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Voltage-dependent inhibition of recombinant NMDA receptor-mediated currents by 5-hydroxytryptamine

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1 The effect of 5-HT and related indolealkylamines on heteromeric recombinant NMDA receptors expressed in *Xenopus* oocytes was investigated using the two-electrode voltage-clamp recording technique.

2 In the absence of external Mg^{2+} ions, 5-HT inhibited NMDA receptor-mediated currents in a concentration-dependent manner. The inhibitory effect of 5-HT was independent of the NR1a and NR2 subunit combination.

3 The inhibition of glutamate-evoked currents by 5-HT was use- and voltage-dependent. The voltage sensitivity of inhibition for NR1a + NR2 subunit combinations by 5-HT was similar, exhibiting an e-fold change per $\sim$20 mV, indicating that 5-HT binds to a site deep within the membrane electric field.

4 The inhibition of the open NMDA receptor by external Mg^{2+} and 5-HT was not additive, suggesting competition between Mg^{2+} and 5-HT for a binding site in the NMDA receptor channel. The concentration-dependence curves for 5-HT and 5-methoxytryptamine (5-MeOT) inhibition of NMDA receptor-mediated currents are shifted to the right in the presence of external Mg^{2+}.

5 The related indolealkylamines inhibited glutamate-evoked currents with the following order of inhibitory potency: 5-MeOT = 5-methyltryptamine > tryptamine > 7-methyltryptamine > 5-HT $\gg$ tryptophan = melatonin.

6 Taken together, these data suggest that 5-HT and related compounds can attenuate glutamate-mediated excitatory synaptic responses and may provide a basis for drug treatment of excitoxic neurodegeneration.
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Abbreviations: CNS, central nervous system; HEPES, 4-2-(hydroxyethyl)piperazine-1-ethanesulfonic acid; 5-HT, 5-hydroxytryptamine; IC_{50}, half-maximal inhibitory concentration; 5-MeOT, 5-methoxytryptamine; NMDA, *N*-methyl-D-aspartate

Introduction

Glutamate is a fast excitatory neurotransmitter in the central nervous system (CNS), acting through NMDA and non-NMDA glutamate receptors (McBain & Mayer, 1994). Activation of NMDA receptor currents is conditional, that is, the channels only gate following presynaptic release of glutamate and coincidental postsynaptic membrane depolarization, which relieves voltage-dependent Mg^{2+} block (Mayer *et al*., 1984; Nowak *et al*., 1984). This property as well as high Ca^{2+} permeability render NMDA receptors well suited for the role of mediating synaptic plasticity and long-term potentiation (LTP), which is believed to underlie learning and memory (Bliss & Collingridge, 1993; Castellano *et al*., 2001). However, persistent activation of NMDA receptor channels also mediates enhanced excitatory synaptic transmission and triggers a neurodegenerative cascade precipitated by excessive Ca^{2+} influx. These neurotoxic events underlie a variety of pathological disorders including hypoxic–ischemic neuronal cell death, epileptic seizure activity, Huntington disease, Alzheimer disease and amyotropic lateral sclerosis (Choi, 1990; Olney, 1990; Chapman, 1998; Dingledine *et al*., 1999; Castellino & Prorok, 2000; Cull-Candy *et al*., 2001). Furthermore, NMDA receptors play an important role in the modulation of cell responses to chronic pain sensation and hyperalgesia (Dickenson, 1990; Bennett, 2000).

The secondary structure of the NMDA receptor predicts three transmembrane domains and a re-entrant loop (M1–M4) with an extracellular N-terminus and the C-terminus located intracellularly. The M2 region forms a cytoplasmic re-entrant loop which lines the channel pore (Kuner *et al*., 1996; Dingledine *et al*., 1999). This region harbors a narrow constriction forming the NMDA receptor ion selectivity filter. The voltage dependence of Mg^{2+} block indicates that the binding site is within the membrane electric field (Ascher & Nowak, 1988; Jahr & Stevens, 1990; Wollmuth *et al*., 1998). The binding site, in part, is formed by homologous NR1 and NR2 subunit *N*-site asparagines located near the tip of the M2 loop and an asparagine residue adjacent to the NR2 subunit *N*-site, the $N+1$ site. Mg^{2+} blockade is markedly reduced by mutations of these residues (Kuner *et al*., 1996; Wollmuth *et al*., 1996; Kupper *et al*., 1998).

