

**FLUORINE-18 RADIOLABELLING, BIODISTRIBUTION STUDIES AND
PRELIMINARY PET EVALUATION OF A NEW MEMANTINE
DERIVATIVE FOR IMAGING THE NMDA RECEPTOR**

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ABSTRACT

A synthetic method has been established for preparing [¹⁸F]1-amino-3-fluoromethyl-5-methyl-adamantane ([¹⁸F]AFA). Biodistribution of the radiotracer in mice showed high brain uptake. The peak uptake (3.7% ID/g organ) for the brain occurred at 30 min after injection. Accumulation of radioactivity in mouse brain was consistent with the known distribution of the NMDA receptors. The binding of [¹⁸F]AFA to the phencyclidine (PCP) binding sites of the NMDA receptor complex and the sigma recognition sites in a Rhesus monkey was also examined using positron emission tomography (PET). The regional brain distribution of [¹⁸F]AFA was changed by memantine and by (+)-MK-801, indicating competition for the same binding sites. Treatment with haloperidol caused a marked reduction of radioactivity uptake in all the brain regions examined. (-)-Butaclamol, which has pharmacological specificity for sigma sites, did not have any significant effects.

INTRODUCTION

The N-Methyl-D-Aspartate (NMDA) receptor complex is a ligand-gated ion channel that is permeable to sodium, calcium and potassium ions. It is one of several ionotropic glutamate receptors that is thought to be responsible for excitatory transmission in the central nervous system (1). The receptor has been implicated in various brain physiological and pathophysiological processes. Excessive activation of the receptor leads to large increases in intracellular calcium ions, which ultimately can lead to cell death (2). Over the past years, the NMDA receptor has been the focus of intensive research and the current knowledge on this receptor has considerably increased. A wealth of evidence suggests that the NMDA receptor contributes to brain disorders such as Alzheimer's disease (3), Huntington's disease (4) and epilepsy (5). In addition, the NMDA receptor has been shown to play key roles in synaptic transmission, memory and learning (6).

A large number of compounds based on uncompetitive NMDA antagonists (+)-MK-801 and TCP have been labelled with positron emitting radionuclides such as ^{11}C and ^{18}F for the in vivo studies with PET (7, 8). The results obtained from these studies have not been encouraging because none of these radioligands has proved to be useful for the in vivo imaging of the NMDA receptor complex. Memantine (1-amino-3,5-dimethyl adamantane) is a drug which is used in the treatment of Parkinson's disease and movement disorders (9). Its poor metabolism in man, its ability to readily cross the blood brain barrier ($\log P = 3.3$) and the suggestion that none of the known metabolites of memantine is a potent NMDA antagonist confer memantine distinct advantages amongst the uncompetitive NMDA antagonists. Such data suggest that memantine, labelled with a positron emitting radionuclide, could provide a radioligand with potential for investigating the NMDA receptor complex by PET. Memantine is an adamantane derivative and does not lend itself to facile isotopic labelling with either [^{11}C]carbon or [^{15}N]nitrogen therefore radiolabelling with the positron emitter, fluorine-18 (half-life = 110 min) was undertaken. In a previous work

(10), we synthesized the non-radioactive compound, 1-amino-3-fluoromethyl-5-methyl-adamantane (AFA), and showed in in vitro receptor screening assays that this new compound binds with high selectivity to the PCP binding site of the NMDA receptor complex.

In this paper, we describe the radiosynthesis, biodistribution studies in mice and the preliminary PET investigations of this new compound in a Rhesus monkey.

