([18]F)JHU86358, A NOVEL RADIOLIGAND WITH IMPROVED BRAIN KINETICS FOR PET IMAGING OF EXTRATHALAMIC nAChR

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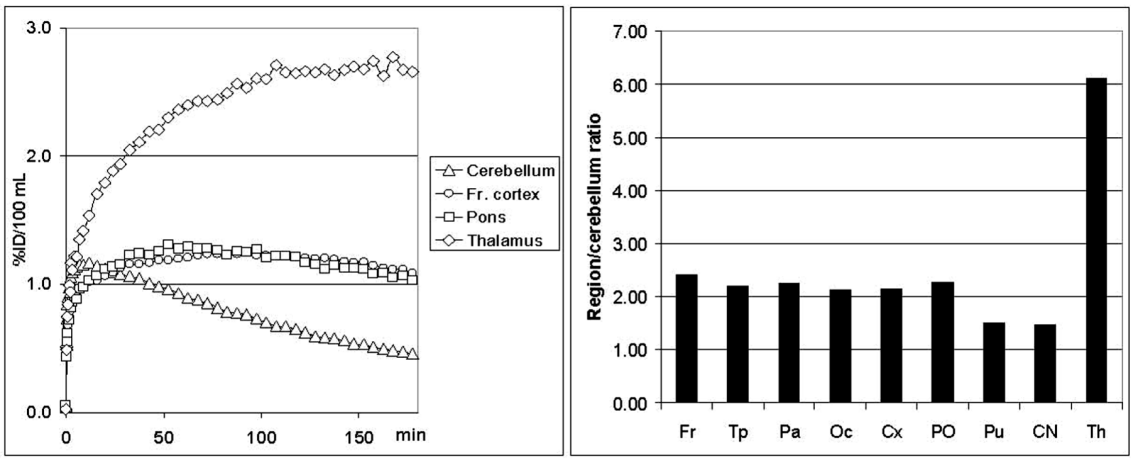
Introduction: Nicotinic acetylcholine receptors located in extrathalamic brain regions (EN) are important therapeutic targets. The ability to image EN in the human brain has been hampered by the lack of suitable radiotracers. The radiotracers for imaging of the EN used previously in animals, display very slow brain kinetics requiring many hours of PET imaging. Here we present a novel high affinity radioligand [18F]JHU86358 with improved brain kinetics and high regional binding potentials for imaging of EN.

Experimental: The in vitro inhibition binding assay of JHU86358, a novel fluorinated 5-aminoalkyl-3,3′-bipyridine synthesized in our lab, was performed in duplicate using rat cortical membranes and [3H]epibatidine. [18F]JHU86358, was prepared by radiofluorination of corresponding bromo precursor using GE box. Regional brain distribution of [18F]JHU86358 was studied by baboon PET.

Results and Discussion: Inhibition binding affinity of JHU86358 was Ki = 51 pM, whereas epibatidine, a potent nAChR ligand, displayed a binding affinity of 43 pM in the same assay. [18F]JHU86358 was obtained with radiochemical yield, radiochemical purity and the average specific activity of 50%, 99% and 580 GBq/μmol, respectively. High radioligand uptake in the baboon brain was observed after injection of [18F]JHU86358 using PET. The highest accumulation of radioactivity occurred in the thalamus. Intermediate uptake was observed in the cortex and pons. The lowest level of radioactivity was in the cerebellum. At 3 h after injection, region-to-cerebellum ratios values of 6.1, 2.4 and 2.1 in thalamus, frontal cortex and pons, respectively, were calculated. The time to reach a steady-state in the cortex and pons was about 2 h and it was more delayed in the thalamus. The distribution and kinetics of [18F]JHU86358 in brain regions were similar to those of 2-[18F]FA and 6-[18F]FA but the tissue/cerebellum ratios of [18F]JHU86358 were about 200-300% higher with the new radioligand. The brain kinetics of [18F]JHU86358 were also faster than those of [18F]NIDA52189 and [18F]NIDA522131 since the latter two required at least 8 h to reach steady-state in the cortex.



Scheme. Left: Time-uptake curves of [18F]JHU86358 in baboon brain. Right: Region/verebellum ratios of [18F]JHU86358 in 180 min after injection.

