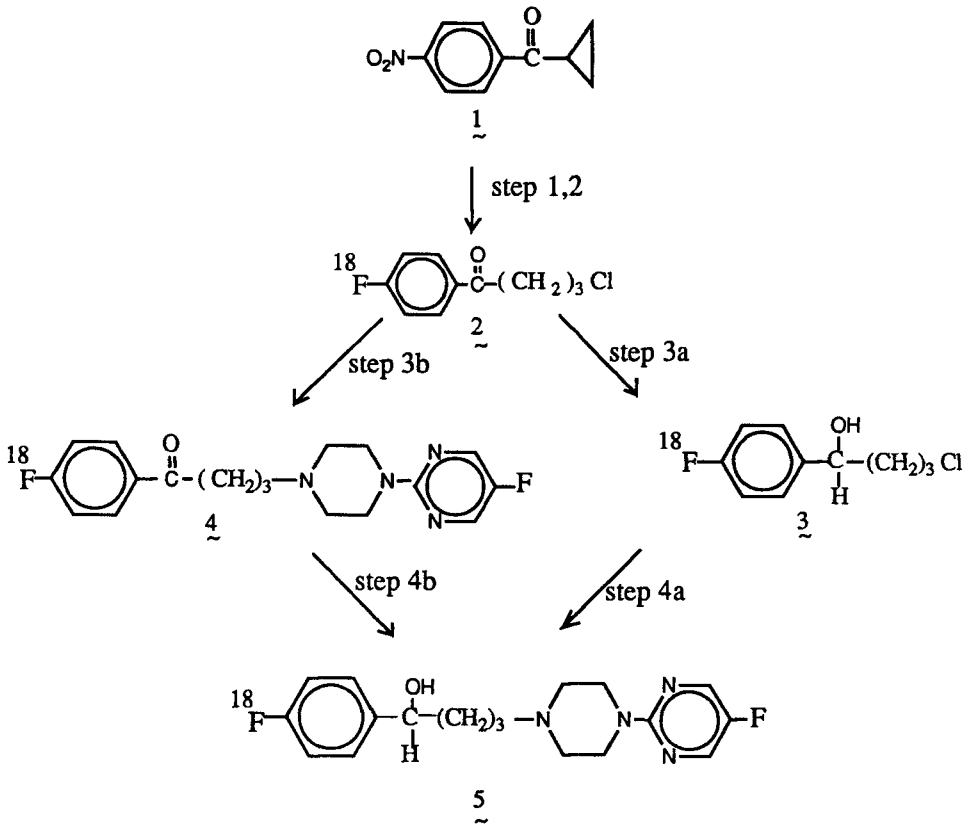
C.-Y. Shiue, L.-Q. Bai, G.G. Shiue, J.A. Rysavy, R.C. Pleus[+], H. Huang, M.P. Frick, J.D. Catt[++] and J. P. Yevich[++]. Center for Metabolic Imaging, Creighton University, 901 Dorcas Street, Omaha, NE 68108. [+]Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198, [++]Bristol-Meyers Squibb Pharmaceutical Research Institute, Wallingford, CT.

BMY 14802, α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol is an agent with antipsychotic properties without the side effects of the existing neuroleptics (1-4). Its mechanism of action is different from conventional antipsychotics and has been shown to bind to σ-receptors with stereoselectivity. The dextrorotatory enantiomer, (+)BMY 14802, is about ten times more potent than its levorotatory isomer (3). In order to study its pharmacokinetic in animals, we have synthesized (±)[^{18}F]BMY 14802 and studied its anatomical distribution in rodents.

Fluorine-18 labeled (±)BMY 14802 (5) was synthesized by two methods (Scheme 1). Nucleophilic substitution of 1 with K[^{18}F]/Kryptofix 2•2•2 followed by hydrolysis, alkylation (5), reduction and purification with HPLC (semi-preparative C_{18} column, 10 x 300 mm, CH_3CN:0.1M HCO_2NH_4, 40:60, 6.5 ml/min) gave compound 5 in approximately 5% yield in a synthesis time of 140 minutes from EOB. The alternative method of the synthesis was to reduce 4-chloro-4'-[^{18}F]fluorobutyrophenone (2) to the corresponding alcohol (3), followed by alkylation and purification with HPLC to give compound 5 in 7% yield in a synthesis time of 130 minutes from EOB. Compound 3 has also been used to prepare reduced haldol (6) in 6% yield. Following tail vein injection, the initial uptake of compound 5 in mouse brain was high but the radioactivity then declined rapidly (6% and 0.3% respectively at 5 and 120 minutes post-injection). The initial uptake of compound 5 in mouse liver was also high, but the radioactivity then declined slower than from the brain (11% and 6% respectively at 5 and 120 minutes post-injection). The radioactivity in the femur did not increase with time, indicating *in vivo* defluorination may not occur (Table 1). The uptakes of compound 5 in rat brain regions with high concentration of σ-receptors (7-9) (cerebellum, brain stem, hippocampus and spinal cord) were significantly reduced by prior treatment with 2.5 mg/kg of haldol (Table 2). These results suggest that α-(4-fluorophenyl)-4-chlorobutanol (3) may be a versatile reagent for the synthesis of other neuroleptics and that compound 5 binds to σ-receptors. The synthesis of (+)- and (-)-[^{18}F]BMY 14802 and their distributions in rodents are in progress.

1. Yevich J.P. and Lobeck G.Jr. - U.S. Patent 4,605,655(1986)
2. Matthews R.T., McMillen B.A., Sallis R. and Blair D. - J.Pharmacol.Exp.Ther. 239: 124-131(1986)
3. Taylor D.P. and Dekleva J. - J. Drug Dev.Res. 11: 65-70(1987)
4. Wachtel S.R. and White F.J. - J.Pharmacol.Exp.Ther. 244: 410-416(1988)
5. Shiue C.-Y., Fowler J.S., Wolf A.P., McPherson D.W., Arnett C.D. and Zecca L. - J.Nucl.Med. 27: 226-234(1986)
6. Ravert H.T., Dannals R.F., Wilson A.A., Wong D.F. and Wagner H.N.Jr. - J.Label.Compds. and Radiopharm. XXIX: 337-343(1991)
7. Tam S.W. - Proc.Natl.Acad.Sci. 80: 6703-6707(1983)
8. Walker J.M., Bowen W.D., Walker F.O., Matsumoto R.R., Costa B. and Rice K.C. - Pharmacol.Rev. 42: 355-402(1990)
9. Gundlach A.L., Largent B.L. and Snyder S.H. - J.Neurosci. 6: 1757-1770(1986)

Scheme 1: Synthesis of (±) [^{18}F] BMY 14802



Step 1= K [^{18}F] /Kryptofix 2.2.2
Step 2= HCl-MeOH
Step 3a= $NaBH_4$ / MeOH
Step 3b= 5-fluoro-2-(1-piperazinyl) pyrimidine
Step 4a= 5-fluoro-2-(1-piperazinyl)pyrimidine
Step 4b= $NaBH_4$ / MeOH

Table 1 Tissue Distribution of (+)[^{18}F]BMY 14802 in Mice
% Dose/Gram (Mean ± SD) (n = 4)

Organ	Time After Injection			
	5 minutes	30 minutes	60 minutes	120 minutes
Blood	2.07 ± 0.55	1.11 ± 0.52	0.50 ± 0.08	0.71 ± 0.48
Brain	6.06 ± 0.88	0.87 ± 0.29	0.38 ± 0.05	0.25 ± 0.04
Heart	3.05 ± 0.41	0.90 ± 0.26	0.46 ± 0.07	0.40 ± 0.07
Lungs	9.93 ± 2.00	2.33 ± 0.80	1.13 ± 0.21	0.81 ± 0.15
Liver	11.60 ± 1.25	6.85 ± 1.91	6.18 ± 1.25	6.82 ± 1.45
Spleen	6.82 ± 1.08	2.15 ± 0.84	1.49 ± 0.45	0.79 ± 0.17
Kidneys	8.22 ± 0.62	4.22 ± 1.15	2.79 ± 0.38	2.02 ± 0.22
Small Intestine	8.07 ± 0.86	6.72 ± 4.47	7.88 ± 2.16	5.16 ± 1.63
Femur	2.39 ± 0.27	0.70 ± 0.05	0.48 ± 0.06	0.62 ± 0.18

Table 2 Distribution of (+)[^{18}F]BMY 14802 in Rat Brain One Hour After Injection (n = 3-4)

Region	Pretreatment*	% Dose/Gram (Mean ± SD)
Striatum	S	0.16 ± 0.03
	H	0.12 ± 0.02
Cerebellum	S	0.15 ± 0.03
	H	0.10 ± 0.01
Hippocampus	S	0.18 ± 0.03
	H	0.12 ± 0.02
Brain Stem	S	0.25 ± 0.04
	H	0.12 ± 0.01
Rest of Brain	S	0.19 ± 0.03
	H	0.11 ± 0.01
Spinal Cord	S	0.26 ± 0.04
	H	0.13 ± 0.02

*Rats were pre-treated with ethanol-saline (2:1)(S) or with 2.5 mg/kg of haldol in ethanol-saline (2:1)(H).

Paper K9

Comparison of *S*-[^{11}C]CGP 12177 metabolism in rat, dog and man using solid phase extraction and HPLC

Luthra S.K., Osman S. Steel. C. J., Law M.P., Rhodes C. G., Vaja V., Ahier R., Brady F., Waters S.L. De Silva R., Hughes J.M.B and Camici P.G.
MRC Cyclotron Unit and Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 OHS, U.K.

The hydrophilic ß-blocker CGP 12177 (CGP) labelled with [^{11}C] has been proposed as a tracer for studying the β-adrenergic receptors *in vivo* by PET (1). Determination of the percentage of unchanged [^{11}C]CGP is required for the correction of the arterial input function which is necessary for kinetic analysis of PET data. This work reports a method for determination of unchanged [^{11}C]CGP in rat, dog and human plasma using an automated solid phase extraction and HPLC.

R,S and *S*-[^{11}C]CGP were prepared as previously described and the chemical structure and the optical purity of the products were verified using mass spectrometry and circular dichroism (2).

Rats (Sprague Dawley), dogs (Greyhounds) and eight human subjects were studied. The rats were injected with 37 kBq of either *R,S*-[^{11}C]CGP or *S*-[^{11}C]CGP. The dogs were given 470±325 MBq (mean ±SD) of *S*-[^{11}C]CGP. The human subjects received an i.v. injection of *S*-[^{11}C]CGP with high specific activity (151±23 MBq containing 6.5±1 µg of unlabelled CGP) followed 30 min later by a second injection of [^{11}C]CGP with low specific activity (298±57 MBq containing 36±5 µg of unlabelled CGP).

Arterial blood samples were taken at 2, 5, 10, 20 min after the first injection and 10, 20 and 40 min after the second injection of *S*-[^{11}C]CGP into heparinised tubes to which was added unlabelled CGP. Cell-free plasma was prepared by centrifugation and analyzed using a solid phase extraction and HPLC (3). The sample enrichment column containing C18 material was washed by pumping 0.01 M di-ammonium hydrogen phosphate at a flow rate of 5 ml per min for 2 min. The sample enrichment column was then back flushed with the eluent for 2 min to load the sample onto the HPLC column ('µ'Bondapak C18). *S*-[^{11}C]CGP was eluted from the HPLC column with a mixture of methanol and 0.01M di-ammonium hydrogen phosphate (55:45) at a flow rate of 3 ml per min. The HPLC eluate was monitored for UV absorbance at 254 nm and radioactivity (using a solid glass scintillant flow-cell connected to a photomultiplier tube). Both the UV and the radioactivity channels were linked to an integrator. The HPLC eluate was collected in fractions of 0.7 ml and counted in a gamma counter. The percentage of unchanged *S*-[^{11}C]CGP was calculated from the integrated radioactivity trace and the activity present in the solid phase extraction eluent. The recovery of [^{11}C] from the sample enrichment and the HPLC columns was greater than 98 % of the total plasma radioactivity.

The time course of unchanged *S*-[^{11}C]CGP in rat, dog and human plasma is shown in Figure 1. No significant metabolism of *S*-[^{11}C]CGP was observed in either man or dog over 80 min. In the rat, the percentage of unchanged *S*-[^{11}C]CGP ranged from 92% at 5 min to 74% at 80 min after injection. In rats, the metabolism of *R,S*-[^{11}C]CGP and *S*-[^{11}C]CGP was identical. In the rats, the results of the solid phase extraction method were also compared with methanol precipitation of plasma and HPLC. There was no difference in the percent of unchanged *S*-[^{11}C]CGP obtained using either method.

In conclusion, although [^{11}C]CGP is partially metabolised in the rat, it remains unchanged in canine (Greyhound) and human plasma up to 80 min after i.v. injection. Our data is in agreement with those reported for metabolism of *S*-[^{3}H]CGP in rats (4) and for metabolism of *S*-[^{11}C]CGP in dogs by Jones *et. al.*, (5), but in contrast to those of Delforge *et al.*, who reported that *R,S*-[^{11}C]CGP was rapidly metabolised in dogs (Beagles) (as determined by Over Pressure Thin Layer Chromatography) (1).