MATERIALS AND METHODS

[^{18}F]Fluoride for nucleophilic labelling was produced by irradiation of 98% enriched [^{18}O]H $_2$ O by the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ reaction. The precursor used for the fluorine-18 radiolabelling was synthesized as described previously (10). (-)-Butaclamol and (+)-MK-801 maleate were purchased from RBI (Research Biochemicals International, Rahn AG, Zürich, Switzerland) and memantine hydrochloride was a gift from Merz + Co. (Frankfurt/M, Germany). Unless otherwise noted, all other reagents and solvents used were of analytical quality or high performance liquid chromatography (HPLC) grade and were purchased from Merck (Darmstadt, Germany) or from Fluka Chemie (Buchs, Switzerland). PET scans were performed using a PRT-2 Prototype rotating tomograph (Siemens-CTI) with a spatial resolution of 6 mm.

Two HPLC systems were used for the semi-preparative purification and the quality control of the product:

Semi-preparative (system A): Consisting of a Waters 510 pump, a Valco 6-port valve with 5 mL loop, a KNAUER UV detector, a Geiger-Müller counter LND 714 with an Eberlein RM-14 instrument and a Waters μ -Bondapak C-18 column, 300x7.8 mm and 0.1% H $_3$ PO $_4$: EtOH (88:12) at 4 mL/min.

Analytical HPLC (system B): Consisting of a Rheodyne injector with 100 μ L loop, a Merck-Hitachi L 6200 pump, a NaI scintillation detector (Scintillation Meter type 540, Mini Instruments Ltd, Burnham on Crouch/UK), a Merck-Hitachi L-4000 UV detector (at 215 nm), a Merck-Hitachi D-2500 Chroma

integrator, a Waters μ -Bondapak C-18 column, 300x4.6 mm and 0.1% H_3PO_4 : EtOH (85:15) at 2 mL/min.

Fluorine-18 labelling

[^{18}F]AFA was prepared according to a modified protocol as previously described (10). Aqueous [^{18}F]fluoride, obtained via $^{18}O(p,n)^{18}F$ reaction was placed into a 10 mL Reacti-vial[®] containing 3 mg K_2CO_3 . A solution consisting of 14 mg Kryptofix 2.2.2 in 0.5 mL CH_3CN was added and the solvent was removed under a stream of nitrogen in a block heater at 100°C, followed by azeotropic evaporation with CH_3CN (2x, 1.0 mL). In 0.5 mL of dry DMSO were dissolved 2 mg of the precursor. The Reacti-vial containing the solution was heated at 130°C for 20 min. The reaction mixture was diluted with H_2O (5 mL) and passed through a Sep-Pak C-18 Cartridge (Millipore Corp.). The cartridge was prewashed with H_2O (5 mL) and the radiolabelled intermediate was eluted from the Sep-Pak cartridge with ether (10 mL). After the evaporation of the solvent under a stream of nitrogen, the residue was treated by heating with 20% HCl (1 mL) for 10 min at 110°C. The resulting product was isolated by reversed-phase HPLC (system A). Besides HPLC, thin layer chromatography (TLC) was used to determine the identity and purity of [^{18}F]AFA ($R_f = 0.38$). For in vivo investigations, the collected product fraction was buffered with 0.6 M phosphate buffer to give, after sterile filtration, an isotonic and injectable radiopharmaceutical.

Biodistribution studies in mice

The biodistribution studies of [^{18}F]AFA was determined in female ICR mice weighing 25-30 g. Three animals at each time point were injected in the tail vein with 1.5 - 3.0 MBq of [^{18}F]AFA and were sacrificed 5, 15, 30, 60, 120 and 240 min post injection (p.i). Blood samples and organs of interest were removed, blotted dry and weighed. Radioactivity was measured on a Packard auto-gamma 500

scintillation counter. After correction for physical decay, percent injected dose per gram organ (% I.D./g) was calculated for each organ. For the brain regions the percent injected dose per gram region of interest was normalized to the whole brain. Whole blood activity was calculated assuming a blood volume V [mL] = $1/15 \times$ body weight [g]. In addition, urine and faeces were collected and monitored for radioactivity. The brain/blood ratios were calculated from the corresponding % ID/g organ values. The brain was dissected and the following regions were isolated: cerebellum, frontal cortex, parietal and occipital cortices, hippocampus and brain stem. In separate experiments, [^{18}F]AFA was co-injected in the tail vein with (+)-MK-801 (0.10 mg/kg) and the animals were sacrificed 60 min p.i. The tissue radioactivity concentrations were assayed as described above.