Conclusion: [18F]JHU86358 holds promise as a radioligand for studying extrathalamic regions in human brain. It shows higher binding potential and shorter time to steady state than previously developed radioligands.

Keywords: nAChR, PET

([11C]GSK189254, A PET LIGAND FOR THE HISTAMINE H_3 RECEPTOR-LABELLING IN TWO DIFFERENT POSITIONS

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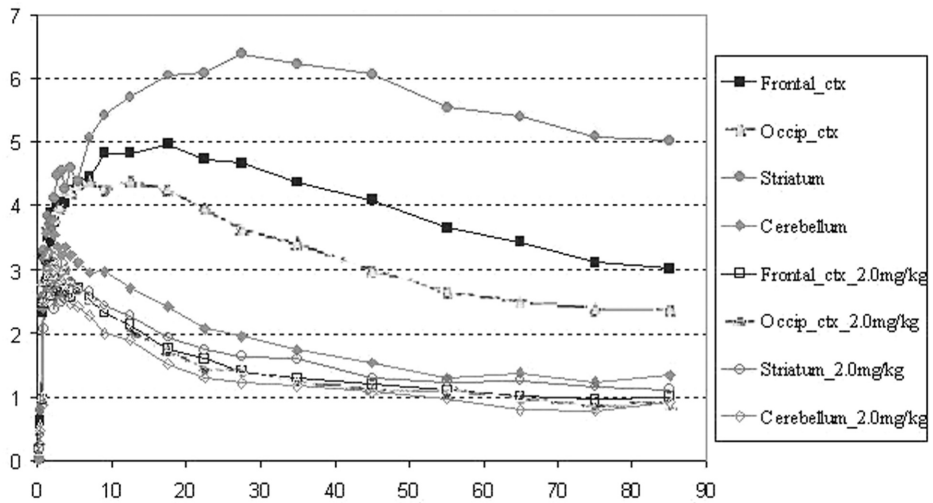
Introduction: Histamine is a neurotransmitter in the central nervous system (CNS) as well as being present peripherally, and exerts its action through four distinct histamine receptor subtypes, H_1, H_2, H_3 and H_4. The histamine H_3 receptor (H_3R) has been reported to regulate the synthesis and release of histamine, as well as other neurotransmitters, such as acetylcholine, noradrenaline, dopamine and serotonin. The CNS H_3R has been implicated in a number of pathologies and therefore there is a considerable interest in the pharmacology of this receptor.

In order to help characterise H_3R status in healthy volunteers and patients, we developed *N*-methyl-6-(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yloxy)-nicotamide ([11C]GSK189254), a potential PET radioligand for imaging and quantifying the H_3R.

Experimental: GSK189254 exhibited a high affinity for the human H_3R (pKi=9.6) and was C-11 labelled both in the *N*-methyl and the carbonyl positions by *N*-alkylation of the carboxamide precursor using [11C]MeI or by Pd-catalyzed aminocarbonylation of the appropriate iodopyridine with [11C]CO, respectively. Using either radiosynthetic route, carbon-11 was introduced in good radiochemical yield, purity and specific activity.

Results and Discussion: *In vivo* brain regional distribution of [11C-*N*-CH_3]GSK189254 was investigated in pig, [11C-*N*-CH_3]GSK189254 readily entered the brain; its regional brain distribution reflected the known distribution and density of the H_3R with a higher accumulation in the striatum, cortices, thalamus and a lower uptake in cerebellum. The cerebellum is known to contain low-levels of H_3R and was used to normalise the tissue concentrations of the radioligand. The highest ratios relative to cerebellum occurred at 85 min post-injection and were 4.7, 2.9, 2.7, 2.1 and 2.0 for the striatum, frontal cortex, parietal cortex, thalamus and occipital cortex, respectively.

Co-administration with ciproxifan (0.06, 0.6 and 2.0 mg/kg), an H_3 antagonist, led to a dose-dependent decrease of [11C-*N*-CH_3]GSK189254 uptake in the H_3-rich regions of the brain. At a dose of 2.0 mg/kg, the distribution of [11C-*N*-CH_3]GSK189254 was homogeneous throughout the brain demonstrating the selectivity of the tracer for the H_3R.



Tissue time-activity curves describing the kinetics of [11C-*N*-CH_3]GSK189254 for selected regions of interest in porcine brain pre & post tratment with ciproxifan (2.0 mg/kg); closed & open markers, respectively.