1. Delforge J. Syrota S., Lancon J-P., Nakajima K., Loc'h C., Janier M., Vallois J-M Cayla J. and Crouzel C. J. Nucl. Med. 32: 739 (1991).
2. Brady F., Luthra S.K., Tochon-Danguy H.J., Steel C.J., Waters S.L., Kensett M.J. *et al* Appl. Radiat. Isot. 42: 621 (1991).
3. Luthra S. K., Osman S., Turton D.R., Vaja V. and Brady F. J. Label Compd. Radiopharm. In Press.
4. A. van Waarde (Personal Communication).
5. Jones H., Rhodes C.G., Law M. P., Becket J. M., Clark C, Boobis A. R. and Taylor G.W. J. Chromatography, 570: 361 (1991).

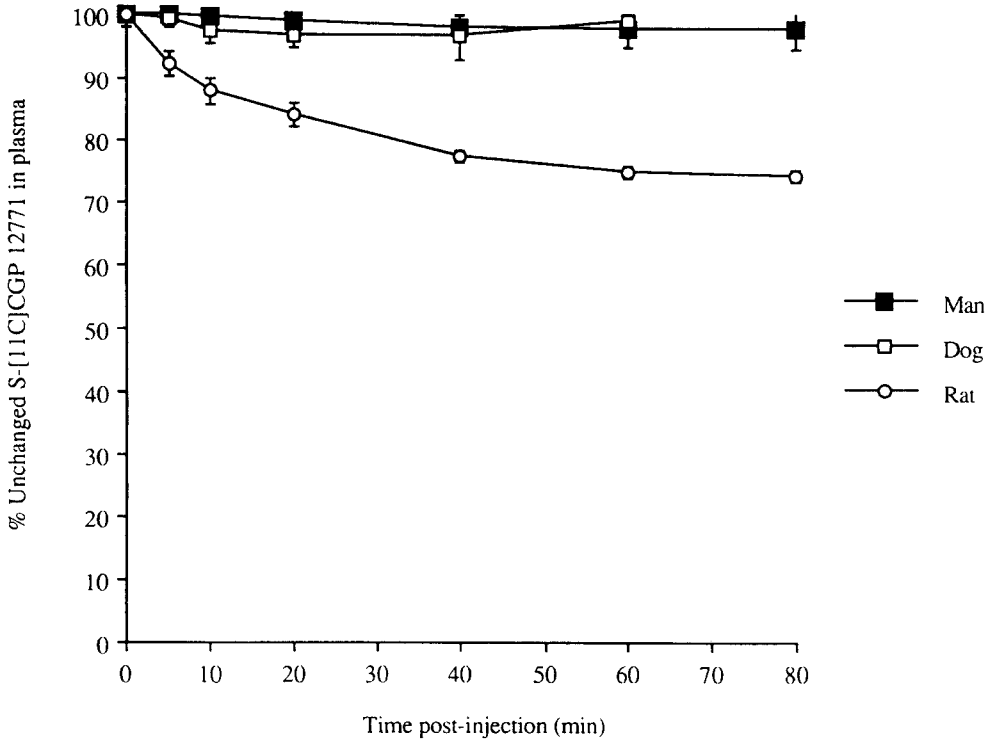


Figure 1 Time Course for S-[11C]SCGP 12177 in rat, dog and human plasma

SYNTHESIS , LABELING AND EXPERIMENTAL STUDY OF NEW ^{125}I-LABELED COMPOUNDS. POTENTIAL APPLICATION FOR DIAGNOSIS AND TREATMENT OF MALIGNANT MELANOMA IN NUCLEAR MEDICINE

M.F. Moreau[1], J.C. Madelmont[1], P. Labarre[1], J. Papon[1], M. Bayle[1], J.Michelot[1], L. Mauclaire[3], A. Veyre[2]
INSERM U 71[1], Centre Jean Perrin[2], Clermont-Ferrand ,Cis Bioindustries[3], Gif/Yvette - France.

Introduction

In view of the poor prognosis experienced by patients with disseminated malignant melanoma, the early detection and treatment of occult metastatic disease is highly desirable. Consequently, an important objective in nuclear medicine is the design and development of melanoma-specific radiopharmaceuticals for both diagnosis and therapy. As concerns the diagnosis, a pharmacological study previously conducted in our laboratory led us to a new potential melanoma-imaging agent : N-(2-diethylaminoethyl) 4-iodobenzamide (BZA) (1-3). The promising results of the experimental study performed in melanoma-bearing mice were validated by the Phase II clinical trial (80 patients) : primary melanoma and metastases were detected by ^{123}I-BZA scintigraphy as early as 6 hr following the injection, the best images having been obtained between 18 and 24 hr. This interval time does not allow the scans to be performed on the same day as the injection of the drug in the patient. Thus, the purpose of this study was to examine the possibility of developing an analogue of BZA which would provide the same scintigraphic information but would require less time between injection and the obtention of quality images. To that effect, we prepared various BZA derivatives (Fig.1) and we examined the consequence of structural modification to the molecule on its melanoma uptake and retention.

Material and methods

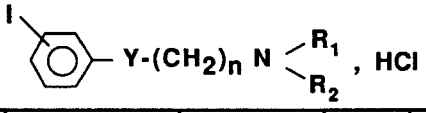
Chemistry: *The non radioactive compounds were prepared according to the classical methods used in organic chemistry by the reaction of iodoaryl activated esters with the proper amines (A to H.) or aminoacid chloride with the iodoaryl amine (L). Identifications were performed by IR, RMN (^{1}H 1D, 2D) and Mass spectroscopy.*

All the compounds were labeled with Iodine-125 by an easy isotopic exchange method. A typical procedure is performed as following : to a solution of iodoaryl amide hydrochloride (10 mg) in 0.05 M citrate buffer at pH 4 (0.5 ml) were added 1 mCi (37 MBq) of Na^{125}I (n.c.a. in NaOH) and 0.5 mg of $CuSO_4$. The reaction mixture was heated about 140°C for 35 to 50 min. Radiochemical purity was determinated by silica gel over pressure TLC with the mixture chloroform-methanol-formic acid

(80-20-0.5 v/v) as eluant, and confirmed by HPLC (HP 1090) using the following systems : reverse phase column Whatman 50DS3 (125 mm x 2 mm) mobile phase constituted of a gradient starting from A.B. (60-40, v/v, A = 0.05 M sodium acetate, B = methanol) to 100 % B in 8 min, followed by 100 % B, at a flow rate of 1.5 ml/min.

All the compounds were shown to contain less than 5 % impurities and can be used without further purification. Specific activities were in the range of 36 mCi (1.3 GBq)/mM.

***Animal experiments** : tissue distribution studies were performed in C57BL6 mice bearing the B16 melanoma, by means of whole body autoradiography and by calculating the concentration of ^{125}I-activity in blood, tumor and selected normal tissues (muscle, brain, lung, liver or intestine). The time course of radioactivity was evaluated in terms of percentage of injected dose per g of tissue (% I D/g), at intervals between 1 and 72 hr post injection. Tumor to blood and tumor to tissue ratios were also calculated, these indexes being of first importance for metastatic melanotic lesions imaging.*



Compound	Iodine	Y	n	R_1	R_2
A	para	CO NH	2	C_2H_5	C_2H_5
B	para	CO NH	2	H	C_2H_5
C	"	"	"	"	$CH(CH_3)_2$
D	"	"	"	CH_3	CH_3
E	"	"	3	"	"
F	"	"	3	C_2H_5	C_2H_5
G	"	"	2	N,R_1,R_2 = N⌐	
H	ortho	"	"	C_2H_5	C_2H_5
I	para	NH CO	2	"	"

Figure 1 : Chemical structures of BZA analogues

Results

Qualitative and quantitative studies showed that five compounds (B C E F I) concentrate in the tumor (between 2 to 4,7 % I D per g 6 hr post-injection). Two labeled derivatives were selected :

Compound E which seemed the most promising for diagnostic 6 hours post administration (tumor/blood : 33, tumor/lung: 9, tumor/brain: 60, tumor/muscle: 60, tumor/liver: 7,5, tumor/intestine: 8) allowed scintigraphic studies in animal.

Compound B which showed a slow tumor clearance (tumor/blood : 110, tumor/brain : 380, tumor/muscle : 270, tumor/lung : 65, tumor/liver : 22, tumor/intestine : 120, with an intratumoral radioactive concentration of 1 % of I D/g 48 hr post injection) could be of interest in the field of therapy. These studies are in progress, on an other hand mechanism melanoma cells binding and the structure activity relationships are under investigations.

References :

1. French patent n° PV 8901898, 02/14/1989.
2. Meyniel G., Michelot J., Moreau M.F., Veyre A.:Perspectives de diagnostic scintigraphique du mélanome malin par un nouveau radiopharmaceutique. C.R. Acad. Sci. 1990 ; t.311, Série III : 13-18.
3. Michelot J., Moreau M.F., Labarre P., Madelmont J.C., Veyre A., Papon J., Parry D., Bonafous J., Boire J.Y., Desplanches G., Bertrand S., and Meyniel G. Synthesis and evaluation of new iodine-125 radiopharmaceuticals as potential tracers for malignant melanoma. J Nucl. Med. 1991; 32 : 1573-1580.

Paper K11

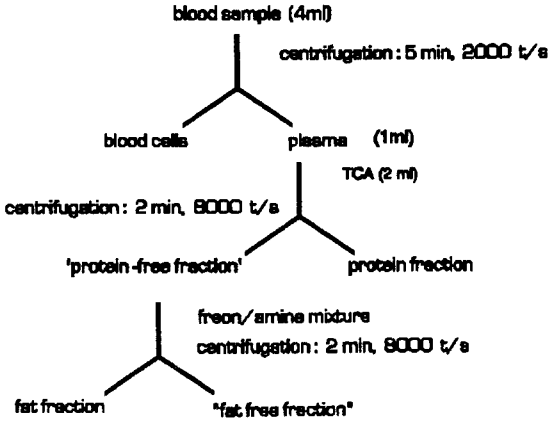
MEASUREMENT OF [METHYL-^{11}C]THYMIDINE AND ^{11}C-METABOLITES IN HUMAN PLASMA

P. Goethals[1],M. Coene[1],M. van Eijkeren[2] and B. Thierens[3]

1. Laboratorium of Analytical Chemistry, Institute for Nuclear Sciences, Proeftuinstraat 86, B-9000 GENT, BELGIUM.
2. Dpt. of Radiotherapy and Nuclear Chemistry, University Hospital, GENT.
3. Medical Physics Laboratory, GENT.

There is no doubt about the clinical usefulness of [methyl-^{11}C]thymidine for tumour visualisation (1), but for quantitative measurement of in vivo cell proliferation by PET, several problems are under discussion. The biodistribution and DNA-incorporation of [methyl-^{11}C]thymidine was already investigated in normal Wister rats. The study showed that [methyl-^{11}C]thymidine was rapid accumulation in fast dividing tissue and the incorporation of an important fraction in the DNA. However accurate quantification by PET requires a detailed knowlege of the in vivo-metabolisation and the fraction of each labeled metabolite in the total blood activity level. A routine method for measuring in human plasma [methyl-^{11}C]thymidine together with its metabolites (thymine, dihydrothymine, β-ureidoisobutyric acid, β-aminoisobutyric acid) was developed. Only a limitted number of plasma samples could be measured due to the short halflife of ^{11}C.

Patients were injected with 20-25 mCi of [methyl-^{11}C]thymidine. Venous blood samples at different intervals (between 2-20 min injection) were taken and prepared for HPLC analyses as pictured in scheme 1.



scheme 1: preparation of the blood sample for HPLC analysis.

For the analysis of "the protein" and "fat free" plasma, 0,5 ml supernatant, spiked with standard solution containing the different metabolites, was applied on a C_{18}-column. The eluent used was isotonic phosphate solution (pH 4,5) containing 3% ethanol. The analyses of 4-6 plasma samples were repeated successively. The effluent was monitorized with an UV- and an activity detector. The fractions containing the different radioactive peaks were collected and measured with a NaI(Tl) detector.

The results are summarized in the following figures. The clearance of total radioactivity from the venous plasma is visualized in fig.1. After an initial rapid decrease, the radioactivity remained nearly constant at a level of 2% of the original injected one L^{-1} plasma. Since no significant activity was observed on the red blood cells, the plasma curve is representative for the blood curve. The radioactivity curve (Fig 1) is the sum of the different ^{11}C-metabolites. Fig.2 illustrates the relative amount of the different ^{11}C-metabolites at different time intervalls. [methyl-^{11}C]Thymidine is rapidly metabolised by elimination of the deoxyribose group and hydrogenation of the double bound (C_5-C_6) in the ring structure to dihydrothymine. During the normal PET-scan period of the patients (5-20 min postinjection), dihydrothymine and the open chain metabolites (β-ureidoisobutyric acid and β-aminoisobutyric acid = ABA) contribute for about 80% in the activity of the blood level as illustrated in Fig.2.

In spite of the rapid in vivo metabolisation, [methyl-^{11}C]thymidine can be applied for studying cell proliferation in tumours since the blood activity level is low in comparison to that of the tumour.

(1): van Eijkeren M., De Schrijver A., Schelstraete k., Poupeye E., Goethals P. and Lemahieu I. Radioactiva isotopes in Clinical medecine and Research 14,(1991)

(2): Goethals P., Poupeye E., Coene M., van Eijkeren M. and Lemahieu I. (abstract) Fourth European Symposium on Radiopharmacy and Radiopharmaceuticals 101, (1991).