PET studies

PET scans were performed in one female Rhesus monkey (*Macaca mulatta*) weighing 4.5 kg. Anesthesia was induced by Nembutal[®] and maintained with a mixture of nitrous oxide and oxygen throughout the whole experiment. Venous blood (1 mL) was taken at 5, 15, 30, 60 and 90 min. Plasma was separated and its radioactivity was analyzed for metabolites using HPLC and TLC. Before each study, the monkey was deprived of food for 12h. For the PET measurement, the head of the animal was placed in the gantry of a ring tomograph and fixed in a stereotactic frame holder (LEKSELL[®]) to ensure identical position of the brain in each scan. After the initial positioning, the animal was not moved for the duration of the scan. The animal was injected intravenously (i.v.) with [^{18}F]AFA (80-100 MBq) and image acquisition started simultaneously in multiple sequences. The following brain regions were delineated: striatum, temporal and frontal cortices and cerebellum. Three types of experiments were performed:

(a) *A baseline study;*

(b) *Blockade experiments* by i.v. pretreatment with different concentrations of memantine hydrochloride (0.5, 1.0 and 2.5 mg/kg respectively, 30 min before PET study) and with (+)-MK-801 maleate (0.5 mg/kg, 5 min prior i.v. injection);

(c) *Blockade studies* by i.v. co-injection of 0.1 mg/kg haloperidol and 0.5 mg/kg (-) butaclamol, 30 min before PET study. The aim of the blocking studies being to determine the extent of specific binding of [^{18}F]AFA. The measured activity values expressed in Bq/ml were normalized to injected activity per gram body weight and plotted versus time.

RESULTS

Radiolabelling

In figure 1 is shown the reaction scheme leading to the final product. The radiochemical yield of [^{18}F]AFA was $22 \pm 7\%$ (decay corrected to end of bombardment) with a total synthesis time of 90 min. The radiochemical purity was greater than 98%.

Biodistribution

The biodistribution of [^{18}F]AFA in mice for selected organs is shown in fig. 2. The regional distribution of radioactivity at 60 min after injection of [^{18}F]AFA showed the following order: lung > kidney > liver > brain > heart > blood. The peak uptake (3.7% I.D/g organ) for the brain occurred at 30 min postinjection (p.i.). Clearance of radioactivity was fast for peripheral organs such as liver, lung and blood. The brain showed a prolonged retention of radioactivity. The highest brain/blood ratio was 9.3 at 120 min after administration of radioactivity.

PET studies

Figure 4 shows the time-activity curve of [^{18}F]AFA uptake in the monkey brain at the level of the frontal cortex before and after pretreatment with various antagonists. The radioactivity time curve obtained from the base-line scan showed high brain uptake and low clearance of the radiotracer, with a plateau from 35 min p.i.. The highest uptake of radioactivity was observed in the cerebellum. Radioactivity uptake in the striatum, frontal cortex and the temporal cortex was similar. The radiotracer cleared rapidly from the blood (data not shown). The

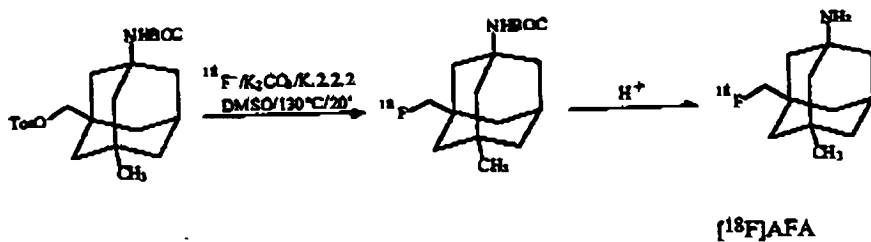


Fig.1. Radiosynthesis of [¹⁸F]AFA.

