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*Report*

# Synthesis and Receptor Binding Studies of (±)1-Iodo-MK-801[1]

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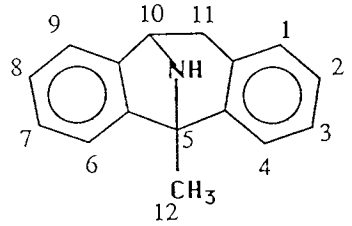
The glutamate analogue *N*-methyl-D-aspartate (NMDA) binds to a subset of glutamate receptors that are coupled to a voltage-sensitive cation channel. This NMDA-linked channel is the likely binding locus of the potent anticonvulsant MK-801. To develop single-photon emission computed tomography (SPECT) probes of this brain channel, we synthesized (±)1-iodo-MK-801 and (±)1-[$^{125}$I]iodo-MK-801. The effect of (±)1-iodo-MK-801 on ligand binding to the NMDA-linked glutamate receptor site was assessed using a rat brain homogenate assay. (±)1-Iodo-MK-801 displaced the dissociative anesthetic ligand [$^{3}$H]*N*-[1-(2-thienyl)cyclohexyl]piperidine ([$^{3}$H]TCP) binding with an $IC_{50}$ of 1 $\mu M$, which is a 10-fold lower binding affinity than that of (±)MK-801. In *in vivo* autoradiographic studies, (±)MK-801 failed to block selective uptake of (±)1-iodo-MK-801 in rat brain. These results suggest that (±)1-iodo-MK-801 may not be a suitable ligand for mapping NMDA-linked glutamate receptor channels.

KEY WORDS: (±)1-iodo-MK-801; radiosynthesis; autoradiogram; receptor binding.

## INTRODUCTION

L-Glutamate, an excitatory amino acid, is the major excitatory neurotransmitter in mammalian brain (1). Evidence from animal studies indicates that selective *N*-methyl-D-aspartate (NMDA) receptor antagonists possess anticonvulsant, anxiolytic, and muscle relaxant properties and can prevent neuronal degeneration caused by ischemia, anoxia, hypoglycemia, and endogenous neurotoxins (2).

MK-801 (I; 5-methyl-10,11-dihydro-5H-dibenzo[*a,d*]-cyclohepten-5,10-imine), a potent anticonvulsant exhibits central sympathomimetic properties in laboratory animals. It also binds noncompetitively to the NMDA receptor in rat brains and can ameliorate glutamate-induced neuronal damage when given before or after an ischemic incident (3,4). In addition, MK-801 is lipophilic and has been shown to penetrate the central nervous system (5). Other selective competitive NMDA antagonists, such as D-2-amino-5-phosphonovaleric acid and D-2-amino-7-phosphonoheptanoic acid, are polar compounds that do not penetrate the central nervous system well. Thus they cannot be used for *in vivo* glutamate receptor mapping by single-photon emission computed tomography (SPECT). The purpose of our study was to develop a SPECT probe of the brain NMDA receptor channel to be used in research on neurodegenerative diseases. An iodine-125 labeled radioiodinated MK-801 tracer was therefore chosen for preliminary autoradiographic evaluation.




Scheme I. MK-801.

## METHODS

### Synthesis

The technique for direct iodination of MK-801 was modified from the Derbyshire technique (6). To a stirred racemic mixture of MK-801 (7) (310 mg, 1.40 mmol), silver sulfate (222 mg, 0.21 mmol), and 85% sulfuric acid (17 ml) was added iodine (181 mg, 0.71 mmol); the resulting mixture was stirred at an ambient temperature for 7 hr. It was then cooled on ice and made alkaline by adding 28% $NH_4OH$. The mixture was extracted with dichloromethane (three times with 50 ml each) and the combined extracts were dried over $MgSO_4$. The organic solvent was filtered and evaporated to dryness *in vacuo*. Flash chromatography with hexane:ethyl acetate (1:1) yielded the major product (25 mg, 5% yield) as a clear oil. Treatment of this oil with ethereal HCl provided the corresponding salt (m.p., 205–207°C). Data for $^{1}$H-NMR,

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$^{13}$C-NMR, and mass spectrum of the title compound were recently reported by us (5). Briefly, significant changes of chemical shift signals in $^{1}$H-NMR and $^{13}$C-NMR were observed compared with 3-bromo-MK-801 and MK-801, respectively.

