

The *N*-methyl-D-aspartate receptor channel blockers memantine, MRZ 2/579 and other amino-alkyl-cyclohexanes antagonise 5-HT₃ receptor currents in cultured HEK-293 and N1E-115 cell systems in a non-competitive manner

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Abstract

The type 3 serotonin (5-HT₃) receptor is a ligand-gated ion channel. In concentration-clamp experiments, we investigated the effects of the uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists memantine, amantadine and MRZ 2/579 on 5-HT receptors stably expressed in HEK-293 cells and on native 5-HT₃ receptors in the N1E-115 cell line. All agents antagonized serotonin (10 μM)-induced inward currents with similar potency to that reported for NMDA receptors. This effect was characterized by inducing a pronounced receptor desensitization, and was probably non-competitive and voltage-independent. In contrast, (S)-ketamine was much weaker as an antagonist of 5-HT₃ receptors than NMDA receptors. Similar effects on 5-HT₃ receptors have been reported previously for a variety of anti-depressants and it is possible that the clinical anti-depressant effects reported for both memantine and amantadine are mediated, at least in part, by antagonistic effects at 5-HT₃ receptors. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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5-HT₃ receptors are ligand gated ionotropic receptors permeable for monovalent cations. Since 5-HT₃ receptors not only have a high density in the area postrema but also in the hippocampal and amygdala regions of the limbic system, it has been suggested that 5-HT₃ selective antagonists may have psychotropic effects [6]. The immediate consequence of neuronal depolarization resulting from 5-HT₃ receptor activation is the release of stored neurotransmitter, particularly dopamine in mesolimbic pathways, which suggests a potentially important role for this receptor system in neuronal circuitry involved in drug abuse. Early animal studies suggested that the 5-HT₃ receptor antagonists, in addition to their well recognised anti-emetic use, might be clinically useful in a number of areas [2]. These included anxiety, schizophrenia, drug and alcohol abuse, depression, cognitive disturbances, Alzheimer's disease, cerebella tremor, Parkinson's disease treatment-related psychosis,

the treatment of inflammatory pain (migraine and irritable bowel syndrome) and appetite disorders [6,7,9,14].

This tempted us to test whether the amino-adamantanes memantine and amantadine [13] and novel amino-alkyl-cyclohexane derivatives such as MRZ 2/579 [12] are, in addition to their well characterized actions as uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists, also antagonists of 5-HT₃ receptors. To this means we assessed the action of several selected agents on serotonin-induced currents mediated via 5-HT_{3A} receptors stably expressed in human embryonic kidney cells (HEK-293 cells) and native 5-HT₃ receptors in the mouse neuroblastoma N1E-115 cell line [1,10].

HEK-293 cells stably expressing the human 5-HT_{3A} receptor [5] were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Germany) with phenol red supplemented with 10% charcoal-stripped steroid-free foetal calf serum, 100 units/ml penicillin and 100 units/ml streptomycin. Mouse neuroblastoma N1E-115 cells, containing both the 5-HT_{3A} and the 5-HT_{3B} splice variants, were purchased from the European collection of cell cultures (ECACC, Salisbury, UK) and stored at -80°C

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until further use. The cells were plated at a density of 100,000 cells cm^{-2} onto plastic Petri dishes (Falcon) and were nourished with NaHCO_3 /HEPES-buffered minimum essential medium (MEM), supplemented with 15% foetal calf serum (Gibco) and incubated at 37°C with 5% CO_2 at 95% humidity. The medium was exchanged completely daily. Once every 3 days, cells were re-seeded onto fresh Petri dishes following treatment with trypsin-EDTA (1% in PBS), resuspension in MEM and centrifugation at 1000 rpm for 4 min.

Patch clamp recordings at -70 mV were made from lifted cells, 2–3 days following seeding with polished glass electrodes (2–6 $\text{M}\Omega$) in the whole cell mode at room temperature (20 – 22°C) with an EPC-7 / EPC-9 amplifier (List). The contents of the intracellular solution were as follows (mM): CsCl (130), HEPES (10), EGTA (10), MgCl_2 (2), CaCl_2 (2), K-ATP (2), Tris-GTP (0.2), D-Glucose (10); pH was adjusted to 7.3 with CsOH or HCl. The extracellular solutions had the following basic composition (mM): NaCl (124), KCl (2.8), HEPES (10), pH 7.3 adjusted with NaOH or HCl.

