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Tryptamine derivatives as non-competitive *N*-methyl-D-aspartate receptor blockers: studies using [^{3}H]MK-801 binding in rat hippocampal membranes

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Abstract

Derivatives of phenylethylamine and tryptamine share structural features with the non-competitive *N*-methyl-D-aspartate (NMDA) receptor blockers phencyclidine, ketamine, and MK-801. Tryptamine and phenylethylamine inhibited the specific binding of [^{3}H]MK-801 to rat hippocampal membranes with IC_{50}'s 190 and 905 μM, respectively. The corresponding amino acids phenylalanine and tryptophan were inactive, their methyl esters, however, were slightly more potent than the amines. The methyl ester of the naturally occurring L-tryptophan was 12 times more potent than the methyl ester of the D-isomer, whereas the corresponding isomers derived from phenylalanine exhibited no stereoselectivity. The potency of tryptamine was increased by substitutions as, e.g. in 5-methyltryptamine (IC_{50} 12 μM) and tryptophan octylester (IC_{50} 5.2 μM). Compounds formed in vivo from L-tryptophan and a lipophilic counterpart may function as endogenous non-competitive NMDA receptor blockers.

Keywords: Tryptamine; *N*-methyl-D-aspartate receptor; Rat hippocampus; [^{3}H]MK-801; Binding site; Endogenous ligand

The ion channel associated with the *N*-methyl-D-aspartate receptor (NMDA)-receptor complex is the target of dissociative anesthetics like ketamine and phencyclidine (PCP). In 1987, Zukin et al. [12], partially purified a fraction from bovine hippocampal extracts mimicking the actions of PCP on the binding of [^{3}H]TCP (the thieno-analog of PCP) and on NMDA-stimulated release of acetylcholine and dopamine. Already 3 years earlier, at a time when PCP still was not known to act via the NMDA receptor complex, Quirion et al. [9] had isolated from porcine brain a peptide with an estimated molecular weight of 3000 Da whose binding and physiological properties were similar to PCP and coined the term α-Endopsychosin. In 1994, Porter and Greenamyre [8] reported about an endogenous inhibitor of [^{3}H]MK-801 binding that could be eliminated from rat brain tissue sections by a prewash; the washing procedure was more efficient in the presence of the co-agonists glutamate and glycine, i.e. when the ion channel was in its open state. And in 1998, combinatorial chemistry led to the discovery of several hexapeptides rich in tryptophan and arginine residues, potently inhibiting glutamate-evoked currents at NMDA receptors expressed in *Xenopus* oocytes [3]. Whether any of these latter peptides (or compounds structurally related to them) were responsible for the endogenous activities observed earlier is unknown.

In search for simple endogenous compounds fulfilling obvious requirements for blocking an ion channel permeable to cations (positive charge and lipophilicity), physiological aromatic amines derived from phenylethylamine and indolethylamine (tryptamine) were screened for their potency as inhibitors of [^{3}H]MK-801 binding. Parts of the present results have been published in abstract form [2].

The CA1/dentate gyrus part of rat hippocampi was dissected from unfrozen rat brains (male Wistar rats, age 4–7 months) and homogenized in cold 50 mM Tris–acetate (pH 7.0) with a glass/teflon Potter-type homogenizer. Whole cell membranes were obtained by centrifugation (10 min 35 000 × *g*) and washed four times, including a 10 min treatment with 0.02% Triton X-100 in the 2nd washing step (37°C water bath). Aliquots of membrane suspension were kept at −80°C for several years without loss of binding. [^{3}H]MK-801 (NEN, 5 nM, 23.9 Ci/mmol) was bound to thawn membranes (equivalent to 1 mg original tissue/vial) in 50 mM Tris–acetate (pH 7.0), in the presence of 10 μM glutamate and glycine (2 h, 23°C water bath). All determi-