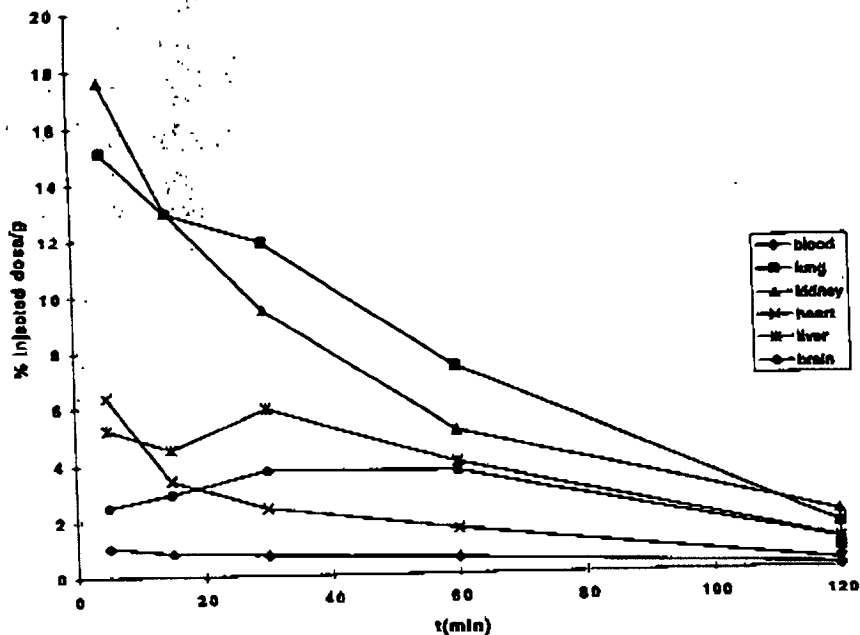


Fig. 2. Biodistribution of [¹⁸F]AFA in organ tissues of mice.

blood uptake 15 min p.i. decreased by $94 \pm 2\%$ compared to the activity concentration measured in the collected blood 2 min following the i.v. injection of [¹⁸F]AFA. Drug treatment did not change the value of the plasma uptake counted under base-line conditions. Metabolic studies indicated that more than 97% of radioactivity in monkey plasma was parent compound. Preinjection with a therapeutic dose of memantine (1 mg/kg) 30 min prior to i.v. administration of

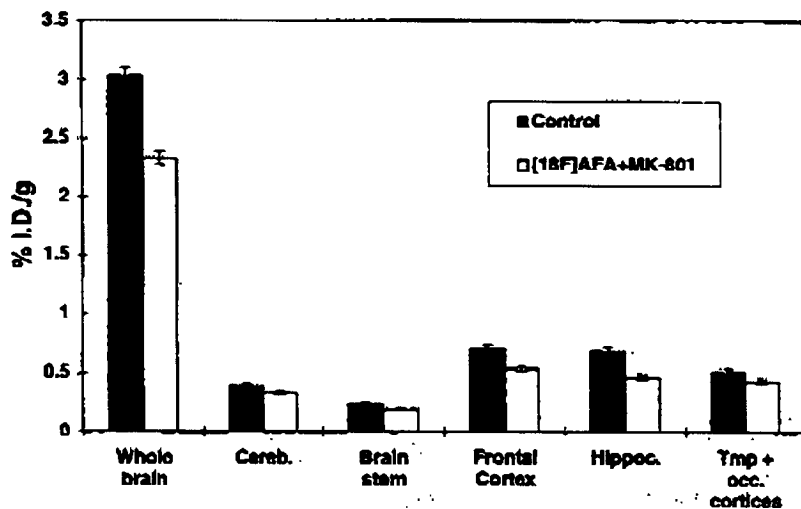


Fig. 3. Percent injected dose per gram (% I.D./g) of [¹⁸F]AFA in organ tissues of mice in control and after co-injection with 0.10 mg/kg of (+)-MK-801. 60 min postinjection (cereb. = cerebellum, hippoc. = hippocampus, temp. occ. = temporal, occipital).