Conclusion: These findings suggest that [11C-*N*-CH_3]GSK189254 is a very promising radioligand for imaging and quantifying the H_3R in pig. Evaluation of this radioligand in other species is in progress.

Keywords: Carbon-11, Histamine H3 Receptor, PET Imaging, Pig, [11C]CO Carbonylation

(^{18}F)MK-9470 AND (^{11}C)CB-119, PET TRACERS FOR CANNABINOID CB1 RECEPTORS

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Introduction: A great deal of effort has been directed towards developing CB1R SPECT and PET ligands. Only a few of the reported compounds have been used in non-human primate PET imaging studies (Horti et al, 2006, J Nucl Med, 1689-1696; Donohue et al, 2006, NeuroImage, T50) and only [^{123}I]AM281 and [^{124}I]AM281 have been used in clinical studies (Berding et al, 2004, Biol Psychiatry, 904-915; 2006, Psychiatry Research Neuroimaging, 249-256). We report here the synthesis of [^{11}C]CB-119 and [^{18}F]MK-9470 (Figure 1), PET tracers suitable for *in vivo* imaging of CB1 receptors.

Experimental: [^{11}C]CB-119 and [^{18}F]MK-9470 were synthesized with high specific activity by alkylation of **1** (Figure 1) with either [^{11}C]methyl iodide or [^{18}F]fluoroethylbromide. Reactions were carried out in DMF with Cs_2CO_3 at 100°C for 5 minutes followed by HPLC purification.

Results and Discussion: Autoradiographic studies of [^{18}F]MK-9470 in rhesus brain showed the expected distribution, and the binding of [^{18}F]MK-9470 was blocked by the addition of AM251. Rhesus PET imaging studies showed very good brain uptake of each tracer and a distribution pattern consistent with that seen in the autoradiographic studies. The brain uptake of these tracers was blocked by pretreatment with MK-0364, a CB1R inverse agonist. Figure 2 shows the baseline and blocked studies in rhesus with [^{18}F]MK-9470.

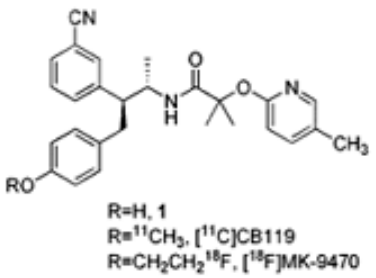

Fig. 1

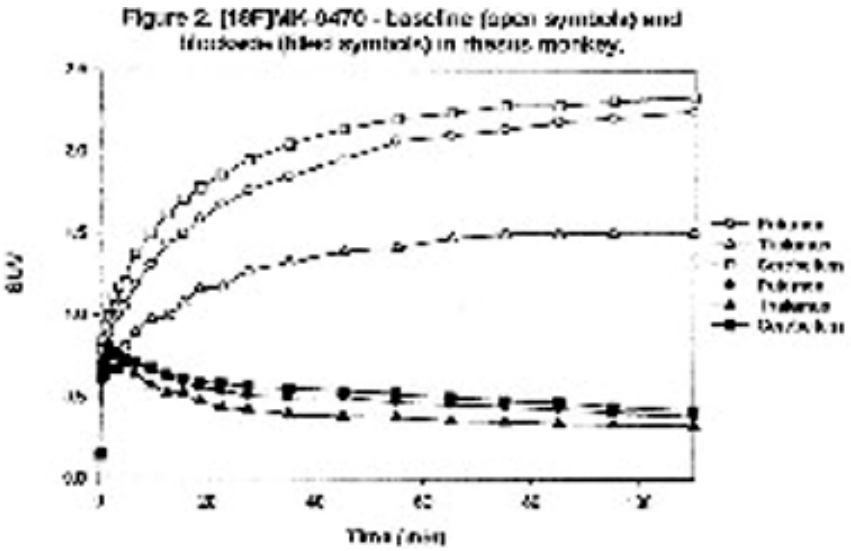

Fig. 2

In vitro metabolism studies using rat, monkey and human liver microsomes and *in vivo* metabolism studies in rhesus showed these tracers have acceptable metabolic profiles.