### Radiosynthesis

Radioiodide labeling was accomplished by a previously described ammonium sulfate-catalyzed solid-phase exchange technique (8). Briefly, 25 μg of (±)1-iodo-MK-801 and 6.5 mCi of $Na^{125}I$ in 300 μl of ethanol and water (1:1) were added to a 3-ml multidose vial containing ammonium sulfate (6.3 mg) and heated to dryness in an oil bath at 180°C. Ten milliliters of air was slowly injected over 1 min and the reaction mixture was maintained at 180°C for 30 min. After being cooled, the reaction mixture was dissolved in acetone (500 μl), and the solution was analyzed by radio-TLC (thin-layer chromatography; silica, $Et_2O/CH_3CN/NH_4OH$, 70:30:5; $R_f$ = 0.28; radiochemical yield, 83%). Evaporation of the acetone left a residue that was extracted with $CH_2Cl_2$ (2 × 1.0 ml) and transferred to a silica Sep-Pak. The Sep-Pak was successively washed with 4 ml each of hexane, hexane and EtOAc (9:1), hexane and EtOAc (3:1), and hexane and EtOAc (1:1) to remove less polar impurities. Subsequent elution with 4 ml of EtOAc yielded 2.8 mCi of desired product; the radiochemical purity was >60 Ci/mmol.

The radioligand was stored in absolute ethanol (5.8 mCi/ml) at −20°C in the dark until needed. Under these conditions, no radiolytic decomposition was observed up to 46 days after synthesis. Just prior to biological testing, the solvent was evaporated, and the compound was formulated in ethanol and 5 m*M* sodium acetate buffer (15:85), pH 4.5.

### *In Vitro* Binding Assay

The affinity of the compounds to the NMDA-linked receptor channel was determined by a modification of a previously described technique (2). Briefly, the binding reaction was initiated by adding [$^{3}$H]TCP (12 n*M* final concentration) to the membrane suspension and allowing the tubes to stand at room temperature for 1 hr. Incubation was terminated by rapid filtration through Whatman GF/B filters presoaked with 0.5% aqueous polyethylenimine. The filters were suspended in 5 ml of Hydrofluor and allowed to stand at room temperature for 1.5 hr before undergoing liquid scintillation counting. Protein concentrations were determined according to the method of Lowry *et al.* (9). In this study, the concentrations of unlabeled MK-801 derivatives displacing 50% of specific [$^{3}$H]TCP binding ($IC_{50}$) were determined.

### Autoradiography of *in Vivo* (±)1-[$^{125}$I]Iodo-MK-801 Binding

Male Sprague–Dawley rats (240–260 g) were anesthetized by inhalation of ether. Polyethylene catheters preloaded with heparin–saline (100 U intravenous/ml) were inserted into the left femoral vein. Fifteen minutes prior to injection of (±)1-[$^{125}$I]iodo-MK-801, the rats were pretreated intravenously with vehicle or 5 mg/kg MK-801. The rats were then injected with a bolus of approximately 200 μCi of (±)1-[$^{125}$I]iodo-MK-801. The rats were killed by decapitation 5 or 30 min after (±)1-[$^{125}$I]iodo-MK-801 injection. The brains were removed by dissection and frozen on dry ice. These sections (20 μm) taken from the frozen tissue were thawed and mounted onto gelatin-coated glass microscope slides and apposed to LKB ultrafilm $^{3}$H. After 3–7 days, the film was developed in Kodak GBX developer to produce the autoradiograms.

