



Electrophysiological Study, Biodistribution in Mice, and Preliminary PET Evaluation in a Rhesus Monkey of 1-Amino-3-[¹⁸F]fluoromethyl-5-methyl-adamantane (¹⁸F-MEM): A Potential Radioligand for Mapping the NMDA-Receptor Complex

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ABSTRACT. The effect of the fluorinated memantine derivative and NMDA receptor antagonist, 1-amino-3-fluoromethyl-5-methyl-adamantane (¹⁹F-MEM), at the NMDA receptor ion channel was studied by patch clamp recording. The results showed that ¹⁹F-MEM is a moderate NMDA receptor channel blocker. A procedure for the routine preparation of the ¹⁸F-labelled analog ¹⁸F-MEM has been developed using a two-step reaction sequence. This involves the no-carrier-added nucleophilic radiofluorination of 1-[N-(tert-butylxy)carbonyl]-3-(toluenesulfonyloxy)methyl-5-methyl-adamantane and the subsequent cleavage of the BOC-protecting group using aqueous HCl. The ¹⁸F-MEM was obtained in 22 ± 7% radiochemical yield (decay-corrected to EOB) in a total synthesis time including HPLC purification of 90 min. A biodistribution study after IV injection of ¹⁸F-MEM in mice showed a fast clearance of radioactivity from blood and relatively high initial uptake in the kidney and in the lung, which gradually decreased with time. The brain uptake was high (up to 3.6% ID/g, 60 min postinjection) with increasing brain-blood ratios: 2.40, 5.10, 6.33, and 9.27 at 5, 30, 60, and 120 min, respectively. The regional accumulation of the radioactivity in the mouse brain was consistent with the known distribution of the PCP recognition site. Preliminary PET evaluation of the radiotracer in a rhesus monkey demonstrated good uptake and prolonged retention in the brain, with a plateau from 35 min onwards p.i. in the NMDA receptor-rich regions (frontal cortex, striata, and temporal cortex). Delineation of the hippocampus, a region known to contain a high density of NMDA receptors, was not possible owing to the resolution of the PET tomograph. The regional brain uptake of ¹⁸F-MEM was changed by memantine and by a pharmacological dose of (+)-MK-801, indicating competition for the same binding sites. In a preliminary experiment, haloperidol, a dopamine D2 and sigma receptor antagonist, decreased the binding of ¹⁸F-MEM from the brain regions examined, suggesting that binding was also occurring to the sigma recognition sites. NUCL MED BIOL 25;4:323–330, 1998. © 1998 Elsevier Science Inc.

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INTRODUCTION

The glutamatergic *N*-methyl-D-aspartate (NMDA) receptor is a major ionotropic receptor type that mediates excitatory synaptic transmission in the mammalian central nervous system (10, 24, 25). The NMDA receptor when activated controls the opening of an ion-channel, which permits the entry of monovalent and divalent cations (mainly Na⁺ and Ca²⁺) into target cells. In recent years, increasing attention has focused on the NMDA receptor because of its involvement in various brain physiological and pathophysiological processes. Excessive activation of the receptor, in response to brain injury, can lead to cell death, probably caused by an excess accumulation of intracellular Ca²⁺ (7). It seems likely that the NMDA receptor contributes impor-

tantly to the etiology and progression of many neurological disease states such as ischemia (34), epilepsy (29), traumatic CNS injury, hypoglycemia (7), Alzheimer's disease (15, 39), and Huntington's disease (46).

In addition, the NMDA receptor has been shown to be essential for neuronal and behavioral plasticity, and hence has effects on learning and memory (25, 26). NMDA receptor-mediated long-term potentiation in the rat hippocampus is now a main experimental model for investigations on learning and memory at a molecular level (3). Thus, there has been great interest in the development of radioligands for imaging the NMDA receptor complex in the living human brain by noninvasive tomographic techniques like positron emission tomography (PET). Because uncompetitive NMDA antagonists (ion-channel blockers) have proved to be important tools for investigating the basic mechanisms of NMDA receptor function (19), several compounds mainly based on the uncompetitive NMDA antagonists phencyclidine (PCP), (+)-MK-801, and ketamine have been labelled successfully with positron-emitting nuclides for *in vivo* studies with PET (2, 5, 14, 17, 21, 30, 36).

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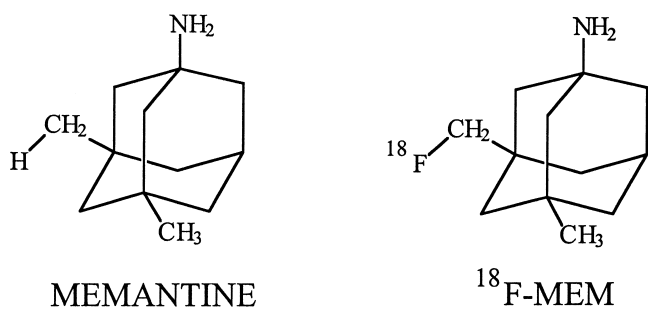


FIG. 1. Structures of memantine and the new [^{18}F]fluorinated memantine derivative ^{18}F -MEM.