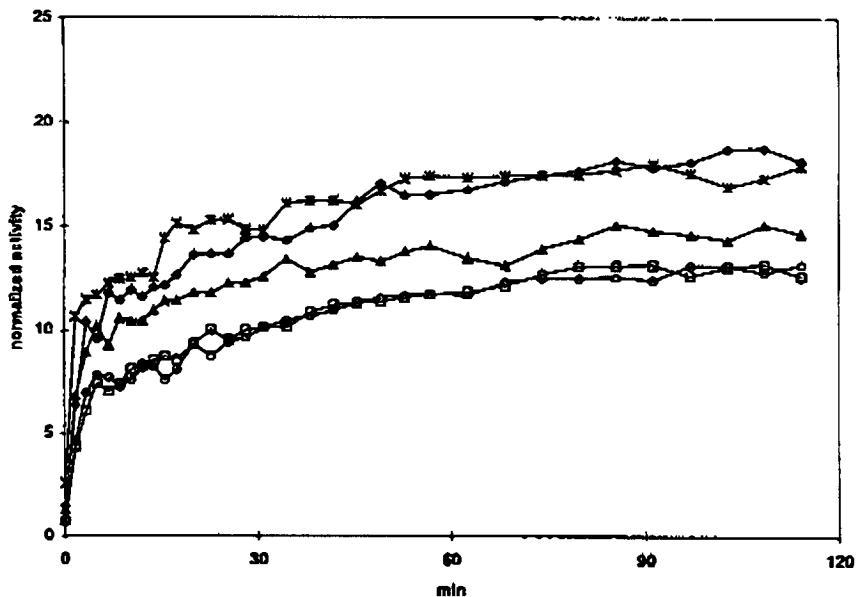


Fig. 4. Time-activity curves of [¹⁸F]AFA obtained by PET studies at the level of the frontal cortex in a Rhesus monkey under baseline and blockade conditions. ◆ Baseline; ▲ 1.0 mg/kg memantine; ○ 0.5 mg/kg (+)-MK-801; □ 0.1 mg/kg haloperidol; * 0.5 mg/kg (-)-butaclamol.

[¹⁸F]AFA led to a reduction of activity uptake by 32% from 60 min p.i. onwards in the examined brain regions (striatum, frontal and temporal cortices and the cerebellum). The pretreatment of the monkey with a lower dose of memantine (0.5 mg/kg) also reduced the brain uptake of the radiotracer but to a lesser extent than the dosage with 1 mg/kg. In contrast, preinjection with 2.5 mg/kg memantine caused an increase in the radioactivity uptake (not shown). A reduction in the binding of [¹⁸F]AFA by pretreatment with (+)MK-801 in all the brain regions was also observed. Like (+)MK-801, haloperidol produced a marked decrease in the uptake of radioactivity in all the brain regions examined. In contrast, (-)-butaclamol, which has pharmacological specificity for sigma sites, did not have any significant effects.

DISCUSSION

The present paper describes the radiosynthesis and the binding characteristics of the new memantine derivative. We have previously shown that AFA is an uncompetitive ion channel blocker at the NMDA receptor complex (10). Though micromolar affinities are considered to be too low for *in vivo* receptor quantification by PET, the promising characteristics shown by non-radioactive AFA prompted us to further evaluate [¹⁸F]AFA and to assess its suitability as an imaging agent for the NMDA receptor.