## RESULTS AND DISCUSSION

### Chemistry

The direct iodination of (±)MK-801 provides a monoiodinated product pattern. The structure of (±)1-iodo-MK-801 has been previously assigned on the basis of the $^{1}$H- and $^{13}$C-NMR spectrum (5).

The radiosynthesis of (±)1-[$^{125}$I]iodo-MK-801 was accomplished by the solid-phase radioiodide exchange method (8). Rapid passage of the reaction mixture through a silica Sep-Pak to eliminate unreacted radioiodide and other radioactive inorganic substances was the only purification necessary. A radiochemical yield of 85% was obtained at a specific activity of 60 Ci/mmol.

### *In Vitro* Receptor Binding Studies

The $IC_{50}$ of the ligands for the NMDA receptor was determined *in vitro* by their ability to displace [$^{3}$H]TCP (12 n*M*) binding in rat brains. These results are summarized in Table I. (±)1-Iodo-MK-801 has a 10 times lower affinity to the TCP binding site than MK-801 does. In addition, the (±)MK-801 enantiomer has a higher binding affinity than racemic MK-801.

### *In Vivo* Autoradiographic Studies

Five minutes after (±)1-[$^{125}$I]iodo-MK-801 tracer injection, the rat brain showed a substantial heterogeneous accumulation of radioactivity, which indicates that (±)1-iodo-MK-801 is lipophilic enough to cross the blood–brain barrier. The radioactivity was mainly in the forebrain area. The cerebellum had the least amount of radioactivity, similar to the results of *in vitro* binding studies (11). The density of uptake in the brain as determined by image analysis of the autoradiograms is shown in Table II.

Thirty minutes after (±)1-[$^{125}$I]iodo-MK-801 was injected, the radioactivity in the brain had decreased and been redistributed. The areas of highest uptake were the CA1 region of the hippocampus and the thalamus. The uptake in the CA1 region was bilaminar and similar to that in *in vitro* studies (11). Rats pretreated with 5 mg/kg MK-801 5 min prior to (±)1-[$^{125}$I]iodo-MK-801 injection did not have uniform dis-

Table I. Affinity of Ligands to NMDA-Linked Glutamate Receptor in Rat Brain

| Compound | $IC_{50}$ ± SD (nM)[a] |
|---|---|
| (+)MK-801 | 28 ± 3 |
| (±)MK-801 | 100 ± 14 |
| (±)1-Iodo-MK-801 | 1000 ± 140 |

[a] Triplicate for each ligand per assay.

Table II. Relative Distribution of (±)1-[$^{125}$I]Iodo-MK-801 in Rat Brain[a]

| Region | Distribution at 5 min (mean ± SD)[b] | | Distribution at 30 min (mean ± SD) | |
|---|---|---|---|---|
| | Unblocked (1) | Blocked (3)[c] | Unblocked (2) | Blocked (4)[c] |
| Forebrain | 611.9 ± 4.0 | 746.0 ± 21.7 | 381.5 ± 5.6 | 334.5 ± 17.2 |
| Cerebellum | 594.1 ± 11.6 | 612.9 ± 4.1 | 333.1 ± 20.0 | 306.8 ± 24.4 |
| Tectum | 770.1 ± 5.7 | 781.2 ± 0.9 | 363.0 ± 3.1 | 318.6 ± 23.4 |
| Cortex | 728.0 ± 16.6 | 800.3 ± 19.3 | 382.7 ± 8.4 | 342.5 ± 15.3 |
| Striatum | 684.7 ± 8.8 | 830.9 ± 8.6 | 365.3 ± 25.0 | 308.3 ± 27.1 |
| Hippocampus | 608.4 ± 12.5 | 837.0 ± 11.1 | 423.4 ± 65.4 | 389.1 ± 17.0 |
| Corpus $CA_1$ | 372.0 ± 7.1 | 541.0 ± 48.0 | 389.6 ± 64.0 | 324.4 ± 12.7 |