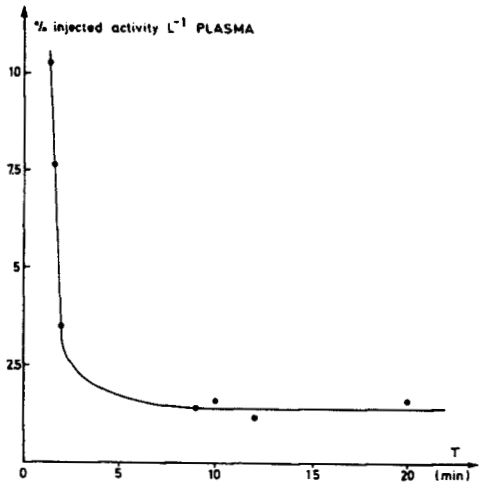


Fig.1 : Clearence of the venous plasma radioactivity after i.v. injection of [methyl-^{11}C]thymidine.

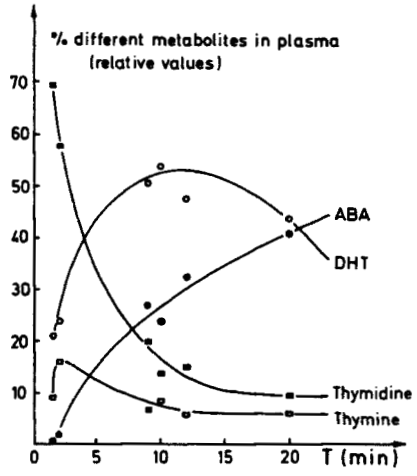


Fig.2 : Percentages of the different metabolites in venous plasma.

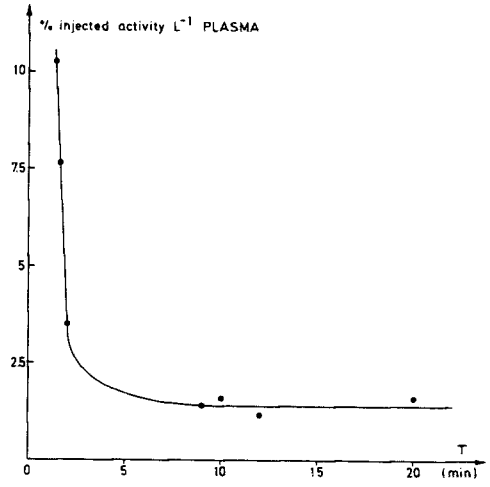


Fig.1 : Clearence of the venous plasma radioactivity after i.v. injection of [methyl-^{11}C]thymidine.

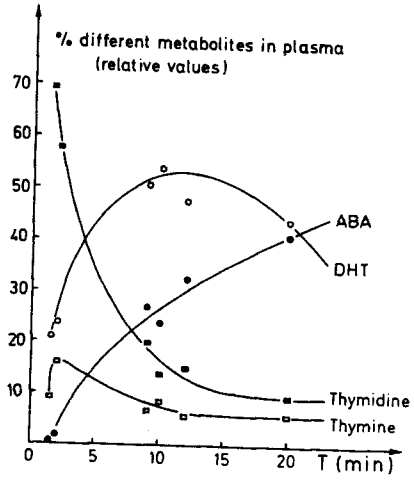


Fig.2 : Percentages of the different metabolites in venous plasma.

Paper K12

KINETIC EVALUATION OF 16α[^{18}F]FLUORO-17β-ESTRADIOL AS A LIGAND FOR IN VIVO ESTROGEN RECEPTOR BINDING IN THE RAT BRAIN

R.M. Moresco, R. Casati, A. Carpinelli, S. Todde, G. Lucignani, F. Colombo, F. Fazio.
University of Milan, CNR-INB, Scientific Institute H San Raffaele, Milan, Italy.

16α[^{18}F]fluoro-17β-estradiol ([^{18}F]FES) is a selective estrogen receptor ligand developed by D.O.Kiesewetter et al. (1) for the in vivo imaging of primary breast cancer and metastasis. Several studies have shown that steroid hormones exert physiological, biochemical and behavioural effects on the central nervous system. Recent works indicate that estrogen receptors are expressed in several brain areas of various animal species. The aim of the present study was to evaluate the suitability of [^{18}F]FES as a ligand for in vivo localization of estrogen receptors in rats brain as a preliminary step to its use in human subjects.

The systemic metabolism and the kinetics of cerebral uptake of the tracer were evaluated in female Sprague-Dawley rats (200-225 g). Polyethylene catheters were inserted into one femoral artery and vein under light halotane anesthesia. After recovery from surgery and anesthesia animals received 1 to 2 mCi of [^{18}F]FES with a specific activity (4307-11090 mCi/umole) and were serially sacrificed.

To estimate the blood clearance of [^{18}F]FES , serial blood samples (100 ul) were collected from the artery up to 60 min after injection. The samples at 1, 5, 15, 24, 44, 60 minutes were analysed for metabolites as follows: after extraction with 9 volumes of ethanol and drying under a helmium stream , samples were injected into HPLC according to the method described by K.A. Weisman et al.(4) (C18 column, 40% acetonitrile in 7.5 mM KH_2PO_4). To estimate the extraction efficiency ethanol extracts and pellets were counted. Results indicate that the tracer is rapidly cleared from the blood compartment and is metabolized into more hydrophilic compounds.

The time course of F-18 uptake in the rat brain [cpm / (uCi injected x g of tissue)] was measured up to 1 hour after injection. Immediately after killing, the brains were rapidly removed and different areas were dissected out (pituitary gland, hypothalamus, hippocampus, striatum and cortex) and placed in preweighed vials for tissue radioactivity measurement. The highest activity accumulation was detected in the pituitary gland, while intermediate levels were observed in the hypothalamus. In the remaining areas: striatum, cortex and hippocampus activity concentration was low ..

Specific uptake was evaluated by sacrificing the animals at the time of maximum uptake (about 27 min.) after coinjection of either 71 ug/kg or 345 ug/kg of cold estradiol with the radiotracer. The inhibition of [^{18}F]FES accumulation was dose dependent in the pituitary and hypothalamus while no effect was observed in the remaining areas .

Results indicate that [^{18}F]FES is a suitable tracer for the visualization of estrogen receptors in the pituitary and hypothalamus. However [^{18}F]FES would probably be inadeguate for the in vivo investigation of estrogen binding sites in brain areas with low receptor levels, such as the hippocampus.

1. Kiesewetter D.O., Kilbourn M.R., Landvatter S.W. et al. , J. Nucl. Med. 25: 1212-1221, 1984.
2. Mintun M.A., Welch M.J., Siegel B.A., et al. Radiology 169: 45-48, 1988
3. McGuire A.H., Dehdashti F., Siegel B.A., et al. J. Nucl. Med. 32: 1526-1531, 1991
4. Weisman R.A., Sahakian J.A., Brodack J.W., et al. J. Label. Compds. Radiopharm. XXX: 426, 1991

Paper K13

ROBOTIC DETERMINATION OF DISCRETE TIME INPUT FUNCTIONS FOR QUANTITATIVE PET: MEASUREMENT OF PLASMA RADIOACTIVITY CONCENTRATION.

D.L. Alexoff, S.J. Gatley, A.P. Wolf, J.S. Fowler, Chemistry Dept., Brookhaven National Laboratory, Upton, NY, USA 11973

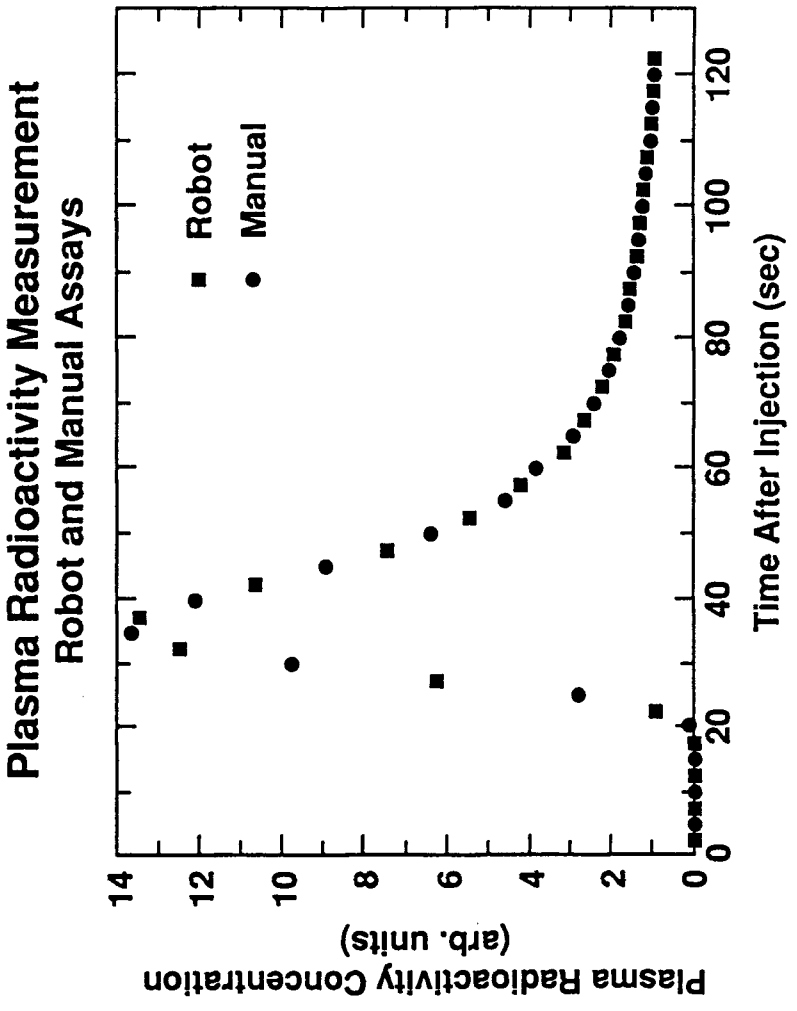
The estimation of kinetic model parameters or other mathematical constructs from PET experimental data usually requires information derived from the time course of radiotracer concentration in the plasma after a bolus injection. This information is often obtained invasively by sampling blood at a peripheral artery and assaying the radioactivity concentration present for each discrete time point. Blood samples, drawn either manually or automatically, are obtained every few seconds during the first minute or two of the PET study in order to minimize errors in peak height and position measurements. This results in large number of samples that must be centrifuged and assayed for plasma radioactivity concentration. In addition, for some radiopharmaceuticals selected samples are subjected to metabolite assay to determine the proportion of unmetabolized radiotracer and pharmacokinectically significant labeled metabolites. These routine tasks require significant personnel commitments and raise safety issues related to handling of radioactivity and potentially infectious materials. Therefore, we have investigated the application of a commercial laboratory robot to the determination of input functions. Recently we described a robotic system for metabolite analysis (J.Nucl.Med., abs. #758, 32:1088, 1991). This present work describes the measurement of plasma radioactivity concentration in whole blood samples.

The laboratory robot system consisted of the following commercially available hardware (PyTechnolog II+ (tm),Zymark, Hopkinton, MA, USA): an Zymate II+ arm with Accutrak(tm), two autochangeable hands (1 ml pipetting and general purpose), a 3000 rpm centrifuge, a Mettler AE200 balance, 2 16x100mm disposable test tube racks, and a System V controller. The system runs under System V ver. 1.51 (Zymark) software and is connected to an IBM PS/2 Model 60 personal computer running DOS 3.30. The PS/2 is also connected (RS-232) to counting equipment that controls a custom engineered, robot compatible NaI crystal "well counter" residing on the robot table. Applications software was written using the EASYLAB (Zymark) robot control software to control container movement and liquid handling, and IBM BASICA programs that synchronize robot and counter functions while acquiring plasma radioactivity and weight data.

Pipetting cell-free plasma from a small volume blood sample where the volume of the sample may vary more than an order a magnitude is the major challenge to robotic control. This problem was overcome by using a gravimetric feedback strategy to control plasma pipetting. Arterial whole blood samples (0.2-0.4 mL) from the femoral artery of a baboon (papio anubis) were obtained with an Ole Dich automated (peristaltic pump) blood sampling every 2.5 seconds for two minutes post injection of 18F-N-methylspiroperidol. Human whole blood samples (arterialized venous, 0.1 - 1.4 mL) were drawn manually by syringe in rapid succession following injection of 18FDG. All blood samples were stored in heparinized 1.5 mL Eppendorf centrifuge tubes that were inserted manually (cap off) down to the bottom of 16x100mm test tubes residing in the robot's sample rack. Samples were centrifuged, pipetted, weighed, and counted automatically by the robot. Plasma aliquots (0.030 mL - 0.3 mL) were weighed and counted in robot-tared 16x100 mm tubes. An iterative, feedback control algorithm was developed to maximize the weight of cell-free plasma. The algorithm uses the subject's hematocrit and the value of the maximum blood volume in all tubes to calculated a "safe" vertical distance just above the cell interface. For samples with a whole blood volume much less than the maximum, 3-4 pipetting iterations were necessary before an adequate plasma aliquot was obtained. For automated blood sampling a single iteration was almost always sufficient to get sufficient plasma (>0.02 mL). In this case, a steady state robotic throughput of about 4 minutes/sample was achieved using a 2 minute counting time. At any one time during this steady state, the robot was centrifuging, counting, and pipetting different samples simultaneously.