Unfortunately, the evaluation of their potential as tracers for PET has not been encouraging.

The development of radiotracers for the NMDA receptor complex appears relatively more difficult compared to radiotracers for common receptor and transporter proteins, owing to the differences in the nature of the binding sites. Whereas the binding sites of common receptors and transporters are located close to the surface of the membrane, the NMDA receptor channels are buried deeper within the membrane, and their accessibility is thus severely restricted by the amount and duration of agonist that is available to activate the channel. The reason for this is that the ion-channel blockers may bind to the open-state of the channel (27).

The starting point for the present work was the finding that the clinically used drug memantine (1-amino-3,5-dimethyladamantane, Fig. 1), with beneficial effects in the treatment of various neurological and psychiatric disorders like Parkinson's disease and spasticity of cerebral and spinal origin (16, 32, 42), is a fast uncompetitive NMDA antagonist with rapid access to open NMDA receptor channels (22, 28). In addition, its ability to penetrate the blood-brain barrier, its poor metabolism in man, and the suggestion that none of the known metabolites is a potent NMDA antagonist confer on memantine distinct advantages among the uncompetitive NMDA antagonists (28). 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 does not lend itself to facile isotopic labelling with either [^{11}C]carbon or [^{13}N]nitrogen.

Consequently, we pursued the labelling of memantine with [^{18}F]fluorine. In a previous work (35), we synthesized 1-amino-3-fluoromethyl-5-methyl-adamantane (^{19}F -MEM), a fluorine analog of memantine. This new compound binds selectively to the PCP binding site located within the NMDA receptor-associated ion-channel. The ^{18}F -labelled analog ^{18}F -MEM was prepared by no-carrier-added nucleophilic radiofluorination, starting from 1-[N-(tert-butyloxy)carbamoyl]-3-(toluenesulfonyloxy)-methyl-5-methyl-adamantane (**1**) and could now be produced routinely in good radiochemical yield and high specific activity for *in vivo* investigations. In this report the direct effect of ^{19}F -MEM at the NMDA receptor channel is demonstrated by patch clamp experiments. Biodistribution of ^{18}F -MEM in mice and preliminary PET studies in a rhesus monkey were undertaken to determine whether ^{18}F -MEM could be a promising tracer for *in vivo* studies of the NMDA receptor complex by PET.

MATERIALS AND METHODS

General

Both 1-[N-(tert-butyloxy)carbamoyl]-3-(toluenesulfonyloxy)methyl-5-methyl-adamantane (**1**) and 1-amino-3-fluoromethyl-5-methyl-adamantane (^{19}F -MEM) were synthesized as described previously (35). TLC and radio-TLC chromatograms were performed on silica gel plates (SIL G/UV₂₅₄, Merck) using $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (7/3) as mobile phase. Visualization of nonradioactive compound (^{19}F -MEM) was achieved by ninhydrin spray. The (+)-MK-801 maleate was 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 were of analytical quality or HPLC grade and were purchased from Merck (Darmstadt, Germany) or from Fluka Chemie (Buchs, Switzerland). The [^{18}F]fluoride for nucleophilic labelling was produced by irradiation of 98% enriched [^{18}O]H₂O by the $^{18}\text{O}(p,n)^{18}\text{F}$ reaction as described previously (33). PET scans were performed using a PRT-2 Prototype rotating tomograph (Siemens-CTI) with a spatial resolution of 6 mm (40). Two systems were used for the isocratic HPLC separations:

SYSTEM A (SEMIPREPARATIVE). This system consisted 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; 300 \times 7.8 mm and 0.1% H₃PO₄: EtOH (88:12) at 4 mL/min.

SYSTEM B (ANALYTICAL). This system consisted of a Rheodyne injector with 100- μL 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, 300 \times 4.6 mm and 0.1% H₃PO₄: EtOH (85:15) at 2 mL/min.

Radiochemical Synthesis of 1-amino-3- [^{18}F]fluoromethyl-5-methyl-adamantane (^{18}F -MEM)

The ^{18}F -MEM was prepared according to a modified protocol previously described (35). Briefly, aqueous [^{18}F]fluoride, obtained via $^{18}\text{O}(p,n)^{18}\text{F}$ reaction as described above, was placed into a 10-mL Reacti-vial® containing 3 mg K₂CO₃. A solution consisting of 14 mg Kryptofix 2.2.2 in 0.5 mL CH₃CN was added and the solvent was removed under a stream of nitrogen in a block heater at 100°C, followed by azeotropic evaporation with CH₃CN (2 \times , 1.0 mL). Two milligrams of the precursor **1** were dissolved in 0.5 mL dry DMSO and added to the residue with a syringe. The Reacti-vial was heated at 130°C for 20 min. The reaction mixture was diluted with H₂O (5 mL) and passed through a Sep-Pak C-18 Cartridge (Millipore Corp.). The cartridge was washed with H₂O (5 mL), and the protected intermediate ^{18}F -BOC-MEM was eluted from the Sep-Pak with ether (10 mL) and collected in a new Reacti-vial. After ether evaporation under a stream of nitrogen, ^{18}F -BOC-MEM was subsequently deprotected by heating with 20% HCl (1 mL) for 10 min at 110°C. The resulting ^{18}F -MEM was isolated by reversed-phase HPLC (system A). Besides HPLC, TLC was used to determine the identity of ^{18}F -MEM ($R_f = 0.38$). 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.