[a] The density of (±)1-[$^{125}$I]iodo-MK-801 uptake in the brain region was determined by image analysis of autoradiograms generated from 3–7 days of contact with transverse brain sections.
[b] Data represent an average of three sections' estimation of optical density from each of three animals.
[c] Blocked groups indicates that rats were pretreated with MK-801 (5 mg/kg, i.v.) prior to injection of tracer.

placement of the radioactivity, but there were some regional changes. For example, pretreating 30 min before injection caused a decrease in $^{125}$I uptake in the striatum. Pretreating 30 min before (±)1-[$^{125}$I]iodo-MK-801 injection also changed the high uptake in the entorhinal cortex to low and the low uptake in the perirhinal cortex to high.

Although the blocking experiments with (±)1-[$^{125}$I]iodo-MK-801 failed to displace uniformly $^{125}$I uptake and in fact increased it in certain areas, we still suggest that the uptake of radioligand is due to receptor binding because (i) the uptake was heterogeneous and distinct between regions, which argues against nonspecific distribution based on blood flow, and (ii) the regional anatomy closely matched that seen in *in vitro* binding experiments with $^{3}$H ligands. If the uptake was determined by receptor binding, then the lack of displacement by 5 mg/kg MK-801 would suggest either that the high dose of displacer has different pharmacodynamics than the radiotracer (because of central or peripheral mechanisms) or that the MK-801 binding site (the NMDA receptor coupled-PCP receptor) is dynamically regulated, with the number of available sites changing according to the dosage of NMDA-linked channel blockers. The latter could be caused by activation of NMDA receptors or by recruitment of cryptic receptors.

In summary, this study demonstrates that foreign isotopes such as $^{125}$I can be incorporated into MK-801 by convenient high-yield methods. *In vivo* and *in vitro* receptor binding studies suggested that (±)1-iodo-MK-801 may not be an ideal ligand for mapping the NMDA-linked glutamate receptor channel. We are now studying other iodine atom positions in the MK-801 molecule.

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## REFERENCES

1. J. C. Watkins and R. H. Evans. *Annu. Rev. Pharmacol. Toxicol.* 21:165–204 (1987).
2. M. E. Van Dort, D. J. Yang, M. R. Kilbourn, D. J. Gole, A. Kalir, D. C. Chu, A. B. Young, E. F. Domino, and D. M. Wieland. In E. F. Domino and J. M. Kamenka (eds.), *Sigma and Phencyclidine-like Compounds as Molecular Probes in Biology*, NPP Books, Ann Arbor, Mich., 1988, pp. 727–734.
3. E. H. F. Wong, A. R. Knight, and G. N. Woodruff. *J. Neurochem.* 50:274–281 (1988).
4. J. A. Kemp, A. C. Foster, R. Gill, and G. N. Woodruff. *TIPS* 8:414–415 (1987).
5. D. M. Wieland, M. R. Kilbourn, D. J. Yang, E. Laborde, D. L. Gildersleeve, M. E. Van Dort, J.-L. Pirat, B. J. Ciliax, and A. B. Young. *Int. J. Appl. Radiat. Isot.* 39:1219–1225 (1988).
6. D. H. Derbyshire and W. A. Waters. *J. Chem. Soc.* 3694 (1950).
7. D. Bender, S. Karady, and T. Rothauser. U.S. Patent 4,477,668 (Oct. 16, 1984).
8. T. J. Mangner, J.-L. Wu, and D. M. Wieland. *J. Org. Chem.* 47:1484–1488 (1982).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall. *J. Biol. Chem.* 193:265–275 (1951).
10. R. L. Albin, A. B. Young, and J. B. Penney. *Neurosci. Lett.* 8(3):303–308 (1988).
11. N. G. Bowery, E. H. F. Wong, and A. L. Hudson. *Br. J. Pharmacol.* 93:944–954 (1988).