Serotonin (5-HT) plays an important role in controlling many behavioral and physiological functions, including eating,

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sleep, sexual behavior, circadian rhythmicity and neuroendocrine functions. Abnormalities in 5-HT synthesis have been linked to the development of several psychiatric disorders including schizophrenia, depression and anxiety disorders (Delgado *et al*., 1990). Serotonergic axons project from the raphe nuclei to specific areas of the CNS such as the spinal cord, hippocampus and cortex (Siegel *et al*., 1998). This neuroanatomical organization, as well as the finding that 5-HT and glutamate are co-released by single raphe neurons (Johnson, 1994), raised the possibility of a direct interaction of 5-HT and NMDA receptors. Indeed, the modulation of NMDA-mediated responses by 5-HT has been described previously (Blank *et al*., 1996; Chesnoy-Marchais & Barthe, 1996; MacLean & Schmidt, 2001). These effects appear to be either presynaptic, mediated by members of the 5-HT receptor family (Wu *et al*., 1991; Elliott & Wallis, 1992), or postsynaptic, possibly *via* a direct effect on the NMDA receptor. In the spinal cord, the interplay between 5-HT and NMDA receptors has been suggested to underlie the control and generation of motor rhythm activity (Chesnoy-Marchais & Barthe, 1996; MacLean & Schmidt, 2001). Furthermore, 5-HT and related indolealkylamines were found to inhibit LTP *via* NMDA receptor-mediated responses in hippocampal slices (Staubli & Otaky, 1994) and modulate somatosensory synaptic transmission (Murase *et al*., 1990).

To evaluate the direct interaction between 5-HT and NMDA receptors, we have investigated the mode of action of 5-HT and several structurally related compounds on heteromeric recombinant NMDA receptors expressed in *Xenopus* oocytes using the two-electrode voltage-clamp recording technique. A preliminary report of some of these results has been presented in abstract form (Kloda & Adams, 2004).

Methods

Preparation of RNA

Clones of rat wild-type NMDA receptor subunits were obtained from Dr J. Boulter (UCLA, Los Angeles, CA, U.S.A.). Plasmid DNA of NR1a, NR2A, NR2B and NR2C were linearized with *Nhe*I, *Eco*RI, *Not*I and *Bam*HI restriction enzymes, respectively. Linear templates were used for *in vitro* synthesis of 5′ capped mRNA with either T3 or T7 polymerase using mMessage mMachine™ Transcription Kit (Ambion, Austin, TX, U.S.A.).

Expression in Xenopus *oocytes*

Mature *Xenopus laevis* female frogs were anesthetized by immersion in 0.2% of 3-aminobenzoic acid ethyl ester solution for 15–30 min. Harvested ovarian lobes were defoliculated by incubation in 2 mg ml^{-1} collagenase dissolved in ND96 media containing (in mM): 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 4-2-(hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), pH 7.5, at room temperature for 2–4 h. Subsequently, oocytes were rinsed and incubated for 10–15 min in Ca^{2+}-free ND96 solution to remove the remaining follicular cells. Selected stage V and VI oocytes were stored at 18°C in ND96 media supplemented with 1 mM sodium pyruvate and 0.01 mg ml^{-1} gentamycin. NR1a and NR2 RNA transcripts were mixed in a molar ratio of 1 : 3 to minimize the formation of NR1a monomers. Oocytes were microinjected with 50 nl of the final RNA mixture (15–30 ng total) into oocyte cytoplasm. Oocytes were incubated in ND96 media at 18°C for 2–5 days prior to electrophysiological measurements.