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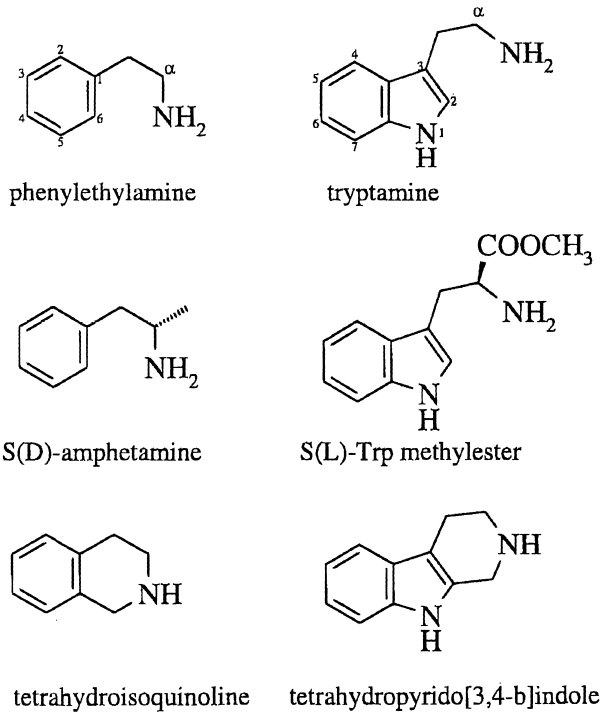

Scheme 1. Structures of test compounds.

nations were performed in duplicates or triplicates. For non-specific binding (NB) glutamate and glycine were replaced by their antagonists D-2-amino-5-phosphono valeric acid (100 μM) and 5,7-dichlorokynurenic acid (10 μM; both Tocris Cookson Ltd). Inhibition of specific [^{3}H]MK-801 binding by test compounds was computer fitted to the formula

$$\text{Bound ligand} = B_o IC_{50}^{n_H}/(IC_{50}^{n_H} + (\text{inhibitor})^{n_H}) + \text{NB}$$

where B_o is the amount of ligand bound specifically in the absence of inhibitor and n_H is the Hill coefficient. Total binding (in the absence of inhibitor) amounted to 1500–2500 dpm/vial, NB to 150–300 dpm/vial. In experiments conducted in parallel under the same conditions, K_D values for the radioligand between 10 and 15 nM were obtained (not shown). Results in Tables 1–4 are given as means ± SD (*n*, number of experiments; if only two experiments were performed, both values are indicated).

Table 1
Inhibition of [^{3}H]MK-801 binding by phenylethyl- and indolethylamines: influence of *N*-methylation, hydroxylation, methoxylation, and ring formation

	IC_{50} (μM)
Phenylethylamine (PEA)[a]	840, 970
N-methyl-PEA	1700, 1800
4-OH-PEA (tyramine)[b]	1100, 1300
3-OH-tyramine (dopamine)[b]	1200, 1100
3-Methoxytyramine[b]	1300, 1300
Tetrahydroisochinoline[a]	530, 520
Tryptamine[a]	190 ± 20 (5)
N-methyltryptamine	780 ± 50 (3)
5-OH-tryptamine[b] (serotonin)	650, 500
5-Methoxytryptamine[b]	1570 ± 150 (3)
Tetrahydropyrido[3,4-b]indol[a]	380, 490

[a] Structure see Scheme 1.
[b] For position of substituent, see Scheme 1.

Table 2
Inhibition of [^{3}H]MK-801 binding by PEA and tryptamine derivatives: influence of α-substitution; Phe, phenylalanine, Trp, tryptophan

	IC_{50} (μM)
R(L)-amphetamine	910, 790
S(D)-amphetamine[a]	420 ± 100 (3)
S(L)-Phe methylester	520, 520
R(D)-Phe methylester	590, 590
S(L)-Phe ethylester	300, 390
S(L)-Phe amide	2410 ± 170 (3)
(±)α-Methyltryptamine[b]	290 ± 20 (3)
S(L)-Trp methylester[a]	140, 120
R(D)-Trp methylester	1250, 1880
S(L)-Trp amide	150 ± 30 (4)
S(L)-Trp ethylester	130, 150
(±)Trp butylester	56 ± 20 (3)
(±)Trp octylester	5.0, 5.4

[a] Structure see Scheme 1.
[b] For position of substituent, see Scheme 1.