Samples from both manual and automated blood sampling were used to validate the robotic method by comparing a plasma input curve generated by an experienced technician (using a 1 min. counting time) with that obtained with the robot. Data generated manually and from the robotic system gave superimposable plasma input functions. The robot with its "intelligent" pipetting was able to compensate automatically for large variations in blood volume and obtain sufficient cell-free plasma from as little as 0.1-0.2 mL whole blood. Robotic throughput (min./sample) was half that of a highly trained technician. For C11 labeled radiotracers, where longer assay times undesirable, longer robot counting times (2-3 minutes) compensate somewhat for reduced throughput and should permit determination of input functions.

This method, combined with a previously described robotic metabolite assay, provides a total solution to automating the determination of plasma input functions of unchanged radiotracer concentration, thereby minimizing worker exposure to radioactive and infectious materials. By itself, this robotic system may by used in clinical PET where quantitation requires only the determination of plasma radioactivity concentration using 18FDG. Supported by USDOE, OHER and NIH Grant NS-15380.


Plasma Radioactivity Measurement
Robot and Manual Assays
Robot
Manual
Plasma Radioactivity Concentration
(arb. units)
Time After Injection (sec)
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120

MEASUREMENT OF LOW-LEVELS OF PET NUCLIDES IN PLASMA METABOLITE ASSAYS: USE OF A MODERN LIQUID SCINTILLATION COUNTER (LSC). SJ Gatley and C MacNabb. Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973.

Quantitative PET studies of biochemistry and physiology require measurement of time-courses in arterial blood plasma. When radiolabeled metabolites of the tracer appear in the plasma, analytical chemistry is also necessary to determine the time-course of the fraction of unchanged tracer. Late samples especially from ^{11}C studies often contain low activities which are further reduced by decay during the analytical procedure and which are of necessity dispersed among several fractions. Furthermore, unless several counters are available each ^{11}C sample can be counted only a short time. Since the precision of the entire PET study cannot exceed that of the input function, it is important to use a counter with high efficiency and low background. The standard counting equipment in our laboratory for large (5-10 mL) samples is a manual 3 inch NaI well-counter. Used without a lower energy threshold to maximize count rates, this has a reasonable efficiency of about 60%, but a background of about 150 cpm, which limits the precision obtainable with necessarily brief counting of low-level activities. The alternative liquid scintillation technology is commonly used for low energy beta particle emitters, but rarely for positron emitters. Modern LSCs do not require chilling of samples to reduce chemiluminescence and will record the counting time of each sample, a necessity for the short-lived positron emitters. LSC cocktails are now relatively non-toxic, environmentally safe, and miscible with an equal volume of water, methanol or acetonitrile, solvents commonly used in HPLC and SEP-PAK analyses. Sample volumes of up to 10mL can be counted in standard 20 mL LSC autochanger vials, whereas most autochanger gamma counters cannot be used with such large samples.

Methods. Counting efficiencies were measured against certified ^{60}Co and ^{137}Cs standards via a Capintec ionization chamber. A Packard Tri-Carb 1600 TR counter was used with UltimaGold (UG) or UltimaGold XR fluid except for determination of Emax, where 10 mL of Econofluor fluid was used with about 0.5 µL of aqueous ^{18}F-FDG or ^{13}N-ammonia solution. Half-lives were determined by repetitive counting with a window from 0 keV to the literature Emax values. For cocaine metabolite assays deproteinized plasma samples were applied in 5 mL water to Waters C-18 SEP-PAK's. These were then successively eluted with 5 mL water, and 20%, 50% and 100% methanol. The fractions were counted after mixing with 10 mL UG XR.

Preliminary tests with ^{13}N (100% β^+) and ^{18}F (97% β^+). Emax values of 1.2 and 0.63 MeV and half-lives of 9.96 min (20 x t1/2) and 109. 6 min (15 x t1/2) were measured, respectively. These values are in good agreement with the literature. Background in a representative experiment was 15.5 ± 3.9 for 99 serial 1 min measurements, showing expected approximately Gaussian properties. Count-rates were linear with ^{18}F activity up to 37 kBq (1 µCi). Counting efficiencies (percent cpm/dpm) were 97±2% for ^{18}F in UG or UG XR over a range of sample volumes and in the presence of limiting proportions of water, methanol or acetonitrile. Initial, rapidly decaying chemiluminescence activities induced by addition of 5 mL of 1N NaOH to 10 mL UG XR were about 2,000 cpm, entirely confined to the 0-3 keV spectral region.

Optimization of counting precision. ^{18}F samples were counted with varying lower threshold. When a 10µL of aq. ^{18}F solution was added to 3 mL of UG fluid in a 7 mL "mini-vial" Eff^2/Bkg, a figure of merit for counting systems, was maximum at about 60 keV. When ^{18}F in 5 mL 50% methanol was mixed with 15 mL UG XR in a standard mL vial, to simulate a typical metabolite fraction, Eff^2/B was maximum at about 15 keV Although Bkg was increased by the larger volume, it was still possible to count with Eff>90% and Bkg<15 cpm.

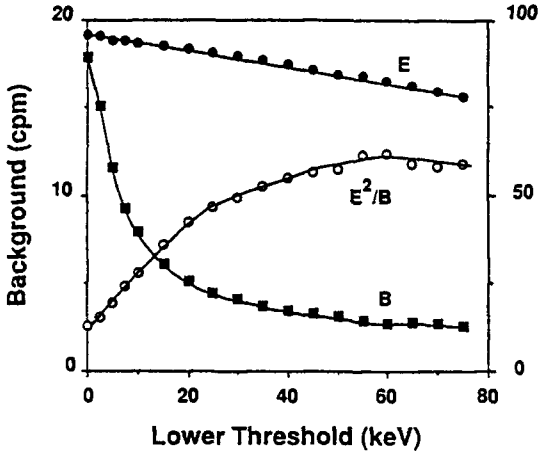
Metabolite fractions. Excellent agreement between NaI and LSC systems was obtained with SEP-PAK assays from representative human or baboon PET experiment with ^{11}C-cocaine, ^{18}F-haloperidol and ^{18}F-N-methylspiperone, and with an HPLC assay of ^{11}C-raclopride.

Conclusions. The relatively high beta particle energy of ^{18}F allows LSC detection very near the theoretical maximum of 97% for this nuclide. ^{11}C (β^+, 99.8%; Emax, 0.96MeV) should therefore have Eff = 100%. The high Eff and especially the low Bkg of LSC translate into a large increase in precision for low-level samples. For a 200 dpm sample counted for 1 minute the uncertainty doubles for a well-counter with Eff = 60% and Bkg = 150 cpm versus LSC with Eff = 95% and Bkg = 10 cpm. Moreover sample sizes as great as 10 mL can be accomodated. Modern LSC systems thus provide an improved technique for assaying non-metabolized tracer fractions in PET. In addition, LSC has uses in other areas of PET work. These include radionuclidic purity: impurities with higher Emax values can be rapidly detected, while the stability, low background and high dynamic range allows half-lives to be rapidly and accurately determined. Furthermore, since detection is virtually quantitative LSC measurements can be used to calibrate other counting equipment.

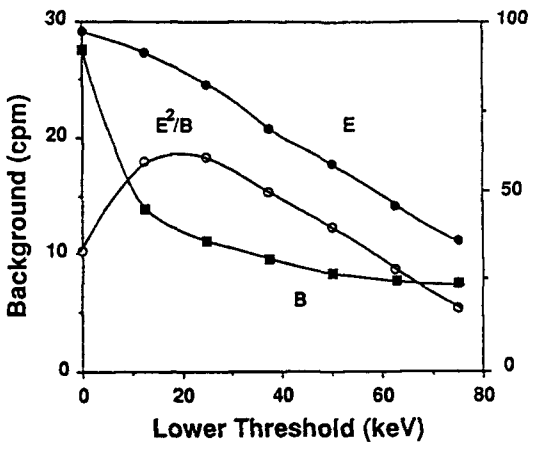
This research was carried out under contract DE-AC02-76CH00016 with the U.S.DOE/OHER.

Supporting data for Gatley and McNabb.

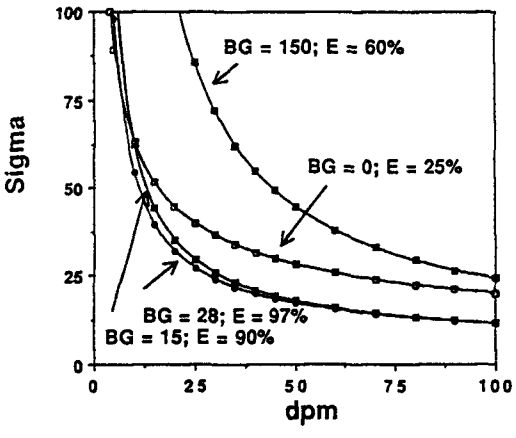
Efficiency and Background for F-18.



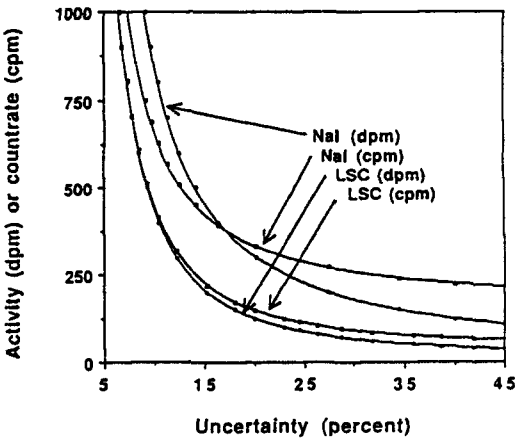
F-18 in 5 mL of 50% Methanol.



Counting uncertainty at low count-rates.



DPM or CPM needed for given uncertainty level.



REFERENCES

1. Wolk, CP, Austin, SM, Bortins, J and Galonsky A (1974): Autoradiographic localization of ^{13}N after fixation of ^{13}N-labeled nitrogen gas by a heterocyst-forming blue-green alga. J Cell Biol. **61**: 440-453.

2. D. N. Abrams, S. A. McQuarrie, C. Ediss and L. I. Wiebe (1976). Cerenkov counting and liquid scintillation counting for the determination of fluorine-18. in Liquid scintillation: science and technology., ed A. A. Noujaim, C. Ediss and L. I. Wiebe. pp 167-171, Academic Press, New York.

3. Gatley SJ, MacGregor RR, Fowler JS, Wolf AP, Dewey SL and Schlyer DJ (1990): Rapid stereoselective hydrolysis of (+)-cocaine in baboon plasma prevents its uptake in the brain: implications for behavioral studies. J. Neurochem. 54:720-723, .

4. Lederer, CM and Shirley, VS (1978). Table of Isotopes. 7th Ed, Wiley, New York.

An automated system based on solid phase extraction and HPLC for the routine determination in plasma of unchanged [^{11}C]-L-deprenyl; [^{11}C]diprenorphine; [^{11}C]flumazenil; [^{11}C]raclopride; and [^{11}C]Scherring 23390.

Luthra S.K., Osman S., Turton D. R., Vaja V., Dowsett K. and Brady F.

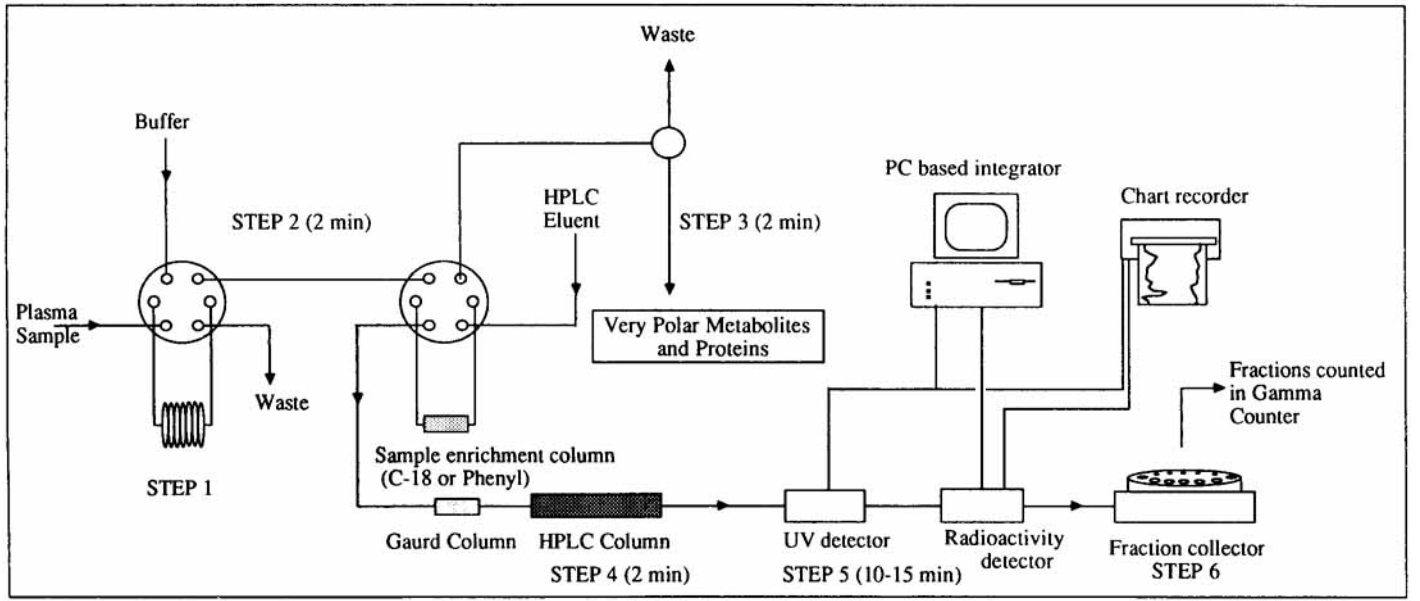
MRC Cyclotron Unit, Hammersmith Hospital, DuCane Rd., London W12 OHS, U.K.