Electrophysiology

Oocytes were placed in the recording chamber (0.1 ml volume) and continuously perfused at >1 ml min^{-1} with a Ca^{2+}- and Mg^{2+}-free solution containing (in mM): 115 NaCl, 2.5 KCl, 1.8 $BaCl_2$ and 10 HEPES, pH 7.3, unless otherwise stated. In a series of experiments, $MgCl_2$ (0.1 mM) was added to the external solution. Membrane currents were recorded using a two-electrode virtual ground voltage clamp circuit with a GeneClamp 500B amplifier (Axon Instruments Inc., Union City, CA, U.S.A.), filtered at 200 Hz and digitized using a Digidata 1200 A interface and pClamp software (Axon Instruments Inc.). Electrodes were filled with 3 M KCl and had resistances of 0.2–1 MΩ. Current amplitude was determined by the steady-state plateau response elicited by 100 μM glutamate in the presence of 10 μM glycine at a holding potential of -70 mV unless otherwise indicated. Current–voltage (I–V) curves were obtained by applying voltage ramps from -120 to $+40$ mV during steady-state responses. Net currents were obtained by subtracting currents recorded in the absence (control) from those in the presence of agonists.

Data analysis

Concentration–response curves obtained for inhibition of glutamate-evoked currents by 5-HT and related compounds were fit to the equation:

$$I_{\mathrm{Drug}} = I_{\mathrm{Control}}/(1 + (\mathrm{IC}_{50}/[\mathrm{antagonist}])^{H}) \qquad (1)$$

where, I_{Control} is the steady-state current evoked by glutamate, I_{Drug} is the current amplitude after steady-state block, IC_{50} is the half-maximal inhibitory concentration of drug and H is the Hill coefficient.

The voltage dependence of the ratio of the current amplitude obtained in the absence and presence of 5-HT (Λ) was used to estimate voltage dependence of block δ (i.e. the apparent fractional electrical depth experienced by the blocker) and the voltage-independent affinity of the blocker, $K_{0.5}$ (0 mV) (Woodhull, 1973) according to the Boltzman type equation:

$$B = B_{\max}/[1 + \exp\{(E - E_{0.5})z\delta F/RT\}] \qquad (2)$$

where B is the fraction blocked, $B_{\max}$ is the maximal fraction blocked, $E_{0.5}$ is the voltage required for half-maximal block, E is the holding potential, z is the charge of the blocking molecule and R, T and F have their normal thermodynamic meanings. δ and $K_{0.5}$ (0 mV) can be determined using the following equation (see also Antonov & Johnson, 1996):

$$z\delta = (RT/F)/V^{\mathrm{e}} \qquad (3)$$

where V^{e} is the voltage required for an e-fold change in the membrane potential. V^{e} can be calculated as a reciprocal of the slope of the plot $\Lambda - 1$ *versus* membrane potential.

Assuming that the blocking particle is impermeant,

$$K_{0.5}(0\ \mathrm{mV}) = [5 - HT]\ \exp(E_{0.5}z\delta F/RT) \qquad (4)$$

where, $E_{0.5} = b/\alpha$ (b is the y-axis intercept and α is the slope of the fitted line of the linear regression function).

Chemicals

The following chemicals were purchased from Sigma Chemical Co., Castle Hill, NSW Australia: HEPES, 3-aminobenzoic acid ethyl ester, collagenase, pyruvic acid, gentamycin, glutamate, glycine, tryptophan, 5-HT, tryptamine, 5-methoxytryptamine (5-MeOT), 5-methyltryptamine, 7-methyltryptamine and melatonin. All other chemicals were analytical grade.

Results

Expression of heteromeric NMDA receptor subunits in *Xenopus* oocytes yielded functional glutamate-activated channels. No glutamate-evoked currents were recorded from noninjected oocytes or oocytes injected with sterile water. 5-HT (0.1–1 mM) applied alone or together with glycine (10 μM) did not activate detectable currents in any of the oocytes tested.