Patch Clamp Studies

Patch clamp recordings were made from cultured superior collicular and hippocampal neurons obtained from (E20) rat embryos at room temperature (20–22°C) with the aid of an EPC-7 amplifier at a membrane potential of –70 mV as described (28). Patch clamp electrodes were pulled and polished with a horizontal puller (DMZ) and had an internal tip diameter between 1.0 and 1.2 μm and a tip resistance of 4–5 MΩ. Cells were continuously superfused via one of eight channels of a custom-designed fast superfusion system with a common outflow. Test substances then were applied by rapidly switching channels. Complete exchange of the superfused solution was achieved within 10 to 20 msec. The application of the solutions and the synchronized on-line electronic acquisition of data were controlled by the program TIDA for Windows. Only results from stable cells were accepted for inclusion in the final analysis; *i.e.*, following recovery of responses to NMDA by at least 75% of their depression by ¹⁹F-MEM.

The contents of the intracellular (electrode) solution were as follows [mM]: CsCl [120], TEACl [20], EGTA [10], MgCl₂ [1], CaCl₂ [0.2], Glucose [10], ATP [2], cAMP [0.25]. The extracellular solutions had the following basic composition (mM): NaCl [140], KCl [3], CaCl₂ [0.2], Glucose [10], HEPES [10], Sucrose [4.5] and Glycine [0.001]. Neurons were pharmacologically isolated from one another by the inclusion of 0.3 μM tetrodotoxin (TTX) to block voltage-activated sodium currents. Test substances were added to this solution in concentrations detailed in results and pH corrected, when necessary, to 7.35.

Biodistribution Studies in Mice

Biodistribution studies were carried out in female ICR mice (25–30 g, obtained from Animal Research Institute of the University of Zurich) according to the regulations for animal research from the Veterinary Health Authorities of the Canton Aargau, Switzerland. After a single intravenous (IV) administration of 1.5–3.0 MBq of ¹⁸F-MEM (in 0.1–0.2 mL solution), the animals were held in metabolic cages. The animals (3 mice per time point) were sacrificed 5, 15, 30, 60, 120, and 240 min postinjection (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 (%ID/g) was calculated for each organ. For the brain regions the %ID/g 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 feces 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, ¹⁸F-MEM was co-injected (IV) 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 Scans

The PET scans were performed in one female rhesus monkey (*Macaca mulatta*) weighing 5 kg. Anesthesia was induced by Nembutal® and maintained with a mixture of N₂O and O₂. Before each study, the monkey was deprived of food for 12 h. The study was conducted according to the regulations for animal research from the

Veterinary Health Authorities of Cantons Zurich and Aargau, Switzerland.

For 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 IV with ¹⁸F-MEM (80–100 MBq), and image acquisition started simultaneously in multiple sequences. Images were recorded for periods of 60 sec (from time 0 min up to 15 min), 180 sec (from time 15 min up to 45 min) and 5 min (from 45 min up to 120 min). The following brain regions were delineated: striatum, temporal and frontal cortices, cerebellum, and white matter. Three types of experiments were performed: (i) a baseline study, to obtain ¹⁸F-MEM pharmacokinetics in the control state; (ii) blockade experiments by IV pretreatment with 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 IV injection of ¹⁸F-MEM); (iii) a blockade study by IV co-injection of 0.1 mg/kg haloperidol and ¹⁸F-MEM. The aim of the blocking studies being to determine the extent of specific binding of ¹⁸F-MEM to the NMDA receptor ion-channel and the sigma opiate binding sites. The measured activity values expressed in Bq/mL were normalized to injected activity per gram body weight and plotted versus time. Finally, venous blood samples (1.0 mL) were collected at 5, 15, 30, 60, and 90 min after tracer administration, and aliquots (0.25 mL) of whole blood and plasma were assayed for radioactivity in a cross-calibrated counter and the values decay corrected.

RESULTS AND DISCUSSION

Patch Clamp

The most important properties of NMDA receptors and of the associated ion-channels (NMDA channels) have been established by means of electrophysiological studies (1). Thus, the direct effect of channel blockers at the NMDA receptor channel has been clearly demonstrated for memantine by patch clamp experiments (4, 28).