The determination of the fraction of the unchanged [^{11}C] labelled compounds in plasma is required for the correction of the arterial plasma input function in PET studies. Here we describe an automated system, based on solid phase extraction and HPLC, for routine serial measurement of unchanged [^{11}C] labelled compounds in plasma.

Figure 1 shows the components and the steps in the operation of the automated metabolite analysis system. These steps are controlled and co-ordinated by a PLC (Toshiba EX-20 programmable controller) which operates the valves, pumps and relays according to a programmed time sequence. After manual injection of serial plasma samples, timers within the programme control the rate of loading of plasma onto the sample enrichment column and transfer of the samples from enrichment column to an on line HPLC column. The PLC also starts a computer integrator at the begining of the HPLC analysis. The ratio of the unchanged [^{11}C]labelled compound in plasma at a given time is calculated from the integrated radioactivity trace and the activity present in the solid phase extraction eluent. Time courses for the percentage of unchanged [^{11}C] labelled compounds (± SD) in human plasma are shown in Figure 2. [^{11}C]Raclopride (15 studies) metabolises slowly, 75% of plasma activity was [^{11}C]raclopride at 1 h after injection. The results obtained are similar to previously reported data for [^{11}C]raclopride using protein precipitation and subsequent TLC or HPLC or Sep-Pak separation (1). The fraction of unchanged [^{11}C]diprenorphine in plasma (70 studies) was found to decrease from 65% at 5 min to 35% by 45 min. Similar results for [^{11}C]diprenorphine have also been reported in baboons (2). The metabolism of [^{11}C]flumazenil (12 studies), [^{11}C]deprenyl (20 studies) and [^{11}C]SCH23390 (2 studies) was very rapid. The fraction of unchanged[^{11}C]flumazenil, [^{11}C]deprenyl and [^{11}C]SCH23390 in plasma was found to be 30%, 16% and 10% respectively at 30 min after injection. Rapid decrease of [11C]flumazenil present in plasma, determined using chloroform extraction and TLC (3) and of [^{11}C]SCH23390 using HPLC analysis were also observed in Cynomolgus monkeys and human subjects (4, 5).

There are several advantages in the use of the automated system described. It allows the direct use of plasma and permits fast processing of serial samples. Subsequent samples can be loaded and processed on the sample enrichment column while the HPLC anlaysis of the previous sample is in progress, thus saving time. The system can be operated by one person only. It can be routinely used for the analysis of a wide range of [^{11}C] labelled radiopharmaceuticals used in PET studies with only adjustments to the composition of the HPLC mobile phase according to the compound being analysed.

1. Luthra S.K., Turton D. R., Price G., Ahier R., Martin F., Hume S and Cremer J. J. Label. Compds. and Radiopharm. XXX: 378 (1990).
2. Shiue C-Y., Bai L-Q., Teng R-R., Arnett C. D., *et. al.*, Nucl. Med. Biol., 18: 281 (1991).
3. Barre L., Debruyane D., Abadie P., Moulin M. and Baron J.C. Appl. Radiat. Isot. 42: 435 (1991).
4. Sedvall G., Farde L., Barner A., Hall H., and Halldin C. Psychopharmacology, 103: 150(1991).
5. Halldin C., Swahn C-G., Farde L., Litton J-E., and Sedvall G. Eur. J. Nucl. Med. 18: 526 (1991).



Cell-free plasma containing stable compound of interest (to facilitate UV monitoring and to enhance recovery from sample enrichment column) is loaded onto the sample loop (Step 1). The sample is transferred onto a sample enrichment column (stainless steel, 5 x 0.3 cm i.d.) containing either C-18 or Phenyl material depending on the C-11 labelled compound being analysed (Step 2). The sample enrichment column is then washed with 0.01 M di-ammonium hydrogen phosphate buffer to remove very polar metabolites and proteins (Step 3). It is then back flushed with HPLC eluent (a mixture of methanol and either 0.01M di-ammonium formate or 0.01M di-ammonium hydrogen phosphate) onto the HPLC column: "μ" Bondapak C-18; 30 x 0.78 cm i.d.; particle size 10 μm; flow rate of 3 mL per min (Step 4). The HPLC eluate is monitored for UV absorbance and radioactivity (Step 5). The radioactivity detector consists of a high efficiency glass scintillant flow cell connected to a photomultipier tube. Both the UV monitor and radioactivity detector are connected to an integrator (Lablogic) which allows the data to be recorded. After monitoring, fractions of eluent are collected in a fraction collector and counted using a gamma counter (Step 6), thus allowing overall recovery to be determined .

Figure 1 Automated System for Metabolite Analysis

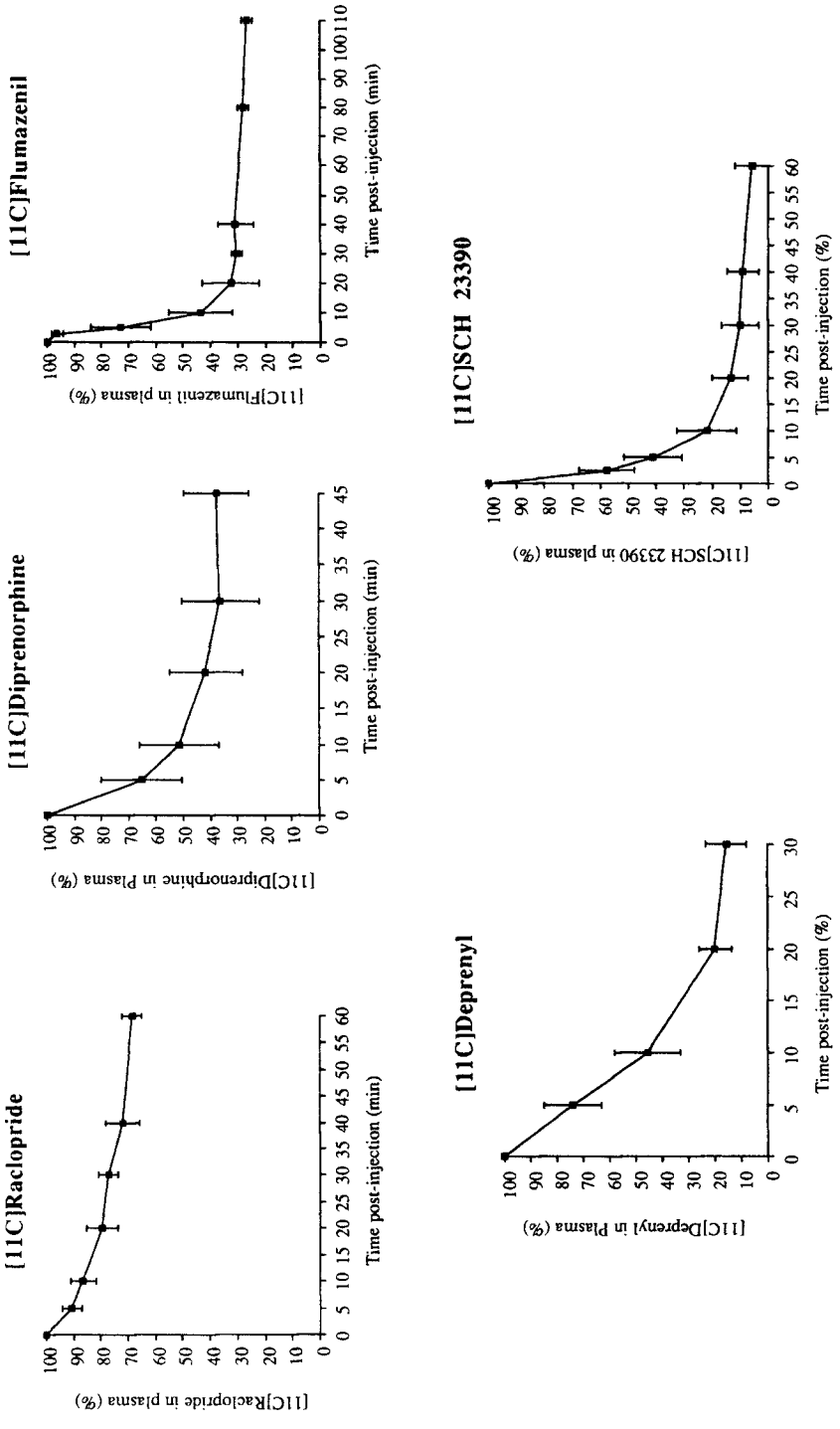


Figure 2 Time Course for unchanged [11C]labelled compounds in plasma

Paper K16

A practical synthesis of 5-[^{18}F]fluorouracil using HPLC and a study of its metabolic profile in rats.

G.D. Brown, H.R. Khan, C.J. Steel, S.K. Luthra, S. Osman, , S.P. Hume, D.W.O.Tilsley, S.L.Waters, K.G.Poole, P. Price and F. Brady.
MRC Cyclotron Unit Hammersmith Hospital, Ducane Road, London W12 OHS

There has been a resurgence of interest in PET studies with 5-[^{18}F] fluorouracil (5-^{18}FU) recently following suggestions that the therapeutic efficacy of 5-FU is modulated by agents such as folinic acid (1) and N-(phosphono-acetyl)-aspartate (PALA) (2). Previously reported methods of synthesis of 5-^{18}FU (3 - 6) in our hands failed to deliver 5-^{18}FU of adequate purity for PET studies. Oberdorfer *et al* (7) reported similar difficulties and proposed a method for synthesising high purity 5-^{18}FU. The latter method however, in common with some earlier procedures utilitses sublimation as part of the isolation process. Sublimation is not easily amenable to remote handling or automation however.We report here a rapid and efficient synthesis of high purity 5-^{18}FU utilising only HPLC for purification. The time course for plasma metabolites of 5-^{18}FU in rats was also measured using HPLC.

[^{18}F]F_2 was passed through a suspension of uracil (30 mg) in acetic acid (15ml) containing sodium acetate (10 mg) at 25^{0}C over ca 20 min. The reaction mixture was transferred to a rotary evoporator and acetic acid removed under vacuum at 90°C. The residue was washed with ethanol (7 ml) which was then evaporated.

A solution (2 mL) of KH_2PO_4 (0.07M), with pH adjusted to 7.0 with Na_2CO_3, containing ethanol (5%) was added to the residue and the mixture heated at 90 °C for 10 min. The solution was transferred to a stainless steel loop and automatically injected onto an HPLC column (Technicol Nucleosil-5 C18; 250 mm x 22 mm). The column was eluted with the above eluent at 9 mL min^{-1}. The 5-[^{18}F]5-fluorouracil peak which eluted at 14 min was collected and Millipore filtered to provide a solution ready for human injection. The formulated product was analysed by HPLC (Waters 'μ' Bondapak C18; 10 μg; 250 x 3.9 mm); eluant 0.01M $(NH_4)_2HPO_4$ at 0.8 mL min^{-1} with ratioactivity and UV monitoring at 260 nm. 5-^{18}FU eluted at 5.3 min and uracil at 6.0 min. Radiochemical yield was typically 26 % (EOB), and specific activity 11.8 MBq/μmol (EOB).The chemical and radiochemical purities were better than 98%.The formulated product was also characterised by mass spectrometry (chemical ionisation and electron impact) and after decay by ^{19}F NMR in DMSO (5-fluorouracil ,δ -59.0 rel. to hexafluorobenzene).

Sprague-Dawley rats were injected with 5-[^{18}F]fluorouracil (37 MBq). Post-injection blood samples were taken over 40 min. Cell-free plasma was prepared by centrifugation. After methanol precipitation, the supernatant was rotary evaporated and the residue resuspended in 0.01M $(NH_4)_2HPO_4$ and analysed by HPLC ('μ'Bondapak C18; 300 x 7.8 mm i.d.) at a flow

rate of 3 ml min^{-1} and monitored for radioactivity and UV at 280 nm. The 5-[^{18}F]FU content of plasma decreased rapidly, only ca 3% remaining at 20 mins whereas α–[^{18}F]fluoro-β-alanine is the major component after only 10 mins increasing to 90% after 40 min. (Fig 1). Predosing the rats with either folinic acid or PALA had no significant effect on this metabolic profile.