The effect of extracellular 5-HT on heteromeric recombinant NMDA receptors was examined in voltage-clamped *Xenopus* oocytes injected with NR1a + 2A, NR1a + 2B and NR1a + 2C receptor combinations and voltage clamped at either −120 or −70 mV. Bath application of 5-HT (0.01–1 mM) inhibited glutamate-induced currents in a concentration-dependent manner (Figure 1a). The inhibition by 5-HT was strongly voltage dependent, whereby 40 mV depolarization shifted the concentration–response curve to the right and increased the half-maximal inhibitory concentration (IC_{50}) approximately 10-fold. The IC_{50}'s obtained for 5-HT inhibition of NR1a + 2A, NR1a + 2B and NR1a + 2C subunit combinations expressed in oocytes held at −120 mV were 59 ± 1.2, 49 ± 2.9 and 44 ± 2.4 μM, respectively, and increased to 479 ± 9 μM for NR1a + 2A, 286 ± 15 μM for NR1a + 2B and 345 ± 5 μM for NR1a + 2C in oocytes held at −70 mV ($n \geqslant 5$) (Figure 1b). The inhibitory effect of 5-HT was not dependent on the glutamate/glycine concentration, eliminating a possible interaction with the agonist-binding sites (Figure 1c).

I–V curves obtained using voltage ramps applied during steady-state glutamate responses markedly rectified in the presence of 5-HT, whereby inhibition of glutamate-evoked currents by 5-HT increased with membrane hyperpolarization and was not observed at positive holding potentials (Figures 2a, 3a and b). In oocytes held at −70 mV, extracellular Mg^{2+} (0.1 mM) inhibited glutamate-evoked currents by approximately 50%, similar to that obtained in the presence of 0.3 mM 5-HT. Co-application of Mg^{2+} (0.1 mM) and 5-HT (0.3 mM) further increased the inhibition of glutamate (100 μM)-evoked currents through NR1a + 2B NMDA receptors by approximately 20%. Similar results were obtained for the inhibition of NR1a + 2A and NR1a + 2C NMDA receptor subunit combinations (Figure 2b).

Competitive interaction between Mg^{2+} and 5-HT for block of NMDA receptors was investigated by examining the degree of block by 5-HT and the most potent indolealkylamine, 5-MeOT, in oocytes held at −70 mV in the presence of 0.3 mM Mg^{2+} in the bath solution (Figure 2c and d). The concentration–response curves for 5-HT and 5-MeOT inhibition of glutamate-evoked currents were shifted to the right in the presence of Mg^{2+}. The IC_{50} for 5-HT inhibition increased approximately three-fold from 0.29 mM in Mg^{2+}-free solution to 1.1 mM in the presence of Mg^{2+} (0.3 mM). Similarly, the