After the whole-cell configuration was established, the cells were lifted from the glass substrate and serotonin (10 μM), memantine and derivatives were applied at the indicated concentrations using a fast superfusion device. A piezo translator-driven double-barreled application pipette was used to expose the lifted cell either to serotonin-free or serotonin-containing solution for control experiments. A 2 s serotonin pulse was delivered every 60 s. The putative antagonists were dissolved in aqua-bidest and diluted with bath solution to the desired concentration. Current signals were recorded using Pulse + PulseFit (Heka 8.31, Heka Elektronik, Lambrecht, Germany) and IgorPro v.3.03 (Wavemetrics, Lake Oswego, Oregon) software on a MacIntosh G3 computer. Only results from stable cells were accepted for inclusion in the final analysis, i.e. showing at least 50% recovery of responses to serotonin following removal of compounds. Despite this, recovery from drug actions wasn't always 100% because of rundown in some cells ($\leq 10\%$ over 10 min). When present, this was always compensated by basing the % antagonism at each concentration on both control and recovery and assuming a linear time course for this rundown. All antagonists were assessed at steady-state blockade with three to six concentrations on at least five cells. Equilibrium blockade was achieved within two to five agonist applications, depending on antagonist concentration.

Memantine, amantadine and MRZ 2/579 antagonized serotonin-induced inward currents in HEK-293 expressing $5\text{-HT}_{3\text{A}}$ receptors (Figs. 1 and 2, Table 1) with similar potencies to those previously reported for NMDA-induced inward currents [12]. These effects were characterized by the apparent induction of pronounced receptor desensitization (e.g. Figs. 1 and 2) and the effects summarized in table 1 represent potencies against 'steady-state' i.e. desensitized responses at the end of the 2 s application period. NB:

true steady-state responses were not determined as the characteristics of the receptor desensitization didn't allow this. Serotonin-induced inward currents in N1E-115 cells are well characterized as being mediated via the only ionotropic serotonin receptor, namely the 5-HT_3 receptor (for example see Refs. [1,10]) although the splice variants involved are not known. In the present study, memantine, amantadine, MRZ 2/579 and nine other amino-alkyl-cyclohexanes had similar potencies when tested on serotonin responses of

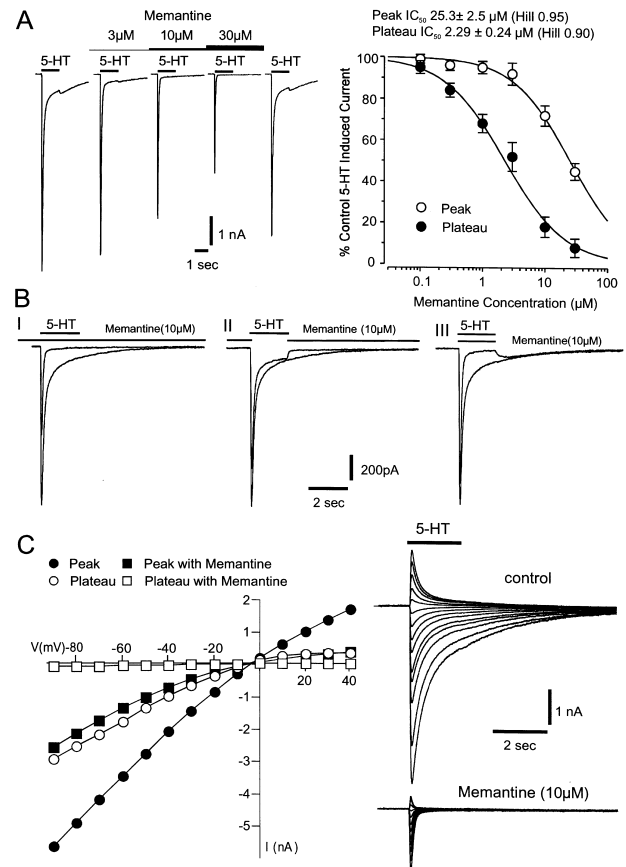


Fig. 1. Effect of memantine at the $5\text{-HT}_{3\text{A}}$ receptor expressed in HEK-293 cells. (A) Left: representative traces for control and different concentrations of memantine on $5\text{-HT}_{3\text{A}}$ -gated currents. Application duration was 2 s. (Right) concentration-response relationship of the antagonism of memantine for peak and 'plateau' currents. The antagonistic effect on 'plateau' currents was about 10-fold more potent, indicating a pronounced induction of receptor desensitization. (B) Representative experiment showing different application modalities, obtained from the same cell. (BI) Pre-exposure to 10 μM memantine and simultaneous application of 10 μM serotonin and 10 μM memantine. (BII) Pre-exposure to 10 μM memantine without simultaneous application of 10 μM serotonin and 10 μM memantine. (BIII) Co-application of 10 μM memantine and 10 μM serotonin. (C) The effect of memantine on $5\text{-HT}_{3\text{A}}$ currents was voltage-independent. (left) The mean amplitude of the peak and 'plateau' currents evoked by 10 μM serotonin in the absence and presence of memantine (10 μM) are plotted against holding potential ($n = 5$). (Right) voltage-independence of the serotonin-evoked cation current at different holding-currents.