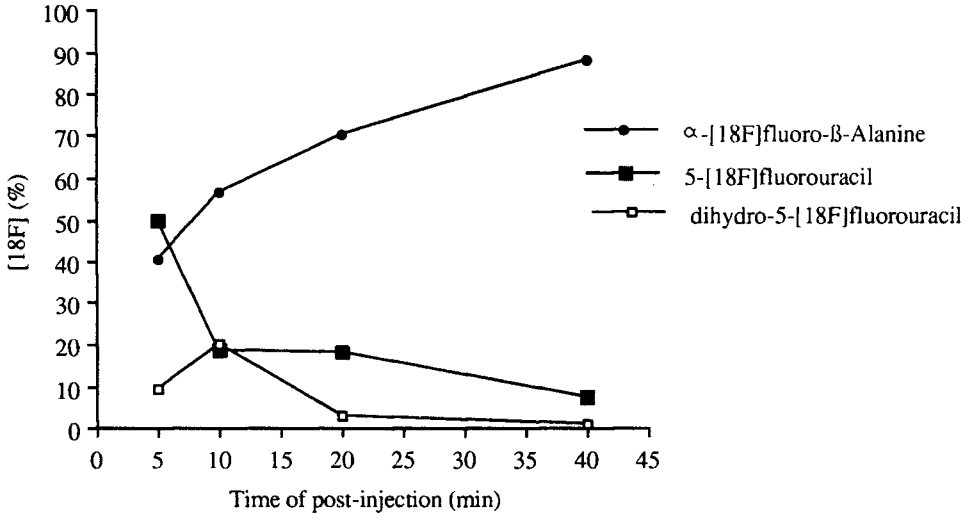


Figure 1 Time Course for 5-[18F]Fluorouracil metabolites in rat plasma

1. Grem J.L., Hoth D.F., Hamilton J.N., King S.A., Leyland-Jones B. Cancer Treat. Rep. 71, 1249 (1987).
2. Grem J.L., King S.A., O'Dwyer P.J., Leyland-Jones B. Cancer Research 48, 4441, (1988).
3. Fowler J.S., Finn R.D., Lambrecht R. M., Wolf A.P. J. Nucl. Med., 14, 63, (1973).
4. Vine E.N., Young D., Vine W. H., Wolf W. Int. J. Appl. Radiat. Isotop., 30, 401, (1979).
5. Shiue C-Y., Wolf A.P., Friedkin M. J. Label. Compds. Radiopharm. 21, 865, (1984)
6. Diksic M., Farrokhzad S., Yamamoto Y.L., Feindel W. Int. J. Med. Biol., 11, 141, (1984)
7. Oberdorfer F., Hofman E., Maier-Borst W., J. Label. Compds. Radiopharm., 27, 137, (1988).

[^{18}F]ALTANSERINE: A SHORT SYNTHESIS OF THE NITRO PRECURSOR AND PRELIMINARY METABOLIC STUDIES IN RAT.

M. Monclus, F. Biver, S. Goldman and A. Luxen
PET/Biomedical Cyclotron Unit, Université Libre de Bruxelles, Erasme Hospital, Brussels, Belgium.

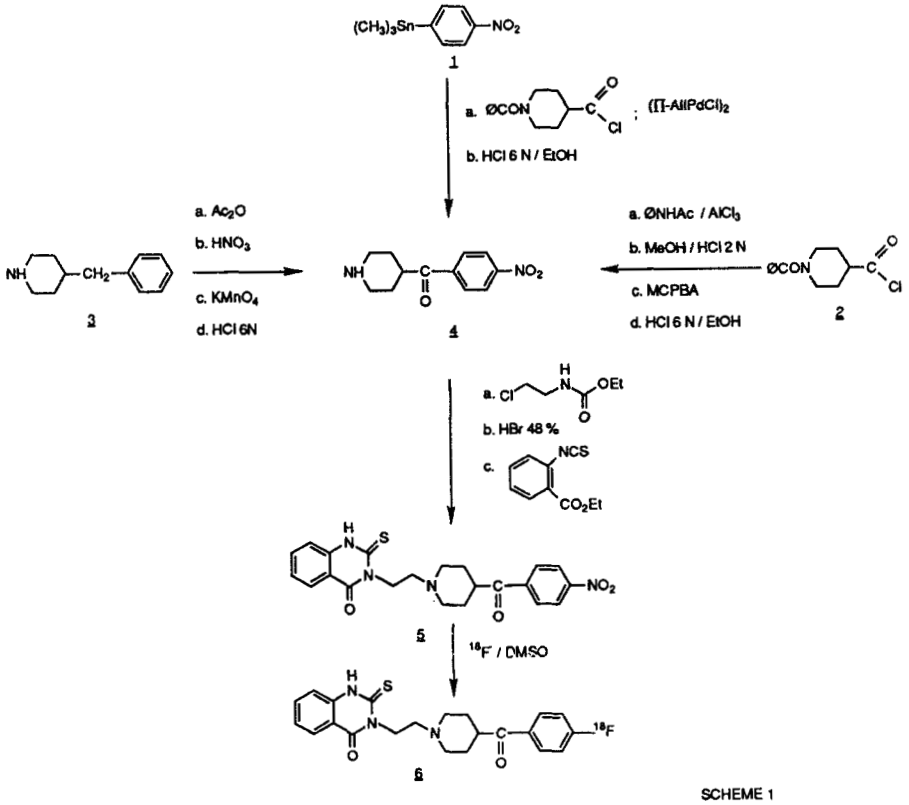
Recently altanserine (see scheme 1, 6) labeled with ^{18}F has been described as a potential ligand for the mapping of 5-HT$_2$ receptors in human using PET (1). [^{18}F]altanserine was prepared via a radiofluorodenitration of 5 using nca [^{18}F] fluoride in DMSO (2, 3). Although the radiosynthesis is easy and reliable, access to the nitro derivative 5 is difficult and the synthesis is very long and time consuming.

Synthesis of the nitro precursor.
We report here a new and shorter route for the preparation of the nitro derivative 4 (Scheme 1). According to the literature, this key compound has been obtained in low yield through a Friedel-Craft reaction followed by an oxidation of the amino function (4). A second route including the nitration of the commercial compound 3 and an oxidation of the benzylic methylene has been reported (3) but the oxidation step described is not reproducible. Therefore, we developed a synthesis of a asymmetric ketone using the coupling of a tin derivative (5, 6) and an acid chloride in THF catalyses by transition metal complex (7). Coupling 1 and 2 in THF at room temperature in presence of π-allyl palladium chloride dimer affords 4 in 45% yield after acid hydrolysis. The nitro derivative 5 is then easily obtained as described (2).

Determination of the unchanged [^{18}F]altanserine in plasma.
The measurement of the in vivo biochemical parameters with PET requires an accurate determination of the plasmatic levels of metabolites and unchanged [^{18}F]altanserine. We describe and compare here two different extraction procedures as well as TLC and HPLC analysis of the extract.
After IV injection of 0.4 mCi of [^{18}F]altanserine in rat, arterial blood samples were collected at 10, 30 and 60 min. Metabolites and unchanged [^{18}F]altanserine were extracted using two different methods, *1*- deproteinization under basic conditions (8), adsorption on ®Extrelut column, extraction with ether and concentration; *2*- deproteinization under acidic conditions (HOAc/NH_4OAc buffer), dilution 10 fold with water, adsorption on a C-18 SEP-PAK light, washing of the support with 0.01M NaOH, extraction with MeOH and concentration. Radioactivity recovery yields were superior to 90% and 85% in method 1 and 2, respectively.
Two different HPLC conditions (injection volume 20 ml) were used for the separation of the radiolabeled ligand and the metabolites, *1*- Merck Select B 4.6x250 mm, 5µm; eluent: 55% 100 mM HOAc/100mM NH_4OAc, 32% THF, 13% MeOH; flow rate: 0.8 mL/min; *2*- Ultrasphere ODS, 4.6x250 mm, 5µm; eluent: 80% 0.1% Et_3N, 20% MeOH; flow rate: 1mL/min. Fractions (0.5 mL) were collected and counted in a gamma counter. Radio-TLC plates (Merck silicagel) eluted with AcOEt-CH_2Cl_2 (60-40) were analyzed on a Berthold TLC analyser. Whatever the conditions used on HPLC, we found similar results: a fraction of about 5, 10 and 20 % of metabolites is present at 10, 30 and 60 min, respectively. These results are different from the data obtained by TLC where a fraction of metabolites of 15, 25 and 40% is observed at 10, 30 and 60 min, respectively. The causes of these disparities are now under study.

Acknowledgment: This research was supported by the National Fund for Scientific Research (FNRS 9.4520.87 and 2.4555.88).

1. Sadzot B. et al, J. Nucl. Med. 32, 980 (1991)
2. Lemaire C. Ph.D. Dissertation, University of Liège (1991).
3. Lemaire C. et al, J. Label. Compds. Radiopharm. 26, 336 (1988)
4. Crouzel C. et al, J. Label. Compds. Radiopharm. 25, 403 (1988)
5. Azizian H. et al., J. Organometal. Chem. 215, 49 (1981)
6. Kashin A. et al., Zh. Org. Khim. 17, 905 (1981)
7. Beletskaya I., J. Organometal. Chem. 250, 551 (1983)
8. Kurowski M., J. Chromatogr.341, 208 (1985)



SCHEME 1

Paper K18

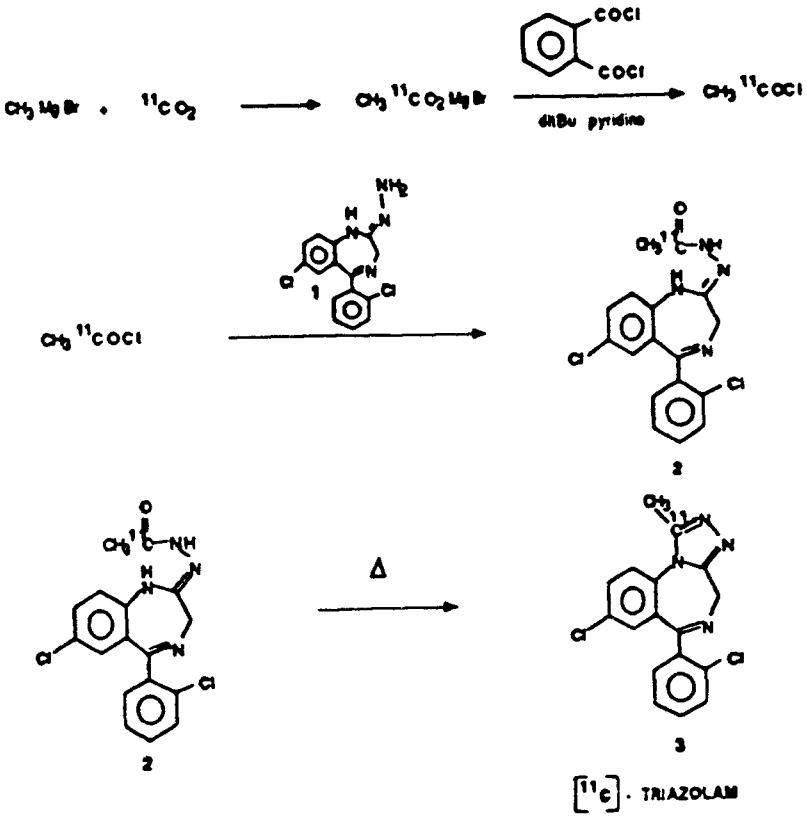
RADIOSYNTHESIS AND BABOON PET EXPERIMENTS OF [^{11}C]TRIAZOLAM :

C. LE BRETON, M. BOTTLAENDER, C. CROUZEL, M. VARASTET, °R SITBON, B. MAZIERE and M.MAZIERE.
Service Hospitalier Frédéric Joliot, DRIPP, CEA, 91401 ORSAY Cedex (France)
°Upjohn, France, Tour Franklin, 92081 PARIS LA DEFENSE (France)

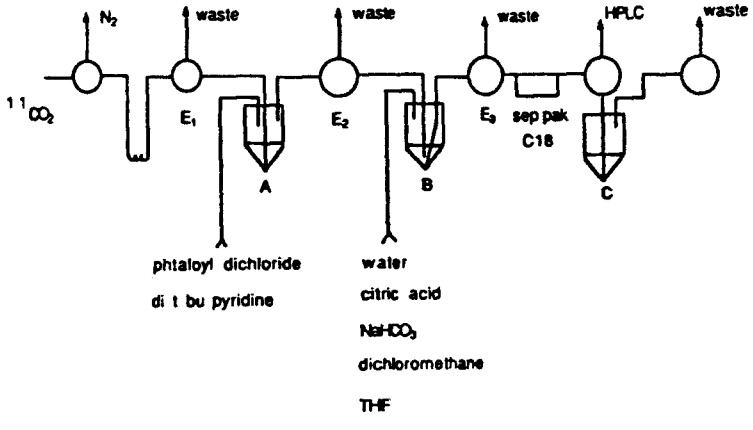
In order to study in vivo the pharmacokinetics and the specificity of the binding with the benzodiazepine receptors (BZR), triazolam, was labelled with carbon-11 by the slightly modified method of Banks et al. (1) : the reaction of the cold precursor, amidrazone, with [^{11}C]acetyl chloride (reactor B) is followed by pyrolysis (reactor C) of the resulting 1-acetyl hydrazine (scheme 1 and 2). [^{11}C]acetyl chloride is produced (reactor A) by the method of Luthra et al. (2). [^{11}C]triazolam is purified by HPLC : Silica columm (Partisil, 50x0.9 cm), solvent : acetonitrile, propanol-1, chlorofrom, water, acetic acid (650, 290, 40, 20, 0.5), flowrate : 8 ml/min, retention time of triazolam : 8 min. 6-60 mCi of [^{11}C]triazolam (RAS 100-400 mCi/μmol) are obtained by a remote controlled system in 55-65 minutes after EOB (scheme 2).