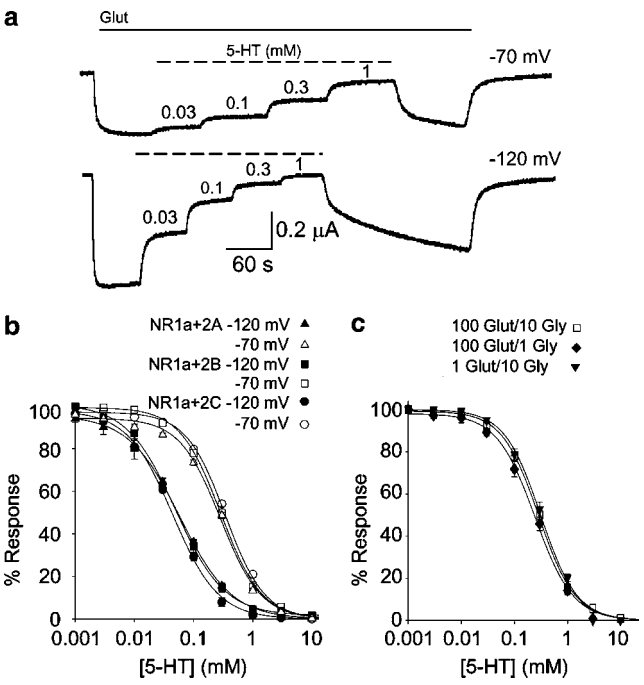

Figure 1 Inhibition of glutamate-evoked currents through recombinant NMDA receptors by 5-HT. (a) Representative current traces showing the concentration-dependent inhibition of glutamate (100 μM)-evoked currents by 5-HT. Currents were recorded from the same oocyte expressing NR1a + 2B NMDA receptor subunits voltage clamped at either −120 or −70 mV. Glutamate was applied as indicated by the solid horizontal lines and the dashed lines indicate the application of 5-HT. (b) Concentration–response curves obtained for 5-HT inhibition of glutamate-induced currents derived from the data including that shown in panel a. Steady-state currents induced by 100 μM glutamate were measured in the presence of various concentrations of 5-HT in oocytes expressing either NR1a + 2A (triangles), NR1a + 2B (squares) or NR1a + 2C (circles) NMDA receptor subunits. Oocytes were voltage clamped at holding potentials of −120 and −70 mV and $n \geqslant 5$ oocytes at each concentration tested. (c) The effect of various glutamate/glycine concentrations on the concentration–response curves obtained for 5-HT inhibition of NR1a + 2B subunits expressed in oocytes voltage clamped at −70 mV. The IC_{50}'s obtained were 286 ± 15 μM for 100 μM glutamate/10 μM glycine, 243 ± 29 μM for 100 μM glutamate/1 μM glycine and 312 ± 0.29 μM for 1 μM glutamate/10 μM glycine, $n = 3$–5 oocytes at each concentration.

IC_{50} for 5-MeOT inhibition increased approximately 10-fold from 18 μM in Mg^{2+}-free solution to 170 μM in the presence of Mg^{2+}, indicating competition between the indolealkylamines and Mg^{2+} for the binding site. Interestingly, the shift of the 5-HT concentration–response curve was parallel, whereas the slope of the 5-MeOT concentration–response curve increased two-fold from $H = 1.2$ in Mg^{2+}-free solution to $H = 2.8$ in the presence of Mg^{2+}, suggesting that there may be either more than one binding site for 5-MeOT or an allosteric effect in the presence of Mg^{2+}.

The voltage dependence of 5-HT block of NMDA receptor channels composed of different NR2 subunits was further analyzed according to the Woodhull (1973) model. *I–V* curves obtained from oocytes expressing NR1a subunit in combination with either NR2A, NR2B or NR2C subunits are shown in Figures 2a and 3a, b. A linear plot of the ratio of glutamate-evoked current amplitude obtained in the absence and presence of 0.3 mM 5-HT (A)−1 as a function of voltage