Conclusion: The details of these preclinical *in vitro* and *in vivo* studies will be presented showing that these two tracers are useful for imaging cannabinoid CB1 receptors in vivo.

Keywords: Cannabinoid, CB1, PET, Fluorine-18, Carbon-11

IN-VITRO CHARACTERIZATION, ^{18}F-SYNTHESIS AND BIODISTRIBUTION OF A PYRAZOLO(1,5-*a*)PYRIDINE BASED DOPAMINE D4 RECEPTOR RADIOLIGAND CANDIDATE

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Introduction: The dopamine D4 receptor (D4R) has been implicated in the genesis of neuropsychiatric disorders, such as attention deficit and hyperactivity disorder (ADHD). As a part of our drug discovery and SAR investigations, we characterized FAUC 213 as a selective D4 "full" antagonist (Ki=2.2nM). The aim of this study was the synthesis, in-vitro characterization and ^{18}F-labelling of derivatives of FAUC 213 as D4R radioligands for PET.

Experimental: Four pyrazolo[1,5-*a*]pyridines with 2- and 4-fluoroethoxy substituted phenylpiperazinyl moieties were synthesized by reductive amination or Mannich reaction. Receptor binding assays were performed using human D2R, D3R and D4R expressed in CHO-cells and porcine striatal membranes (D1R) with [^{3}H]spiperone and [^{3}H]SCH23390. Kryptofix222-assisted ^{18}F-labelling was performed using 2-tosyloxyethyl precursor molecules (T=85°C, CH_3CN). LogP values were determined by radioligand extraction with octanol/PBS (pH 7.4). Sprague-Dawley rats were used for biodistributional studies (5, 10 and 30 min p.i.). In-vitro and ex-vivo autoradiography of brain slices were performed on a μ-imager (Biopspace).

Results and Discussion: All pyrazolo[1,5-*a*]pyridine derivatives demonstrated high D4R affinities (1.3-28 nM) in vitro. 4-(2-fluoroethoxy)phenyl substituted candidates revealed increased D4 receptor selectivity (D2/D4>2000) and Ki(α1)>4000nM, whereas substitution in 2-position was less suitable (D2/D4=150). 2-(4-(2-[^{18}F]Fluoroethoxy)phenylpiperazin-1-ylmethyl)-pyrazolo[1,5-*a*]pyridine (**[^{18}F]NM41**, (Ki(D4R)=13nM, D2/D4=2300) was labelled with an optimized RCY of 87±3% (t=10 min, n=5). LogP was 2.0±0.1 (n=9) and stability in human serum was >98% (37°C, t=90 min). In vitro rat brain autoradiography demonstrated uptake in cortical areas, hippocampus and hypothalamus that was inhibited by 1μM FAUC213. Biodistributional studies showed highest uptake in the liver (4.0%ID/g) followed by kidney (0.8%ID/g). Regional brain uptake was 0.36-0.70%ID/g (30 min p.i.) with highest uptake in the cortex (0.70%ID/g). Pre-injected rats (0.3 mg/kg haloperidol) showed decreased cortical uptake (0.19%ID/g). HPLC analyses of blood and tissue samples revealed high in vivo stability of **[^{18}F]NM41** (>98%).

Conclusion: In vitro uptake of **[^{18}F]NM41** in brain cortex was in accordance with the known D4 receptor distribution in the rat brain. Further in vivo studies are needed to characterize the binding of **[^{18}F]NM41** to interfering receptors more precisely.

Acknowledgement: This study was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG, PR 677/2).

Keywords: F-18, D4 Receptor, Brain Uptake, Autoradiography, PET

RADIOSYNTHESIS AND PRELIMINARY EVALUATION OF 2-(6-CHLORO-2-(4-IODOPHENYL)IMIDAZO(1,2-*a*)PYRIDIN-3-YL)-*N*-ETHYL-*N*-([11]C)METHYL-ACETAMIDE (([11]C)CLINME), A NOVEL RADIOLIGAND FOR IMAGING THE PERIPHERAL BENZODIAZEPINE RECEPTORS WITH PET