¹⁹F-MEM, like memantine (28), phencyclidine (PCP), and MK-801 (11), use dependently blocked current responses of cultured neurons to NMDA. The blockade of whole-cell current responses to NMDA was concentration-dependent (Fig. 2A). The calculated IC₅₀ values using the four-parameter logistic equation (28) were 5.96 ± 0.67 μM (peak: max. response; Hill 1.10) and 6.89 ± 1.58 μM (plateau: response after desensitization; Hill 1.16) (Fig. 2B). The IC₅₀ values of other known uncompetitive antagonists such as memantine, PCP, ketamine, and dextromethorphan were 2.92 ± 0.31 μM, 1.04 ± 0.16 μM, 1.56 ± 0.10 μM, and 6.10 ± 3.60 μM, respectively. It has been demonstrated by autoradiographic and biochemical techniques that compounds that bind to PCP binding site within the NMDA receptor channel (*e.g.*, TCP and memantine) bind to two different sites in rat brain (41, 45). It has also been shown that PCP and its congeners have moderate affinity for the σ-opiate receptor (37). Whether ¹⁹F-MEM also binds to the σ-opiate or to other PCP-sensitive receptor sites could not be answered directly by patch clamp experiments. However, provisional unpublished data indicate that ¹⁹F-MEM does bind to the sigma sites (³H]-DTG, IC₅₀ = 2.7 μM, Hill 0.8, Panlabs) with similar potency to the MK-801 site (³H]-MK-801, Ki = 3.1 ± 0.3 μM, Hill 1.1, Merz).

The blockade of whole-cell current responses to NMDA by ¹⁹F-MEM was voltage-dependent (Fig. 2C), (at +70 mV, strong