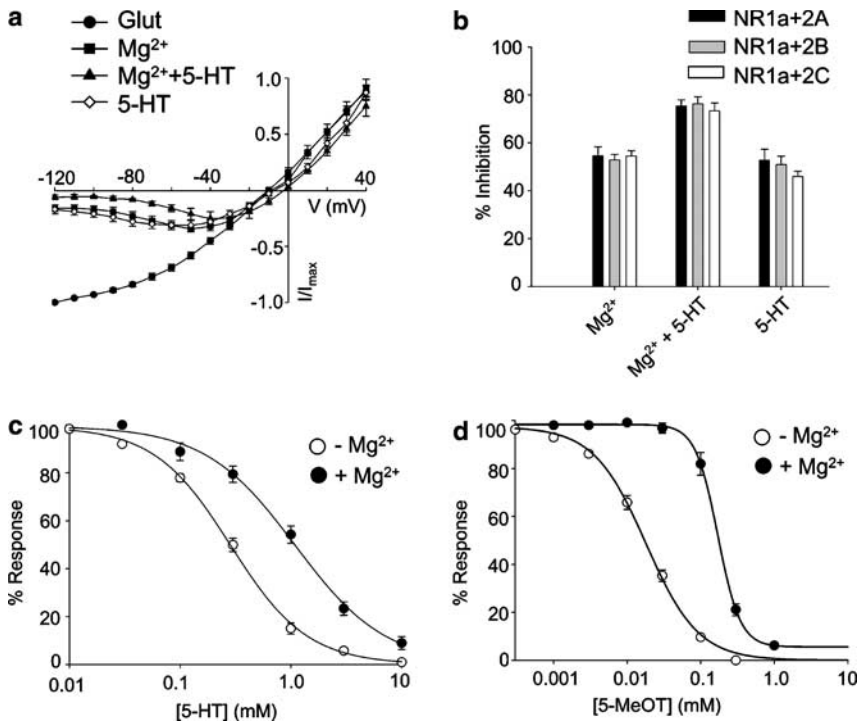

Figure 2 Competitive inhibition of NR1a + 2B NMDA receptors by 5-HT and Mg^{2+}. (a) Normalized *I–V* relations obtained for glutamate-activated NR1a + 2B NMDA receptors in the absence and presence of either 0.3 mM 5-HT or 0.1 mM Mg^{2+}, and a combination of both 0.3 mM 5-HT and 0.1 mM Mg^{2+}. Currents were evoked by voltage ramps applied during steady-state responses to the glutamate (100 μM) before and after addition of the blockers ($n = 5$). (b) Bar graph summarizing the inhibition by 5-HT, Mg^{2+} and both 5-HT and Mg^{2+} of NR1a + 2A ($n = 3$), NR1a + 2B ($n = 5$) and NR1a + 2C ($n = 3$) NMDA receptors in oocytes voltage clamped at −70 mV. (c, d) Concentration–response curves obtained for 5-HT (panel c) and 5-MeOT (panel d) inhibition of NR1a + 2B NMDA receptors in the absence (open symbols) and presence (closed symbols) of 0.3 mM Mg^{2+}. The IC_{50}'s obtained for 5-HT inhibition were 286 ± 15 μM ($H = 1.3 \pm 0.1$) in the absence and 1.1 ± 0.02 mM ($H = 1.1 \pm 0.2$) in the presence of Mg^{2+}. The IC_{50}'s obtained for 5-MeOT inhibition curve were 18 ± 1.8 μM ($H = 1.2 \pm 0.1$) and 171 ± 5.3 μM ($H = 2.8 \pm 0.1$) in the absence and presence of Mg^{2+}, respectively.

revealed an e-fold change per 20.2 ± 2.5 mV for NR1a + 2A, 19.5 ± 2.9 mV for NR1a + 2B and 28.9 ± 3.0 mV ($n = 4$) change in the membrane potential for NR1a + 2C (Figure 3c). The slope of ($\Lambda - 1$) as a function of membrane potential was used to determine the voltage dependence of block, δ, and affinity of the blocker at 0 mV, $K_{0.5}$ (0 mV) (see Table 1).

The three NMDA receptor subtypes did not exhibit significant differences in either δ or $K_{0.5}$ (0 mV). However, the NR1a + 2C NMDA receptor appears slightly less sensitive to block by 5-HT ($\delta = 0.9$ and $K_{0.5}$ (0 mV) = 1.7 mM) compared to the NR1a + NR2A receptor ($\delta = 1.3$ and $K_{0.5}$ (0 mV) = 4.2 mM) and NR1a + 2B receptor ($\delta = 1.4$ and $K_{0.5}$ (0 mV) = 3.0 mM) (Table 1). In control experiments, the parameters for voltage-dependent Mg^{2+} block of NR1a + 2B NMDA receptor were $\delta = 1.2$ and $K_{0.5}$ (0 mV) = 8.7 mM, which are similar to those published previously (e.g. Ascher & Nowak, 1988). Comparison of the voltage dependence of 5-HT and Mg^{2+} block of NR1a + 2B NMDA receptor revealed no statistically significant difference for δ, but a lower $K_{0.5}$ (0 mV) compared to that obtained for Mg^{2+} (Table 1).