2-Methyl-5-OH-tryptamine (2-methyl-serotonin) was obtained from Tocris Cookson Ltd. 5-Fluoro-α-methyltryptamine was a kind gift from M. Bös, Hoffmann-La Roche Ltd., Basel. 1-Methyltryptamine, 5-methyltryptamine, and 6-methyltryptamine were provided by Research Biochemicals International (RBI) as part of the Chemical Synthesis Program of the NIMH, Contract N01MH30003. All other test compounds were obtained from Aldrich or from Sigma.

Tryptamine inhibited [^{3}H]MK-801 binding more potently (IC_{50} 190 μM) than phenylethylamine (IC_{50} 905 μM, Table 1, Fig. 1). *N*-methylation, hydroxylation, methoxylation, and ring formation had only a minor influence; *RS*-synephrine, *RS*-octopamine, *RS*-phenyl-ethanolamine, *R*-adrenaline, and *R*-noradrenaline produced less than 50% inhibition at 3 mM (not shown). α-Substitution was tolerated in both structural leads (Table 2). Remarkably, in α-carboxy-substituted tryptamine the *S*-configuration (as in natural L-tryptophan) was preferred to the *R*-configura-

Table 3
Inhibition of [^{3}H]MK-801 binding by tryptamine derivatives: influence of substitutions of the aromatic ring system (for position of substituents, see Scheme 1)

	IC_{50} (μM)
1-Methyltryptamine	130, 91
5-Methyltryptamine	12 ± 6 (4)
6-Methyltryptamine	260, 175
7-Methyltryptamine	640, 660
2-Methyl-5-OH-tryptamine	150 ± 10 (3)
(±)5-F-α-methyltryptamine	130, 115

Table 4
Polyamine-sensitive inhibition of [^{3}H]MK-801 binding by phenyl- and indolethylamines; ratio: IC_{50} (100 μM spermine)/IC_{50} (no spermine); Trp, tryptophan

	Ratio
Phenylethylamine[a]	2.2, 1.8
Tryptamine[a]	3.6 ± 0.3 (5)
5-Methoxytryptamine[b]	1.3 ± 0.1 (3)
5-OH-tryptamine[b]	2.4, 2.3
2-Methyl-5-OH-tryptamine[b]	3.7 ± 0.02 (3)
R(D)-Trp methylester	2.1, 1.6
S(L)-Trp methylester[a]	5.1, 6.1
S(L)-Trp amide	5.9 ± 0.3 (4)
5-Methyltryptamine[b]	1.6 ± 0.1 (3)

[a] Structure see Scheme1.
[b] For position of substituent, see Scheme 1.

tion by a factor 12; with derivatives of phenylalanine, no such stereoselectivity was observed. In addition, the amide of *S*(L)-phenylalanine was only a very weak non-competitive NMDA receptor blocker (IC_{50} 2.4 mM), whereas the amide of *S*(L)-tryptophan was even slightly more potent (IC_{50} 150 μM, Table 2) than tryptamine. Short peptides with tryptophan amide at the C-terminus have recently been described as NMDA receptor antagonists [3]. No commercially available peptide containing phenylalanine or tryptophan (Phe-Gly, Phe-Leu, Phe-Gly-Gly, Trp-Gly, and pGlu-Ser-Leu-Arg-Trp-NH_2) produced 50% inhibition at 1 mM (not shown). However, tryptophan esters of increasing lipophilicity exhibited increasing potency, with the octyl ester of tryptophan as the most potent compound (IC_{50} 5 μM, see Table 2). A similar increase in potency should also be expected for esters of phenylalanine, however butyl or octyl esters of phenylalanine were not available for testing.