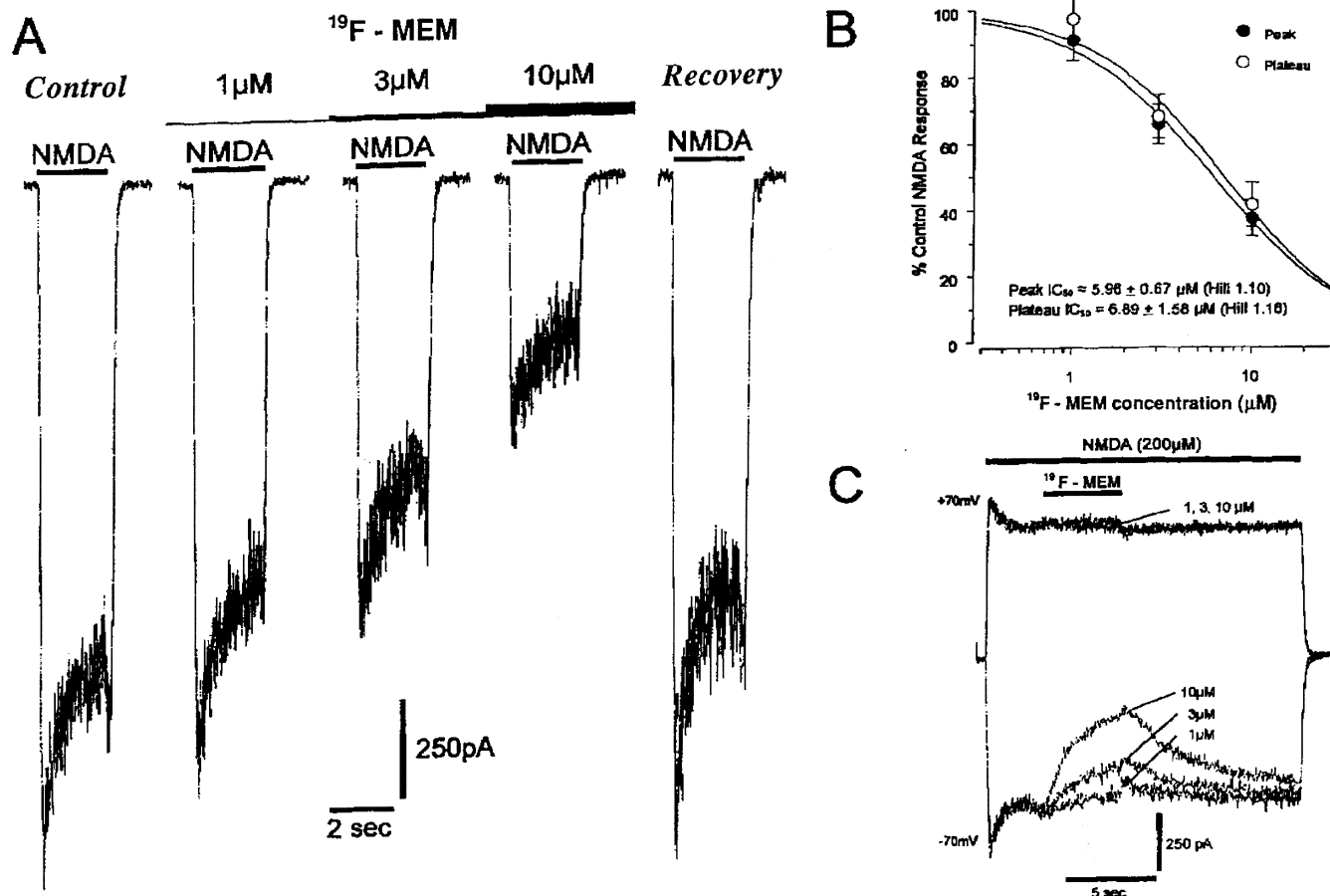


FIG. 2. Evidence for open NMDA receptor channel blockade by ^{19}F -MEM.

depolarization: no blockade; at -70 mV, concentration-dependent blockade).

Radiosynthesis

1-Amino-3- ^{18}F fluoromethyl-5-methyl-adamantane (^{18}F -MEM) was prepared by the no-carrier added (n.c.a.) nucleophilic radiofluorination of 1-[N-(tert-butyloxy)carbamoyl]-3-(toluenesulfonyloxy)methyl-5-methyl-adamantane (**1**) in DMSO using K^{18}F /kryptofix 2.2.2 as fluorination agent (9), followed by the deprotection of the resulting ^{18}F -BOC-MEM intermediate by addition of aqueous HCl (Fig. 3).

Among the alkylsulfonates, the triflate-leaving group has been

reported to react rapidly with activated [^{18}F]fluoride (6, 20). Unfortunately, owing to its high instability, 1-[N-(tert-butyloxy)carbamoyl]-3-(trifluoromethylsulfonyloxy)methyl-5-methyl-adamantane, the triflate analog of compound **1**, does not appear to be a suitable precursor for the desired radiofluorination. Compound **1** with the tosylate-leaving group is, however, stable; prolonged storage up to 6 months at 4°C did not result in its decomposition. The radiosynthesis via the tosylate pathway method (12, 13) was therefore undertaken. ^{18}F -MEM was obtained in $22 \pm 7\%$ (decay-corrected to EOB) radiochemical yield after reversed-phase HPLC (System A) in a total synthesis time of 90 min. Low radiochemical yields of ^{18}F -MEM ($<10\%$) were obtained using K^{18}F /Kryptofix 2.2.2 in acetonitrile or tetra-

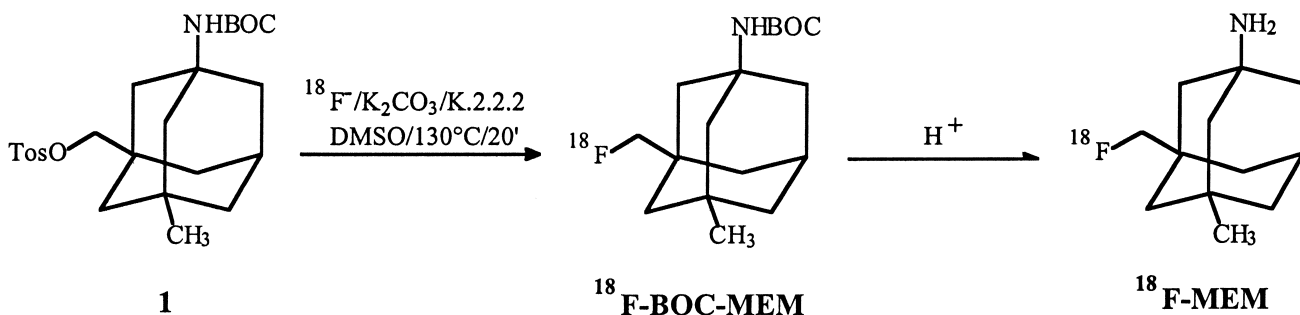


FIG. 3. Radiosynthesis of ^{18}F -MEM.

TABLE 1. Organ Distribution of ¹⁸F-MEM in ICR Mice and the Corresponding Brain/Blood Ratios