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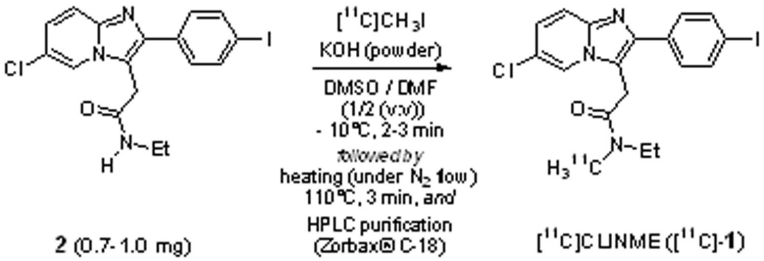
Introduction: [11C]PK11195 is not only the oldest, but also the most widely used PET radiotracer for *in vivo* imaging the peripheral benzodiazepine receptors. Already used for two decades in humans, this ligand yet, suffers from low brain uptake, extensive binding to plasma proteins and relatively high non-specific binding. Within a new 2-(iodophenyl)imidazo[1,2-*a*]pyridineacetamide series, CLINME (**1**) was considered an appropriate candidate for PET-imaging (1). It was labelled with carbon-11, imaged and pharmacologically evaluated in a rodent model of neuroinflammation (unilaterally, AMPA-induced, striatum-lesioned rats).



Experimental: Radiosynthesis: (a) trapping at -10°C of [11C]MeI in DMSO/DMF (1/2 (v:v), 0.3 mL) containing **2** (0.7-1.0 mg) and powdered KOH (3-5 mg); (b) heating at 110°C for 3 min under a N_2 stream; (c) dilution of the residue with 0.6 mL of the HPLC mobile phase and (d) purification using semi-prep HPLC (Zorbax SB18). PET-imaging (Focus 220 Concorde) includes control kinetics and displacement experiments with PK11195 and CLINME (1 mg/kg).

Results and Discussion: Typically, starting from a 1.5 Ci [11C]CO_2 production batch, 120-150 mCi of [11C]-**1** were obtained (RCY: 16-23%, decay-corrected) within 24-27 min (formulation incl.) Specific radioactivities: 0.9-2.7 Ci/micromol at EOS. In PET experiments, [11C]CLINME showed a higher contrast between the lesioned area and the corresponding area in the intact contralateral hemisphere when compared to [11C]PK11195 (ratio ipsi/contra at 20 min post-injection: [11C]CLINME: 2.2 ± 0.3, n = 4; [11C]PK11195, 1.7 ± 0.1, n = 5). Furthermore, [11C]CLINME was totally displaced by PK11195 or CLINME. Immunohistochemical analyses correlate with PET-imaging and showed strong activation of microglia in and around the lesion.

Conclusion: The results obtained demonstrate the potential of [11C]CLINME to image neuroinflamation.

Acknowledgement: Supported in part by FAST FRO40051.

Reference: [1] Katsifis A et al., Int. Appl. 1999: WO 9951594.

Keywords: Carbon-11, Methyl Iodide, CLINME, PBR

IN VIVO EVALUATION OF A ^{18}F-LABELLED IMIDAZOPYRIDAZINE, FOR THE STUDY OF THE PERIPHERAL BENZODIAZEPINE BINDING SITES USING PET

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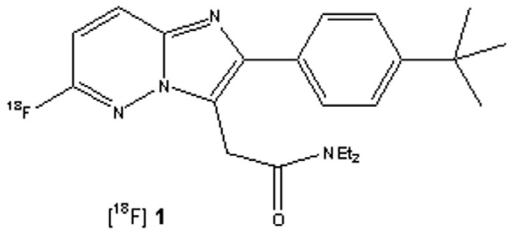
Introduction: Peripheral benzodiazepine binding sites (PBBS) have become attractive targets for the study of a number of clinical conditions including neurodegeneration, inflammation and cancer. Significant upregulation of these binding sites in a number of disease states has prompted the development of radioligands for PET and SPECT imaging. A number of iodinated and fluorinated imidazopyridazines have been developed as potential candidates for the PBBS.

The aim of this study was to evaluate a lead F-18 imidazopyridazine: 2-(2-(4-*tert*-butylphenyl)-6-fluoroimidazo[1,2-*b*]pyridazin-3-yl)-*N*,*N*-diethylacetamide **1** as a probe for the study of PBBS using PET.