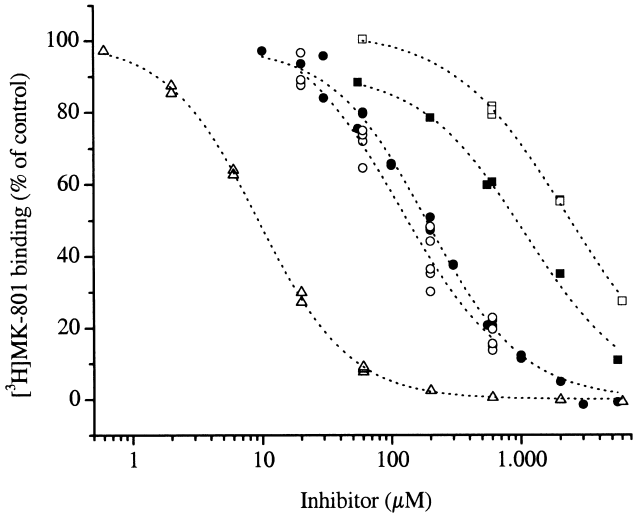

Fig. 1. Inhibition of specific [^{3}H]MK-801 binding by tryptamine (filled circles), phenylethylamine (filled squares), tryptophan amide (open circles), phenylalanine amide (open squares), and 5-methyltryptamine (open triangles); data pooled from 2–4 experiments.

The more promising tryptamine/tryptophan lead was followed up in more detail. Relying on commercially available and on donated compounds, the attempt was made to establish structure/activity relationships of substituted tryptamines as non-competitive NMDA receptor blockers. Methyl-substitution of the aromatic ring system of tryptamine yielded compounds with remarkably differing potencies (Table 3). Among five of the six possible monomethyl derivatives, 5-methyltryptamine was identified as the most potent compound (IC_{50} 12 μM). Potential in vivo effects of 5-methyltryptamine as an NMDA receptor antagonist, however, would be surpassed by its several-fold higher (nanomolar) potency as a 5-hydroxytryptamine(5-$HT)_{2C}$ agonist. Methylation of the indole nitrogen (yielding 1-methyltryptamine, Table 3) was tolerated without loss of potency. Methyl substitution in position 2 of 5-HT increased the (weak) potency of 5-HT as a non-competitive NMDA receptor blocker by a factor 3.8 (compare Table 3 to Table 1). Other derivatives of tryptamine (as 2- and 4-methyltryptamine) and equivalent derivatives of phenylethylamine were not available.

Since inhibition of [^{3}H]MK-801 binding by phenylethyl- and indolethylamines was observed in the presence of saturating concentrations of glutamate and glycine (10 μM each), the inhibition was non-competitive with these co-agonists. The most likely mechanism of action for these compounds is therefore direct blockade of the ion channel, but interaction with a number of other sites postulated to mediate the effects of protons [6], polyamines, and zinc cannot be excluded. Our observation that the polyamine spermine attenuated the inhibitory potency of most tryptamine derivatives (Table 4) suggests some involvement of the polyamine regulatory site of the NMDA receptor complex, and further studies are in progress to substantiate this observation.

In rat brain, 0.04–0.08 nmol tryptamine/g tissue (nmol/g roughly equivalent to μM) have been found in the cerebellum and 0.37–0.39 nmol/g in the hypothalamus [5,10]. In human cerebrospinal fluid (taken from the cisterna cerebello-medullaris), mean tryptamine concentrations in controls between 0.21 and 1.0 μM have been reported, and in schizophrenia patients between 2.0 and 5.5 μM [11], approaching concentrations of some tryptamine derivatives described above as sufficient to block the NMDA receptor complex in vitro. Still higher tissue levels have been described for tryptophan, with 16 nmol/g tissue in the whole rat brain [4,5] and 25 nmol/g in the rat hypothalamus [5]. Based on psychotomimetic effects after exogenous administration of some tryptamine derivatives, their endogenous counterparts have been suspected for a long time to be involved in the symptomatology of psychosis [1]. Although the most likely target mediating the psychotic symptoms is the 5-HT_2 receptor, also inhibition of the NMDA receptor has psychotomimetic consequences [7].

The present observation of a stereoselective effect of non-acidic derivatives of L-tryptophan point to a potential role of L-tryptophan containing compounds (peptides, esters) as endogenous non-competitive NMDA receptor blockers in some brain regions. It might be worthwhile to look for physiologically relevant quantities of such compounds in the brain.

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