	% ID/g organ (median, n = 3)					
	5 min	15 min	30 min	60 min	120 min	240 min
Blood	1.04 ± 0.16	0.84 ± 0.06	0.74 ± 0.16	0.58 ± 0.20	0.11 ± 0.01	0.10 ± 0.02
Lung	15.10 ± 1.32	12.96 ± 1.30	11.86 ± 1.62	7.38 ± 2.22	1.60 ± 0.41	0.74 ± 0.27
Kidney	17.58 ± 0.70	13.00 ± 2.67	9.44 ± 2.30	5.10 ± 1.11	2.02 ± 0.63	0.99 ± 0.24
Heart	6.36 ± 0.92	3.44 ± 0.46	2.42 ± 0.73	1.62 ± 0.21	0.24 ± 0.05	0.22 ± 0.03
Liver	5.23 ± 0.15	4.53 ± 1.27	5.91 ± 0.60	3.97 ± 0.71	1.07 ± 0.25	0.54 ± 0.13
Muscle	2.20 ± 0.37	2.00 ± 0.28	1.80 ± 0.37	1.40 ± 0.31	0.30 ± 0.04	0.20 ± 0.04
Bone	0.92 ± 0.20	0.73 ± 0.31	0.80 ± 0.32	0.60 ± 0.14	0.47 ± 0.06	0.21 ± 0.05
Bladder/urine	n.d.	n.d.	n.d.	13.13 ± 3.8	14.90 ± 2.4	20.60 ± 3.6
Feces	n.d.	n.d.	n.d.	0.08 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
Brain	2.50 ± 0.13	2.96 ± 0.25	3.76 ± 0.45	3.67 ± 0.21	1.02 ± 0.14	0.58 ± 0.01
Brain/blood ^a	2.40 ± 0.30	3.52 ± 0.31	5.10 ± 0.51	6.33 ± 1.35	9.27 ± 0.27	5.80 ± 0.42

n.d., Not determined.

^a % ID/g organ/% ID/g blood.

butylammonium [¹⁸F]fluoride (6). Additionally, TLC (Gelman ITLC-SG, CH₂Cl₂/MeOH 70:30) and analytical HPLC revealed a virtually >99% radiochemical purity.

Biodistribution Studies in Mice

The distribution of radioactivity in the various tissues of female ICR mice following IV administration of ¹⁸F-MEM is summarized in Table 1. The negligible activity in the bone indicates that no significant *in vivo* defluorination of the tracer had occurred. The ¹⁸F-MEM showed a relatively fast blood clearance. Significant uptake was initially observed in the lung, kidney, liver, and heart, but decreased gradually with time. The high accumulation of activity in these organs could be explained in part by the high lipophilicity of ¹⁸F-MEM (logP = 2.6) and by

the good perfusion of these organs. Also, ¹⁸F-MEM showed high brain uptake (up to 3.6% ID/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 hippocampus. Within 120 min, about 15% of the injected activity was found in collected urine, whereas only 0.04% of the injected dose was eliminated by hepatobiliary excretion. Co-injection of ¹⁸F-MEM with (+)-MK-801 (0.10 mg/kg) reduced the initial ¹⁸F-MEM uptake by 32%, 26%, 25%, and 15% 60 min p.i. in the hippocampus, frontal cortex, occipital cortex, and cerebellum, respectively (Fig. 4). In addition, the blockade by MK-801 led to an increase of the renal excretion of radioactivity. More than 20% of the injected activity was found in urine (data not shown).

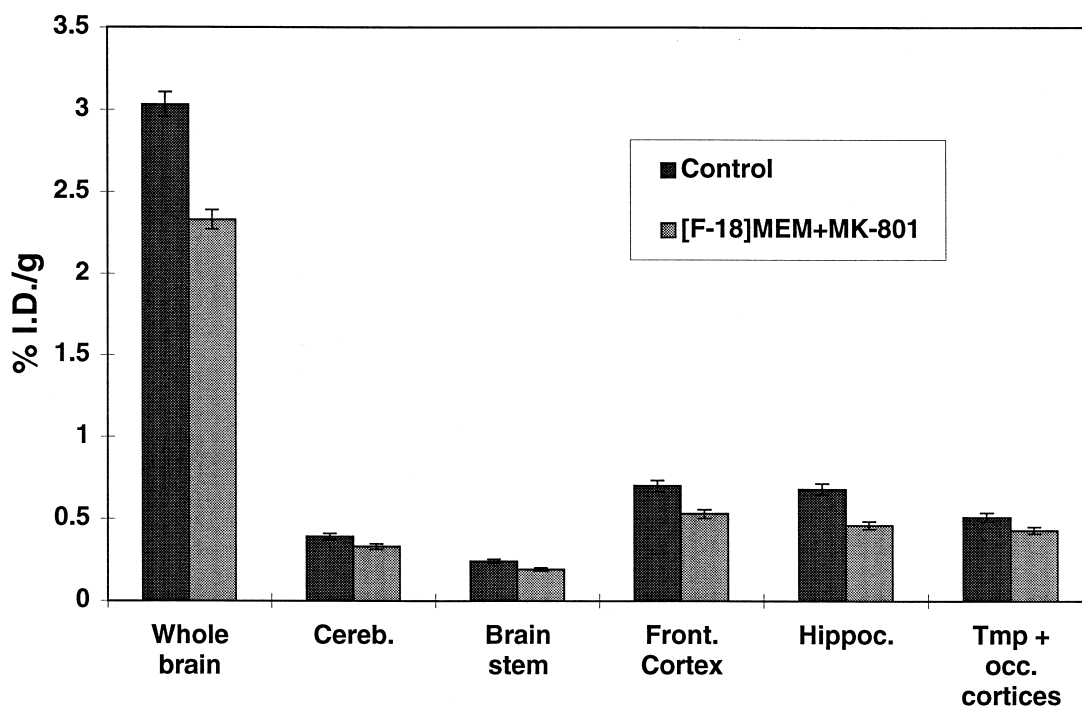


FIG. 4. The % ID/g of ¹⁸F-MEM in organ tissues of mice in control and after co-injection with 0.10 mg/kg of (+)-MK-801, 60 min p.i.