[^{18}F] **1**

Experimental: [^{18}F]**1** has been prepared by nucleophilic substitution of the Br precursor with ^{18}F-fluoride in the presence of K_{222} and K_2CO_3 in DMF at 150°C for 5 mins.

The biodistribution of [^{18}F]**1** was undertaken in SD rats and analysed up to 4 h p.i. in the brain and peripheral tissues. The specificity and selectivity of the tracer was assessed by pre-treatment with PBBS and Central Benzodiazepine Receptor (CBR) specific ligands (1 mg/kg) 5 min prior to injection of [^{18}F]**1.**

Results and Discussion: In vitro binding of **1** indicated an IC_{50} of 29 nM for PBBS and 340 nM for the CBR. [^{18}F]**1** was synthesised in 40-50% radiochemical yield with >95% radiochemical purity and a specific activity of 40-80 GBq/μmol (un-optimised). The in vivo biodistribution of [^{18}F]**1** showed high uptake in tissues of known PBBS. In the adrenals was found an uptake of 13% ID/g at 30 min p.i and maintained over the 4 h. In kidney, heart and lung the activity (4, 8 and 16% ID/g) peaked at 15 min p.i. and decreased over time to less than 2.3% at 4h. Bone uptake ranged from 1 (at 15 min) to 3.3% at 4h. The uptake in the olfactory bulbs ranged from an initial 0.63% at 15 min to 0.25% at 4h p.i. The concentration in the blood decreased from 0.4% at 15 min to 0.07% at 4h. Pre-treatment with PK 11195 and Ro 5-4864 decreased the uptake in the brain and peripheral organs except in the adrenals which showed an activity increase. Flumazenil had no effect in the uptake of [^{18}F]**1** in the brain or peripheral organs.

Conclusion: These results demonstrate the specific PBBS uptake of [^{18}F]**1** in vivo. This suggests that [^{18}F]**1** warrants further investigation as a potential PET marker for the PBBS.

Keywords: Peripheral Benzodiazepine Binding Sites, Imidazopyridazines, Fluorine-18, PET

AN INVESTIGATION INTO SMALL MOLECULE CATALYSED MEMBRANE DIGESTION: TOWARDS A DEEPER UNDERSTANDING OF RADIOLIGAND NON-SPECIFIC BINDING

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Introduction: The phenomenon of non-specific binding is poorly understood, yet is probably one of the most common reasons for the failure for a putitive PET radioligand candidate. Current practice suggests that successful ligands have an octanol-water partition coefficient (logP, logD) of around 1.5 - 3 with an appropriate affinity for the receptor or enzyme of interest. However, many exceptions to this rule exist, suggesting that other factors contribute to the non-specific binding phenomenon.

To address this issue we set out to study the interaction of various ligands and drugs in an experimental system and correlate these measures with the reported literature values of these ligand's non-specific binding.

Experimental: The following CNS radioligands were studied in the vesicular bilayers of artificially self-assembled membrane constructs made with phospholipids commonly found in mammalian tissues: haloperidol (logP=2.24), spiperone (logP=1.73), WAY (logP=3.69), FLB 457 (logP=1.50), raclopride (logP=0.90), flumazenil (logP=1.29), diprenorphine (logP=0.72), R-rolipram (logP=1.72), DASB (logP=3.03) and MDL (logP=2.28).

The molecule-membrane interactions were quantified using spectroscopic techniques such as small-angle X-ray scattering, solid-state NMR, HPLC and fluorescence microscopy.

In vivo non-specific binding distribution volumes for the above compounds were obtained from literature reports.

Results and Discussion: The ligands were shown to reside at the membrane polar-apolar interface. In addition, it was also demonstrated the ligands had a profound effect on the bilayers by catalytically hydrolyzing the double-chained phospholipids into single-chained 'lyso' phospholipids and their associated fatty acids. Kinetic studies were undertaken to determine the rate of the lipid hydrolysis under different conditions such as membrane composition, ligand counterion, and buffer composition.

Conclusion: These studies have shown that both binding and hydrolysis rates are correlated to the in vivo non-specific binding distribution volumes of the PET radioligands. The degree of membrane hydrolysis with respect to time may possibly be used to further understand the phenomenon of non-specific binding on a mechanistic level, as well as in the design of radiolabelled probes for in vivo receptor imaging using PET.

Keywords: Non-Specific Binding, Lipid Hydrolysis