The cerebral pharmacokinetics of [^{11}C]triazolam was studied in Papio papio baboons (n=4, weight=10 $\pm$ 0.8 kg) using Positron Emission Tomography (PET) in 10 experiments. Three different types of experiments (control, full saturation and displacement) were performed: in any case [^{11}C]triazolam (8.9 $\pm$ 6.5 mCi) was administered i.v. at the beginning of the PET study (To) and the kinetics of the radioactivity was followed in various brain structures during 120 min. The data obtained in (figure 1):
three control experiments show that, after administration of [^{11}C]triazolam, the brain uptake of the radioactivity is rapid. The binding reaches a maximum at To+25 min and then decreases slowly up to To+120 minutes (T½ = 190 min).
two experiments performed after full saturation of the BZR (by i.v. injection of 1 mg/kg of flumazenil, a specific BZR antagonist, 5 min before the tracer) display a rapid wash out of the radioactivity indicating a saturability of the [^{11}C]triazolam brain binding. The mean non specific binding represents 30% of the total binding.
five displacement experiments show the displaceability of [^{11}C]triazolam brain binding. These displacement experiments were carried out by injecting i.v., twenty minutes after the [^{11}C]triazolam, pharmacological doses of 3 different BZR ligands : triazolam (0.03 mg/kg), flumazenil (0.008 mg/kg) and the BZ_1 specific agonist zolpidem (2.5 mg/kg) (the injected doses corresponded to the previously determined ID_{50} of [^{11}C]flumazenil). After injection, each of these BZR ligands produced a rapid decrease of the radioactivity from the brain demonstrating that [^{11}C]triazolam brain binding is displaceable.

1 - Banks W.R., Tewson T.J. and Digenis G.A., Appl. Radiat. Isot. 41, 8, 719-725 (1990)
2 - Luthra S.K., Pike V.W. and Brady F. J., Chem. Soc. Chem. Comm. 1423-1425 (1985)



SCHEME 1



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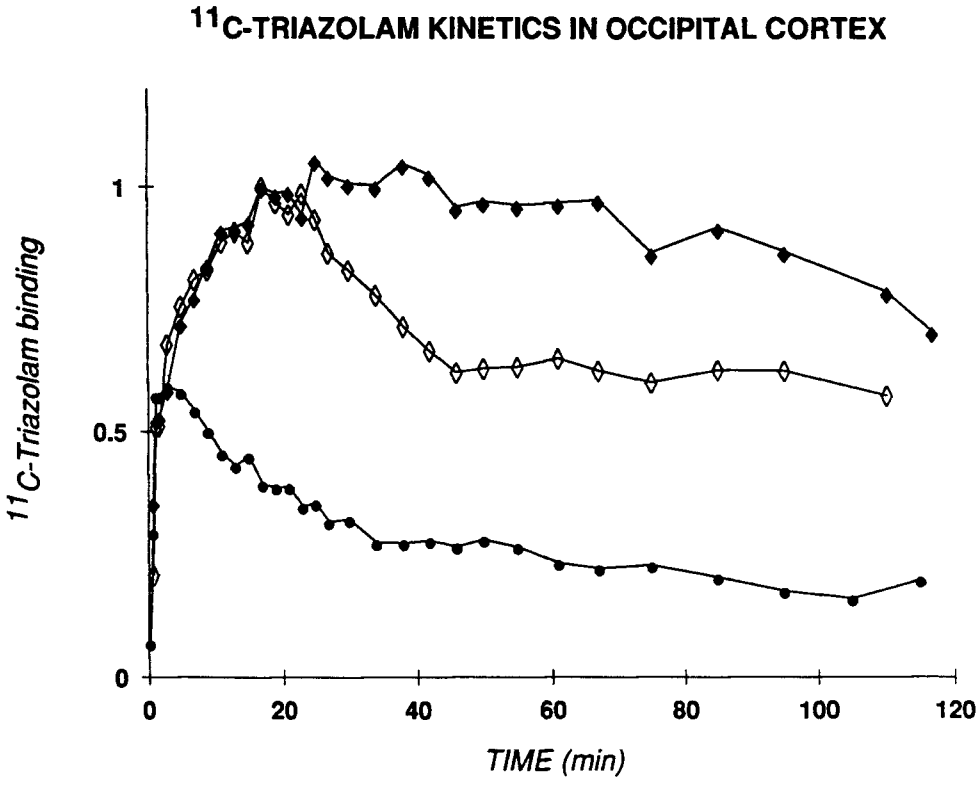


FIGURE 1 :

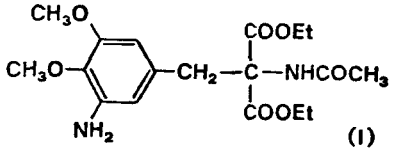
Kinetics of ^{11}C-Triazolam in a control (♦), a full saturation (•) and in a displacement experiment with 0.008 mg/kg of flumazenil (◊) injected i.v. at 20 min in living Papio papio baboons. The binding of ^{11}C-Triazolam is expressed as the percentage of maximal binding

COMPARATIVE METABOLISM OF ENANTIOMERS OF 5- AND 6-FLUORO-DOPA IN AGGREGATING BRAIN CELL CULTURES

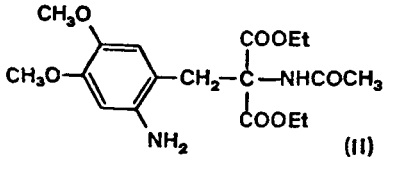
C. Wiese, M. Cogoli-Greuter[1], M. Argentini, R. Weinreich, and K.H. Winterhalter[2],
Paul Scherrer Institute, CH-5232 Villigen, Switzerland,
[1]Institute of Biotechnology and [2]Laboratory for Biochemistry, both Swiss Federal Institute of Technology (ETHZ), CH-8092 Zürich, Switzerland

For understanding and evaluating PET data, an exact knowledge of the biochemical behaviour of the radiolabeled tracer, including its metabolism, is essential. The basic experiments are normally carried out *in vitro* with inactive tracers. In [^{18}F]Fluorodopa measurements, mismatches are obtained between Fluorodopa and its metabolites as well as between cerebral and peripheral activities. In order to exclude the peripheral Fluorodopa amount and to focus the cerebral metabolism, the enantiomers of Dopa, 5-Fluorodopa and 6-Fluorodopa and their metabolites were analyzed after incubation in aggregating cell cultures of fetal rat brains.

5-Fluorodopa and 6-Fluorodopa were synthesized by Schiemann reaction from (I) or (II), respectively



(I)



(II)

The racemic products were separated into its enantiomers by HPLC on a commercially obtainable chiral column (Chiralpak WH, Daicel Chem. Comp.) [1]. The separation was quantitatively.

In order to prepare the aggregating cell cultures of fetal rat brains (mesencephalon - diencephalon - rhombencephalon), hindbrains of 14-day old rat fetuses were mechanically dissociated, and the washed cells ($2.5\text{-}4 \cdot 10^6$ per ml) were inoculated in serum-free medium. The cultures were humidified to 95% saturation, under constant gyratory agitation (68-78 rpm) for maximum 20 or 21 days, respectively [2]. The substrate, containing 50 µM Dopa or Fluorodopa enantiomer, respectively, in medium, was incubated for 30 min

under culture conditions. The cellular aggregates were washed with PBS, homogenized, sonicated and centrifuged. The precipitated proteins were determined by the Folin method. The metabolites were separated by reversed phase HPLC (Plasma Catecholamine Column) and quantified by electrochemical detection.

In Fig. 1, the results are shown. The uptake mechanism of Dopa and all Fluorodopas is enantioselective. Since our cultures are devoid of endothelial cells, this uptake could take place in existing astrocytes. This confirms the hypothesis of astrocytic processes in crossing of blood-brain-barrier [3].

The major metabolites of both L-Dopa and 6F-L-Dopa, are Dopamine (DA) and DOPAC. The ratio DA/Dopa is a parameter to characterize the Dopa isomers with respect to its usefulness for *in vivo* investigations. In L-enantiomers, this ratio follows the sequence L-Dopa>6F-L-Dopa>>5F-L-Dopa.

We found that DA remained as intracellular product for a reasonable long time. This is to explain with a sufficiently long storage in vesicles. Hence, our results confirm the principal correctness of the assumption of a subcellular compartmentalization by vesicles in dopaminergic neurons [4].

With D-Dopa and the D-Fluorodopas, the methylation is the major metabolic step. As purified COMT has a slight enantiospecific preference towards L-Dopa [5], our contradicting finding suggests an intracellular local excess of substrate for COMT.

5F-L-Dopa is mainly 3-0-methylated, 5F-D-Dopa even exclusively. Obviously, AAAD has an extremely low affinity for this enantiomer. This confirms earlier *in vivo* results [6, 7] in an complementary way: those were mixed with a non-quantificable peripheral amount of activity.

[1] T. Mäder et al., Fresenius J. Analyt. Chem., in press (1992).
[2] P. Honegger, in Cell Culture in Neurosci., 223, New York 1985.
[3] E.G. Jones, in Cell and Tissue Biology, 277, Baltimore 1988.
[4] A. Gjedde et al., Proc. Natl. Acad. Sci. USA 88, 2721 (1991).
[5] R.H. Gordonsmith et al., Biochem. Pharmacol. 31, 433 (1982).
[6] P. Cumming et al., J. Neurochem. 48, 601 (1987).

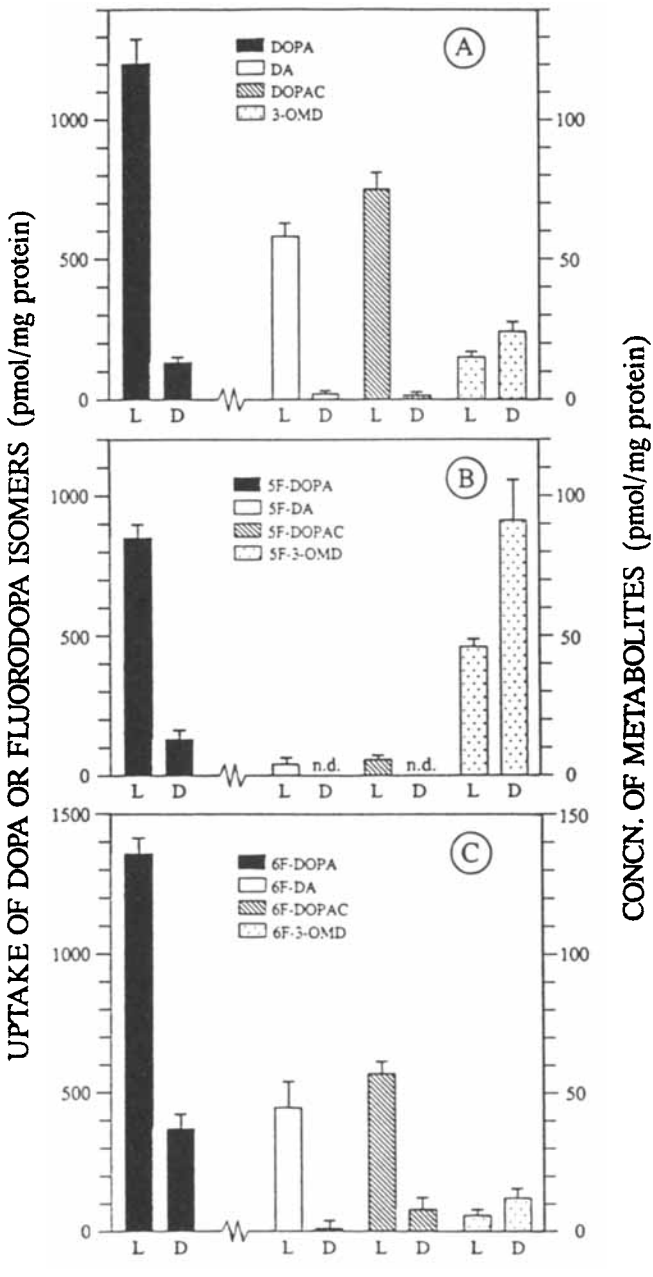


Fig. 1: Uptake and metabolism of the enantiomers of Dopa (A), 5F-Dopa (B), and 6F-Dopa (C) in aggregating brain cell cultures.

Paper K20

ONE STEP DETERMINATION OF IONISATION CONSTANT AND LIPOPHILICITY (LOG P) OF RADIOPHARMACEUTICALS USING THE SHAKE-FLASK METHOD.

C. LOC'H, B. MAZIERE,
Service Hospitalier Frédéric Joliot, CEA, DRIPP, F-91406 ORSAY

Physico-chemical parameter determination is an important part of the development of new radiopharmaceuticals. The knowledge of their ionisation constants and lipophilicity indexes is mandatory to understand their in vivo behaviours such as the non specific binding to plasmatic or tissular proteins or the crossing of the blood brain barrier.
The lipophilicity index of a radiopharmaceutical can be computed using the fragmental or substitutive methods (1,2), or estimated from its partition coefficient between two non-miscible phases measured using the chromatography on reverse phase (3) or the shake-flask technique. Ionisation constant can be determined by potentiometry (if large amounts of materials are available) or by HPLC (4).
Here, we describe an easy way of simultaneously determining Log P and pKa of a radiopharmaceutical using measurements of octanol-water partition coefficients at various pH.
For practical reasons, the equation describing the relation between these two parameters :

$$P_{pH} = P/(1+10^{pKa-pH})$$

(P = Partage coefficient of the non-ionized molecule, P_{pH} = partage coefficient at pH buffer) should be transformed in an hyperbolic function:

$$P_{pH} = (P*10^{pH})/(10^{pKa}+10^{pH})$$

or a linear function:

$$P_{pH}/10^{pH} = (P-P_{pH})/10^{pKa}.$$

The solubility of 1-Octanol in water (0.63μL/mL at 20°C) must be taken in account: $P_{pH} = P_{exp}/(1-0.00063*P_{exp})$

Five phosphate buffers with different pH were prepared from 0.01M solutions of NaH_2PO_4 and Na_2HPO_4. The radiopharmaceutical, dissolved in 2 ml of 1-octanol was agitated, for 30 sec using a vortex system, with 2 mL of each of the 0.01M phosphate buffers. After centrifugation (2 min at 1000g), the aqueous lower phase was withdrawn with a Pasteur pipette. This extraction process was performed 4 more times. For each extractions, the partition coefficients were calculated from the radioactivities measured in aliquots of octanol and buffer solutions. The partition coefficient P and ionisation constant pKa are then computed using a non linear (hyperbolic function) or a linear least square regression programme.