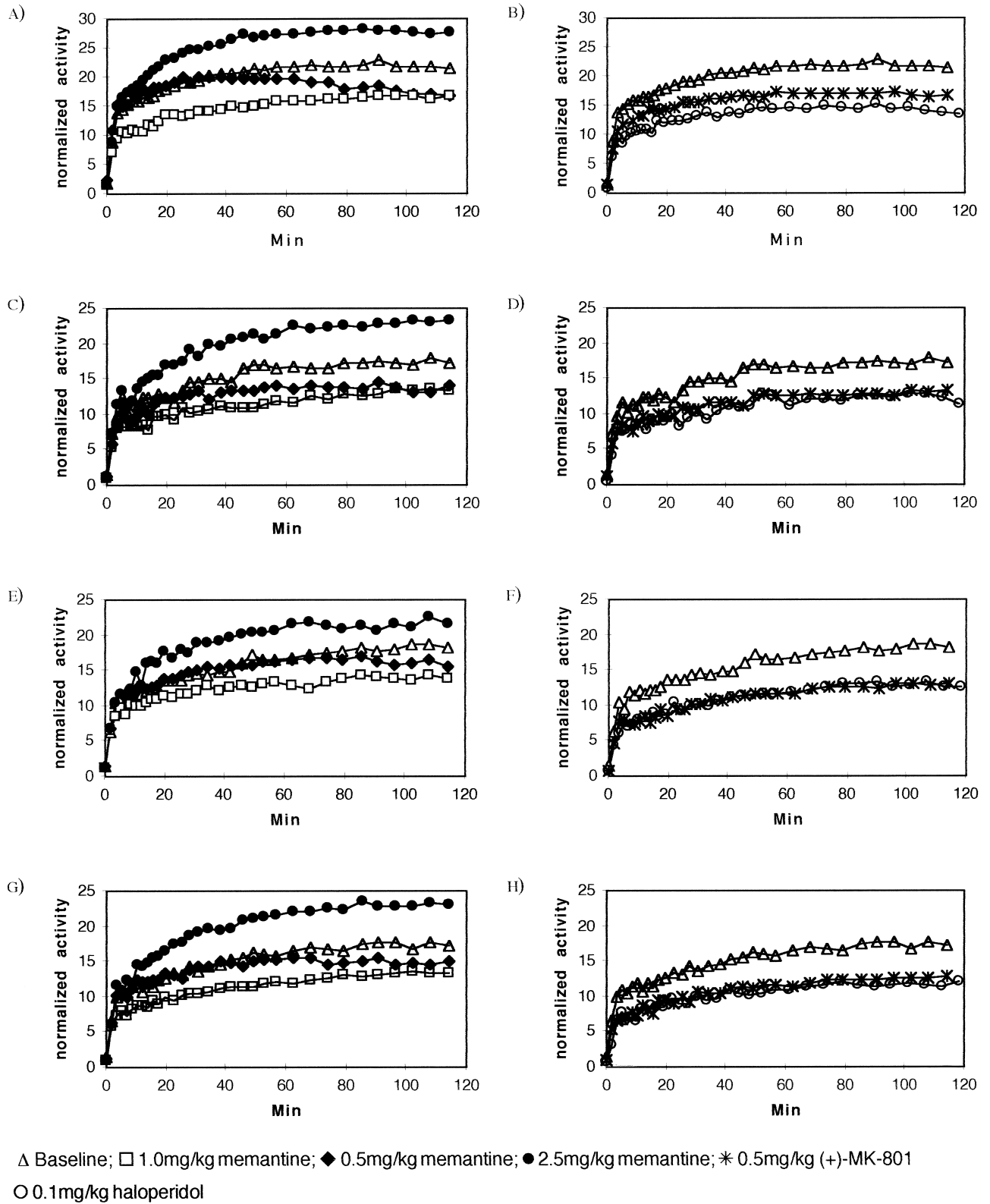


FIG. 5. Pharmacokinetics of ^{18}F -MEM obtained by PET studies in the cerebellum (A, B), striatum (C, D), the frontal cortex (E, F), and the temporal cortex (G, H) in a rhesus monkey under baseline conditions and blockade studies with memantine hydrochloride (left column), (+)-MK-801 maleate, and haloperidol (right column).

PET Studies

The radioactivity time curve obtained from the baseline scan shows high brain uptake and low clearance of the radiotracer from the examined brain regions, with a plateau from 35 min p.i. onwards (Fig. 5). The radiotracer cleared rapidly from the blood. The 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 IV injection of ¹⁸F-MEM. Drug treatment did not change the value of the plasma uptake counted under baseline conditions. Metabolic studies indicated that more than 97% of radioactivity in monkey plasma was parent compound.