The radiolabelling of [¹⁸F]AFA was accomplished by the no carrier added (nca) nucleophilic radiofluorination and using classical nca radiofluorination conditions as previously reported (10). The tosylat group that served as a leaving group appeared to be optimal because of its stability. Best yields were obtained with K¹⁸F/Kryptofix 2.2.2 complex as the fluorination agent (11). Low radiochemical yields of [¹⁸F]AFA (< 10 %) were obtained using K¹⁸F/Kryptofix 2.2.2 in acetonitrile or tetra-*n*-butylammonium [¹⁸F]fluoride (12).

The regional distribution in mice following intravenous administration of [¹⁸F]AFA is shown in fig. 2. High uptake was initially observed in the lung, kidney, liver and in the heart, but the radioactivity levels decreased gradually with

time. The lung is an organ known to take up amines (13). The uptake in the kidney is explained by the excretory function of this organ. [^{18}F]AFA showed high brain uptake (3.6 % I.D./g at 60 min p.i.) indicative of good blood brain barrier penetration, with increasing brain/blood ratios up to 9.3, 120 min p.i. In a regional dissection study, high radioactivity concentrations were observed in the cerebellum, frontal cortex and in the hippocampus, regions known to contain high densities of the NMDA receptor. Within 120 min about 15% of the injected activity was found in collected urine, whereas only 0.04 % of the I.D. was eliminated by hepatobiliary excretion.

Co-injection of [^{18}F]AFA with (+)-MK-801 (0.10 mg/kg) reduced the initial radioactivity uptake by 32%, 23% and 16% and 15% 60 min p.i. in the hippocampus, frontal cortex, occipital cortex and cerebellum, respectively suggesting *in vivo* binding to the NMDA receptor (fig. 3).

The PET studies were performed to assess the pharmacokinetics and the selectivity of [^{18}F]AFA in a nonhuman primate. The good uptake and retention in the frontal and temporal cortices and in the striatal area are consistent with the reported high concentration of NMDA receptors in these brain regions (14). The hippocampus contains the largest number of PCP binding sites (15). However, owing to the resolution of the PET tomograph, delineation of the hippocampus was not possible. Preinjection with therapeutic doses of memantine (1 mg/kg) 30 min prior to i.v. administration of [^{18}F]AFA produced a reduction in the striatum, frontal and temporal cortices and the cerebellum suggesting that part of the binding was due to NMDA receptors. The increased uptake caused by the loading dose of 2.5 mg/kg is probably due to blockade of peripheral organs.

The highest uptake of radioactivity in Rhesus monkey brain occurred in the cerebellum. High concentrations of sigma sites in the cerebellum of Rhesus monkey have been reported (16) therefore the high uptake of [^{18}F]AFA in the cerebellum probably reflects binding to sigma recognition sites. Provisional unpublished data also indicate that non-radioactive AFA does bind to the sigma sites ($[^3\text{H}]$ -DTG, $\text{IC}_{50} = 2.7 \mu\text{M}$, Hill 0.8, Panlabs) with similar potency to the

MK-801 site ($[^3\text{H}]$ -MK-801, $K_i = 3.1 \pm 0.3 \mu\text{mol/l}$, Hill 1.1, Merz). Thus, in preliminary experiments we examined also the extent to which $[^{18}\text{F}]$ AFA binds to the sigma receptors using haloperidol and (-)-butaclamol. Haloperidol has high affinity for the dopamine D_2 receptor and the sigma binding sites ($K_d = 0.95 \text{ nmol/l}$) (17) while (-)-butaclamol has a moderate affinity for the sigma sites ($K_i = 85 \text{ nmol/l}$) (18). Unlike (-)-butaclamol, haloperidol caused a marked decrease in the radioactivity uptake in the cerebellum and other delineated brain regions suggesting that binding was also occurring to the sigma recognition sites. It is unclear why (-) butaclamol failed to show any significant change in the absolute radioactivity uptake.

More detailed studies including further blockade experiments and using different doses of various NMDA and sigma receptor antagonists are ongoing.

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