Using this method, we have determined LogP and pKa for [3H]-Spiperone, [3H]-CGP12177 and for [123I]-Iododenopamine, a new tracer for ß1 adrenergic receptor. The results obtained with this "easy to performed" technique are in good agreement with previously published data (5,6).

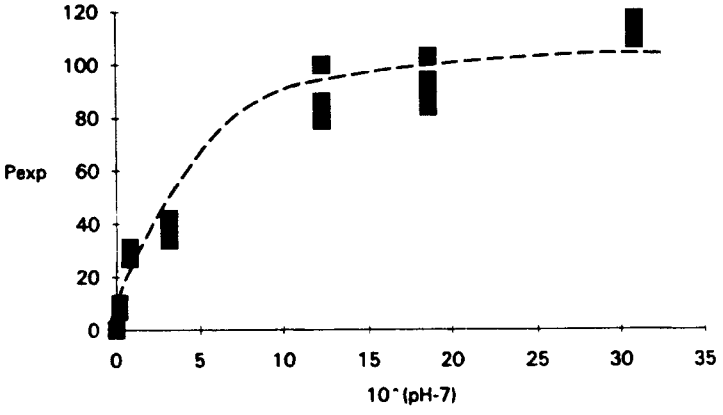
This method, which avoids the use of tedious and time-consuming HPLC techniques, should be recommended for the preliminary measurements of the physicochemical properties of new radiopharmaceuticals.

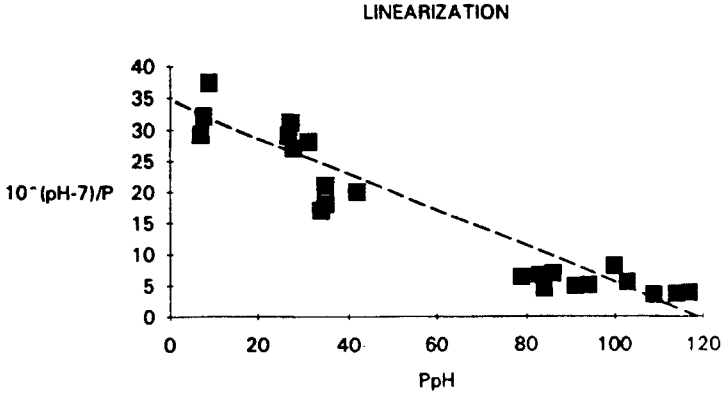
1. Nys G., Rekker R., Eur.J. Med. Chem. 9:361(1974)
2. Leo A., Jow P., Silipo C.et al, J.Med.Chem.,18:865(1975)

3. Hafkenscheid T., Tomlinson E., J. Chrom., 292:305(1984)
4. Stylli C., Theobald E., Appl. Radiat. Isot.,38:701(1987)
5. Moerlein S., Laufer P., Stöcklin G., Int. J. Nucl. Med. Biol.,12:353(1985)
6. Bree F., El Tayar N., Van de Waterbeemd H. et al , J. Recept. Research, 6: 381(1986)

Supporting data

	SPIPERONE	CGP12177	I-DENOPAMINE
Log P	3.55	1.37	2.13
pKa	7.95	8.68	7.84
LogP(7.4)	2.89	0.06	1.56





Paper K21

PREPARATION OF [123I]IODODENOPAMINE : A POTENTIAL RADIOTRACER FOR MYOCARDIAL ß ADRENERGIC RECEPTOR STUDIES

M. BERTAUX, C. BRUTESCO, , C. LOC'H, H.VALETTE, B. MAZIERE
Service Hospitalier Frédéric Joliot, DRIPP, CEA, F-91406 ORSAY

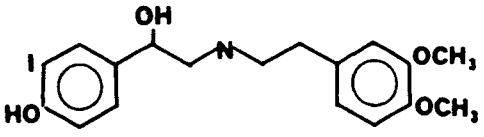
Denopamine (τ-3-4-dimethoxyphenethylaminomethyl-4-hydroxybenzyl alcohol) is a cardiotonic agent with ß1 adrenergic receptor affinity (1). Thanks to an activating hydroxyl group on an aromatic ring, a regioselective labelling of Denopamine with non-carrier-added 123I can easily be obtained by utilizing an electrophilic substitution with Chloramine T (CAT) as the oxydizing agent.

Optimal labelling conditions (time, reagent concentrations) have been determined using sequential radio TLC analysis of samples of the reaction mixture. TLC analysis were performed on RP18 plates and on silicagel plates using respectively water-methanol (70:30) using chloroform-methanol (85:15) as eluates. These analysis show that a satisfactory radiolabelling yield (75%) is obtained in 5 min using a precursor/CAT ratio of 10 (3 μmol of Denopamine and .3μmol of CAT). After having stopped the oxydizing process with $Na_2S_2O_3$, the reaction mixture was poured on a C18 cartridge and polar by-products were eluted with 5 mL water. The radio iodocompound and the excess of precursor were finally eluted with 3 mL ethanol. [123I]-Iododenopamine was purified by HPLC on a reverse phase analytical column (μ-Bondapak C18, 300*3.9 mm, Waters) using acetate buffer-ethanol (85:15) as the mobile phase. Monitoring UV absorption (278 nm) and radioactivity measurements allows the radio-iodopharmaceutical collection. Retention volumes for control (cold) and radioactive iododenopamine are identical (43 mL). The specific activity of the radio-iodocompound, calculated from the HPLC chromatograms, is > 1.5 GBq/μmol; [123]-Iododenopamine chemical and radiochemical purities, assessed by HPLC and radio-TLC, were > 95%. The fundamental physico-chemical properties of [123I]-Iododenopamine have been measured using the shake-flask method partition technique with mixtures of octanol and phosphate buffers at various pH. The partition coefficient for the neutral radiopharmaceutical (log P) is 2.13 and its ionisation constant (Pka) is 7.84.

In vitro pharmacological studies performed on homogenates of rat cardiac membranes have shown that [123I]-Iododenopamine binding to ß receptors is saturable (Bmax = 460 fmol/mg of protein; equilibrium dissociation constant Kd = 51 nmol/L). Biodistribution has been evaluated in rats after IV injection of .5 MBq of radioiodoligand. In the heart, a rapid and high uptake followed by a slow elimination process occured. 15 min post-injection, the heart radioactive concentration was around 1% ID/g with a heart to whole blood ratio of 4. The high radioactive uptake observed in the lung (2% ID/g) cannot be only explained by the lipophilicity of the radioligand (log P7.4 = 1.56). In the brain, the radioactive concentration was < .05 % ID/g.
These preliminary results indicate that [123I]-Iododenopamine is a potentially useful tracer for the clinical SPECT investigation of the myocardial adrenergic function.

1. Naito K., Nagao T., Otsuka M. et al, Japan. J. Pharmacol.,38:235(1985)

Supporting data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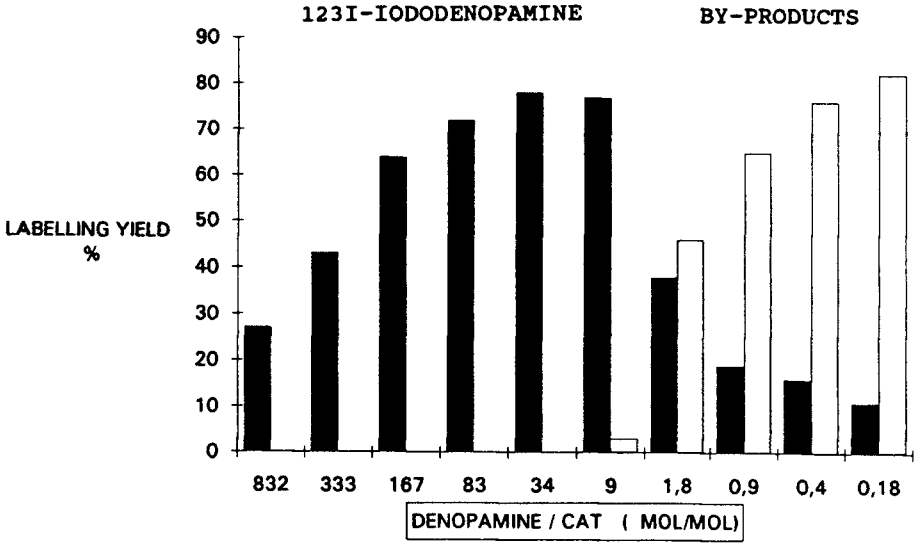
OCH3


123I-IODODENOPAMINE
BY-PRODUCTS
LABELLING YIELD
%
90
80
70
60
50
40
30
20
10
0
832
333
167
83
34
9
1,8
0,9
0,4
0,18
DENOPAMINE / CAT (MOL/MOL)

MEASUREMENT OF 18F-FLUORO-DOPA METABOLISM IN PLASMA USING RADIO-TLC ANALYSIS

C. LOC'H, R.F. WANG, B. MAZIERE. Service Hospitalier Frédéric Joliot, DRIPP, CEA, F-91406 ORSAY

L-18F-Fluoro-DOPA has been extensively used as a tracer for PET investigation of dopamine regional cerebral metabolism (dopamine presynaptic function evaluation). Quantification of this metabolism parameters using a modeling approach requires an accurate knowledge of the input function which is calculated from measurements of sequential unchanged radiotracer plasma concentrations. Reverse phase HPLC analysis (1,2) or alumina adsorption (3,4) have been used to determine unchanged and metabolized 18F-Fluoro-DOPA in plasma samples. For routine clinical investigations with L-18F-Fluoro-DOPA, it was useful to design a more simple and convenient analytical technique.
For this purpose, a new procedure involving radio-TLC analysis on C18 and alumina plates is proposed. The results obtained with these original separation schemes are compared with those of a previously published solid phase extraction technique.

After IV administration in baboons or humans of 200 MBq of the radiotracer, arterial blood samples were collected in heparinized tubes and centrifugated.
For radio-TLC analysis, a 0.2 mL plasma sample, spiked with specimens of the main metabolites of F-Dopa, was deproteinized by addition of 2 mL of methanol and centrifugated. The methanolic supernatant was evaporated and the residue, dissolved in 20 μL of methanol, was analyzed on RP18 plates (EDTA-phosphate buffer (pH3) 0.1M - methanol, 95:5) or on neutral alumina plates (0.5M TRIS HCl buffer (pH=9) - methanol 50:50). The radioactivity distribution along the plates was then measured with a static radiochromatogram analyser and the control cold metabolites were revealed with iodine. With the C18 phase system, 18F-Fluoro-DOPA (rate factor rf=0.85) and 4 radioactive metabolites (L-Dopamine rf=0.75, 3-O-methyl-Dopamine rf=0.65, DOPAC rf=0.45 and homovanilic acid rf=0.21) can be identified in plasma samples. However a non negligible fraction of the radioactivity remains at the deposit point. This fraction which corresponds to adsorbed radioactivity on remaining proteins, should not be taken in account in the evaluation of the unchanged radiotracer fraction. With the alumina system, 18F-Fluoro-DOPA doesn't migrate, while 3-O-methyl-Dopamine (rf=0.75) is separated from homovanilic acid (rf=0.60) and from the other metabolites (rf$\approx$0.4).
For solid phase extraction measurements, 0.2 mL of plasma added with 0.5 mL of 0.5M TRIS-HCl buffer was poured on a prewetted buffer neutral alumina cartridge (Waters). The column was then washed with 2 mL TRIS buffer and 5 mL water, and the remaining radioactivity compared with that of the untreated plasma sample (5).

The data obtained for the determination of the unchanged fraction of 18F-Fluoro-DOPA using the two proposed analytical TLC schemes and the control solid phase extraction are well correlated (Figure 3). RadioTLC analysis appears to be an easy and useful technique for analysing 18F-Fluoro-DOPA and its radioactive metabolites in plasma samples for routine clinical PET studies.

1. Firnau G., Sood S., Chirakal R. et al, J. Nuc. Med. 29:363(1988)

2. Melega W., Luxen A., Permultter M. et al, Bioch. Pharmacol. 39:1853(1990)
3. Boyes B., Cuming P., Martin R. et al, Life Sci. 39:2243(1986)
4. Mac Lellan M., Doudet D., Brucke T. et al, Appl. Radiat. Isot. 9:847(1991)
5. Chan G.L., Pate B., Hewitt K. et al, Proc. 7th Int. Symp. Radiopharmacology, Boston, USA (1991)