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 areas (23, 31). The hippocampus contains the largest number of PCP binding sites (23, 44). However, owing to the resolution of the PET tomograph, delineation of the hippocampus was not possible. Preinjection with therapeutic doses of memantine (0.50 and 1 mg/kg) 30 min prior to IV administration of ¹⁸F-MEM led to a reduction of activity uptake up to 32% from 60 min p.i. onwards in the examined brain regions, including the striatum, frontal and temporal cortices, and the cerebellum (Fig. 5A, C, E, G). In contrast, pretreatment of the monkey with 2.5 mg/kg of memantine caused an increase in the brain uptake of the radiotracer. The reason for this is unclear. However, the inhibition of saturable but not necessarily specific binding sites in the periphery and/or the depolarization of the ion-channels, which occurs at high concentrations of memantine (42), need to be considered as possible factors.

The highest uptake of radioactivity in rhesus monkey brain occurred in the cerebellum. This persistent high-activity concentration observed in the cerebellum is in contrast to the regional radioactivity distribution obtained in the mouse brain as well as the suggested regional distribution of the PCP binding sites (23, 45). Conversely, studies using uncompetitive NMDA radioligands such as [¹⁸F]methyl-MK-801 (2) and S-[¹¹C]ketamine (17) have also documented high uptakes of radioactivity in the cerebellum of monkeys. To our knowledge no species differences in the concentration of the NMDA receptors in the mammalian brain have been reported.

Biochemical binding studies and autoradiographic analysis using [³H]TCP indicated the presence of two different PCP sites with different affinities in both the human brain and the rat brain (41, 45). It has been suggested that PCP and most compounds that interact with the PCP-sensitive sites within the NMDA receptor complex also cross-react with σ -opioid receptors located mostly in the cerebellum (31, 37, 38, 47). One notable exception is the anticonvulsant (+)-MK-801, which is also the most potent uncompetitive NMDA receptor ligand known to date (8, 43, 44). A blockade study with (+)-MK-801 was therefore undertaken. The binding of ¹⁸F-MEM decreased also in all the brain regions in the same range after pretreatment of the monkey with (+)-MK-801 maleate in a pharmacologically active dose (0.5 mg/kg) (18), suggesting competition for the same binding sites (Fig. 5B, D, F, H).

In a preliminary experiment in monkey we examined the extent of specific binding of ¹⁸F-MEM to the sigma receptors using haloperidol, a compound with high affinity for the dopamine D2 receptor and the sigma binding sites. We observed a decrease in uptake, which was similar to that observed using (+)-MK-801 as a blocking agent (Fig. 5B, D, F, H). The reduction in the uptake of radioactivity was, however, more pronounced in the cerebellum, a

region known to contain high concentration of sigma sites. This suggests that binding was also occurring to the sigma recognition sites.

More detailed studies including blockade experiments and the use of different doses of various NMDA and sigma receptor antagonists are therefore currently ongoing and will be reported elsewhere.

CONCLUSION

The new [¹⁸F]fluorinated memantine derivative, 1-amino-3-¹⁸F-fluoromethyl-5-methyl-adamantane (¹⁸F-MEM), is a moderate NMDA receptor channel blocker as attested by patch clamp recording of whole-cell current responses of hippocampal cells to NMDA using the nonradioactive analog ¹⁹F-MEM.

In our study of ¹⁸F-MEM pharmacokinetics in mice and in one monkey, relatively high uptake and retention levels of the radiotracer in the brain were observed. Approximately 75% of the total uptake in mouse brain was found in the hippocampus and in the cerebral cortices, regions known to contain the highest densities of the NMDA receptors. The specificity of the ¹⁸F-MEM binding in mice brain was attested by the reduction of the activity concentration after co-injection of ¹⁸F-MEM with (+)-MK-801. Binding of ¹⁸F-MEM to the PCP site of the NMDA receptor channel was also confirmed by the reduction of radioactivity concentrations in the cortical areas, striatum, and in the cerebellum after pretreatment of the monkey with therapeutic doses of memantine and a pharmacological dose of (+)-MK-801. The binding of ¹⁸F-MEM to the sigma recognition site has also been demonstrated in a preliminary experiment.

However, a serious problem with uncompetitive NMDA receptor antagonists such as memantine and (+)-MK-801 remains to be the dose-dependent blockade of the "open-state" of the ion-channel. Because depolarization of the ion-channel, which occurs above certain concentrations of the channel-blocker, may lead to an enhancement of the radiotracer uptake, the choice of an optimal dosage regimen of these drugs is crucial. Moreover, it seems likely that normal brain physiological variations, resulting in alterations in availability of NMDA binding sites, may also influence uptake of tracers like ¹⁸F-MEM.

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