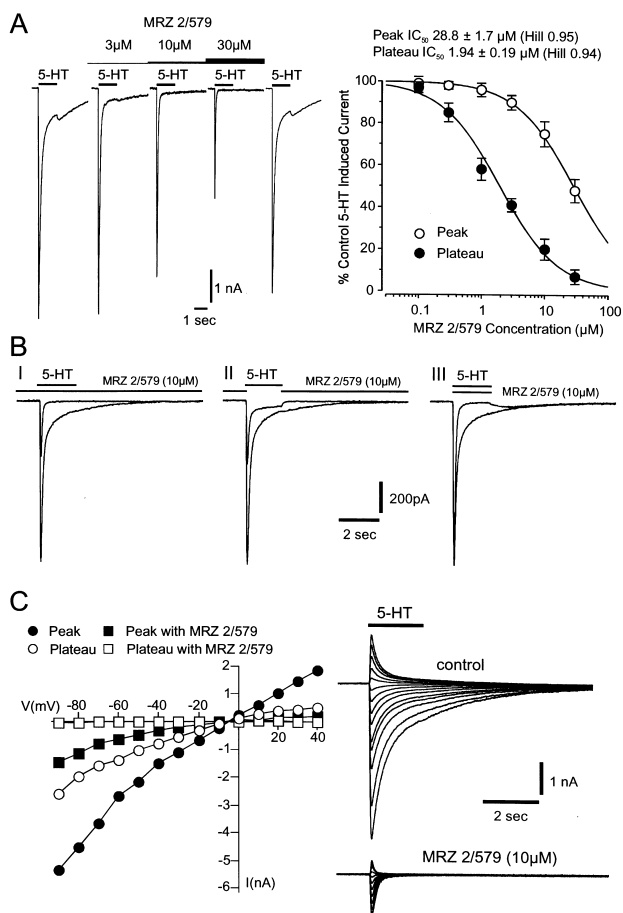


Fig. 2. Effect of MRZ 2/579 at the 5-HT_{3A} receptor expressed in HEK-293 cells. Presentation as for Fig. 1.

these cells (e.g. Fig. 3A). The mode of antagonistic action was further investigated for memantine and MRZ 2/579. The effects of both compounds were voltage-independent (Figs. 1C and 2C). The effects on peak responses were more pronounced following pre-incubation with antagonist (Figs. 1BI/II and 2BI/II). The blockade was apparently not use-dependent (data not shown). These three characteristics argue against open channel blockade by these agents at 5-HT₃ receptors. When co-applied with serotonin, the peak response was least effected and a characteristic “tail” current occurred upon removal of both agonist and antagonist (Figs. 1BIII and 2BIII) which makes a competitive mode of action unlikely. As such, these agents seem to be non-competitive antagonists of 5-HT₃ receptors, but their site of action remains to be determined.

In contrast to the adamantanes and amino-alkyl-cyclohexanes, another uncompetitive NMDA receptor antagonist (s)-ketamine was considerably weaker as a 5-HT₃ antagonist when tested on both 5-HT_{3A} receptors expressed in HEK-293 cells as well as on native receptors in N1E-115 cells.

The present data indicate that amino-alkyl-cyclohexanes and amino-adamantane derivatives are, in addition to their well characterized action as uncompetitive NMDA receptor antagonists, also antagonists of 5-HT₃ receptors. These effects were seen at concentrations similar to, or even lower than, those required for uncompetitive antagonistic effects at NMDA receptors as reported by Parsons et al. [12]. As such, these uncompetitive NMDA receptor antagonists had similar effects on 5-HT₃ receptors as those previously reported for different classes of anti-depressants [3] i.e. they probably antagonized responses by inducing desensitization.

Table 1

Summary of the potencies of amantadine, memantine, (S)-ketamine and 10 amino-alkyl-cyclohexanes on NMDA and 5-HT₃ receptors^a

MRZ 2/	NMDA receptors		5HT ₃ Receptors	
	³ [H]MK-801 (Ki μ M)	PC NMDA (IC ₅₀ μ M)	N1E-115 (IC ₅₀ μ M)	HEK-293 (5HT _{3A} -Receptor) (IC ₅₀ μ M)
579	1.4	1.3	1.69 \pm 0.23 (7)	1.94 \pm 0.19 (8)
601	7.7	10.0	1.27 \pm 0.29 (9)	NT
607	7.7	13.8	22.3 \pm 2.3 (6)	NT
615	2.3	1.30	2.51 \pm 0.15 (5)	NT
616	10.4	33.2	38.7 \pm 3.3 (5)	NT
621	30.6	92.4	20.3 \pm 0.96 (7)	NT
632	2.8	6.4	2.37 \pm 0.32 (5)	NT
633	4.7	13.9	7.70 \pm 0.30 (8)	NT
640	4.8	14.6	10.8 \pm 1.3 (5)	NT
642	10.7	42.5	35.5 \pm 4.7 (5)	NT
Amantadine	25.9	80.8	21.1 \pm 2.8 (8)	31.5 \pm 3.3 (8)
Memantine	2.5	2.3	2.2 \pm 0.15 (10)	2.29 \pm 0.24 (10)
(S)Ketamine	0.5	1.2	89.3 \pm 7.3 (8)	90.40 \pm 2.7 (7)

^a Potencies against 5-HT₃ receptors were assessed as IC₅₀s (μ M) against ‘steady-state’ responses of HEK-293 and N1E-115 cells to serotonin (10 μ M) applied for 2 s. PC is Patch Clamp. NT is not tested. Number in brackets is the number of cells tested. NMDA data taken from [13]

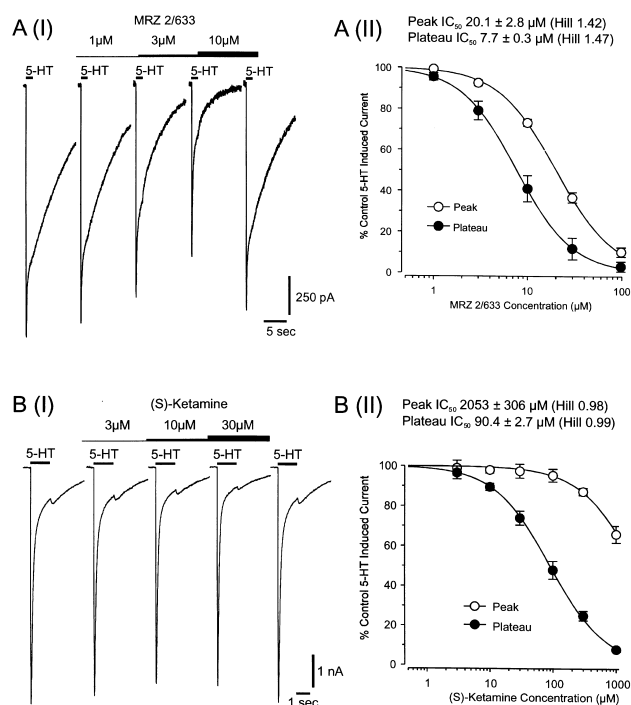


Fig. 3. (A) Concentration-dependence of the blockade of 5-HT₃ receptors by MRZ 2/633 in N1E-115 cells. 5-HT (10 μM) was applied for 2 s every 60 s in the continuous presence of various concentrations of MRZ 2/633. (I) original data for a single cell: 5-HT was applied as indicated by the bars. The left and right panels show control and recovery responses, respectively. The middle three panels show equilibrium responses in the continuous presence of MRZ 2/633 (1, 3 and 10 μM, respectively). (II) Peak and steady-state ('plateau') current responses were normalised to control levels and plotted as means (±SEM) against MRZ 2/633 concentration. Estimation of IC_{50} s and curve fitting were made according to the logistic equation (GraFit, Erithacus Software). (B): Ketamine is a much weaker antagonist of 5-HT_{3A} receptors in HEK-293 cells - presentation as in (A).

5-HT₃ receptor antagonists may, in addition to their well characterized efficacy in emesis and irritable bowel syndrome, also be useful in the treatment of alcohol abuse, anxiety, cognitive deficits and depression. Thus, 5-HT₃ receptors appear to be involved in many neuronal functions including responses to alcohol and other drugs of abuse [8,9,11]. 5-HT₃ receptor antagonists also have a benzodiazepine-like anxiolytic profile in the social interaction test in the rat, the light/dark exploration test in the mouse, the marmoset human threat test and behavioural observations in the cynomolgus monkey [2,14]. Moreover, we suggest that combined antagonistic effects at NMDA and 5-HT₃ receptors for the compounds tested in the present study will lead to positive synergistic effects which could contribute to the therapeutic safety and efficacy of e.g. memantine in Alzheimer's disease by increasing desired effects - cognitive enhancement [16] and antidepressant [3,4,15] whilst further reducing possible negative effects

of NMDA receptor antagonism by e.g. reducing mesolimbic dopamine hyperactivity [7].

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