Divalent cations have been reported to mediate the desensitization of NMDA receptor currents with the repetitive application of the agonist (Vyklicky, 1993). Therefore, the effect of Ba^{2+} ions on the inhibition of glutamate responses by the related indolealkylamine, 7-methyltryptamine, was examined in the presence of 0.9 and 1.8 mM external Ba^{2+}. Reduction of the external Ba^{2+} concentration by half did not significantly affect the inhibition of NR1a + 2B NMDA receptor currents by 7-methyltryptamine. In the presence of 0.9 mM Ba^{2+}, the IC_{50} values for the concentration–response relationships obtained in oocytes voltage clamped at −120 and −70 mV were 43 and 144 μM, respectively. Similarly, the IC_{50}'s obtained for 7-methyltryptamine inhibition of glutamate-evoked currents recorded in the presence of 1.8 mM Ba^{2+} at −120 and −70 mV were 46 and 167 μM, respectively. These data suggest that indolealkylamine block of NMDA receptor-mediated currents is not influenced by the external Ba^{2+} concentration.

The use-dependence of 5-HT inhibition of NMDA receptor-mediated currents is shown in Figure 4a and b. This effect was strongly voltage-dependent, whereby a faster onset of channel block by 5-HT was observed in oocytes held at −120 mV compared to −70 mV. In the presence of 0.2 mM 5-HT, block was achieved instantaneously in response to glutamate in oocytes held at −120 mV, whereas at −70 mV repeated glutamate application was required to reach an equivalent block. In contrast, the recovery from block by 5-HT showed similar time courses in oocytes held at −70 and −120 mV. However, when the oocyte was depolarized to −40 mV for 1–2 min, the amplitude of the glutamate response was restored to the control amplitude within 1 min, independent of glutamate application during the recovery period (Figure 4c).

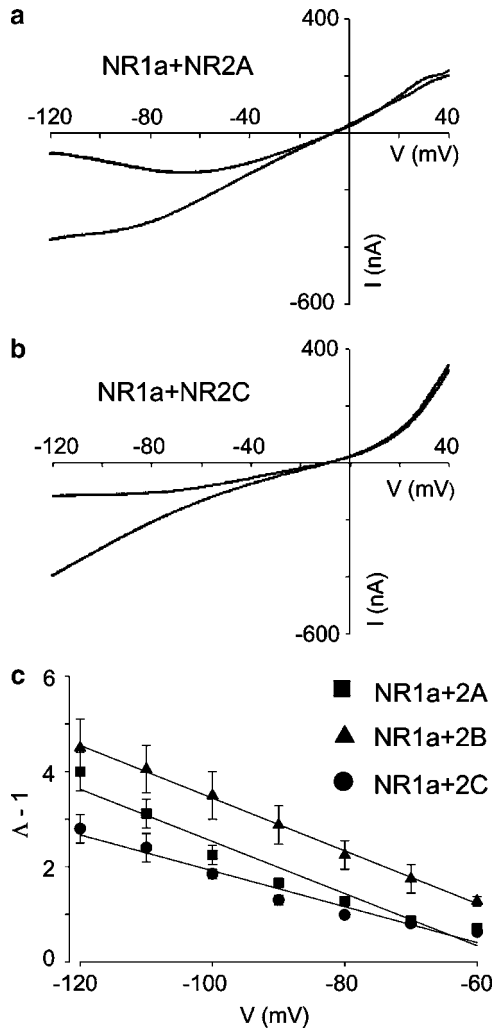

Figure 3 Voltage dependence of 5-HT block of different NMDA receptors. (a, b) Representative *I–V* curves for NR1a + 2A (panel a) and NR1a + 2C (panel b) NMDA receptor subunit combinations obtained in the absence and presence of 0.3 mM 5-HT. Currents were obtained in response to voltage ramps during steady-state responses to the glutamate (100 μM) before and after bath application of 5-HT ($n = 4$). (c) Voltage dependence of inhibition of glutamate-activated currents by 5-HT for different NMDA receptor subunit combinations. The ratio of current amplitudes (Λ) obtained in the absence and presence of 5-HT was determined at different membrane potentials. $\Lambda - 1$ is plotted as a function of membrane potential and data points were fitted by linear regression.

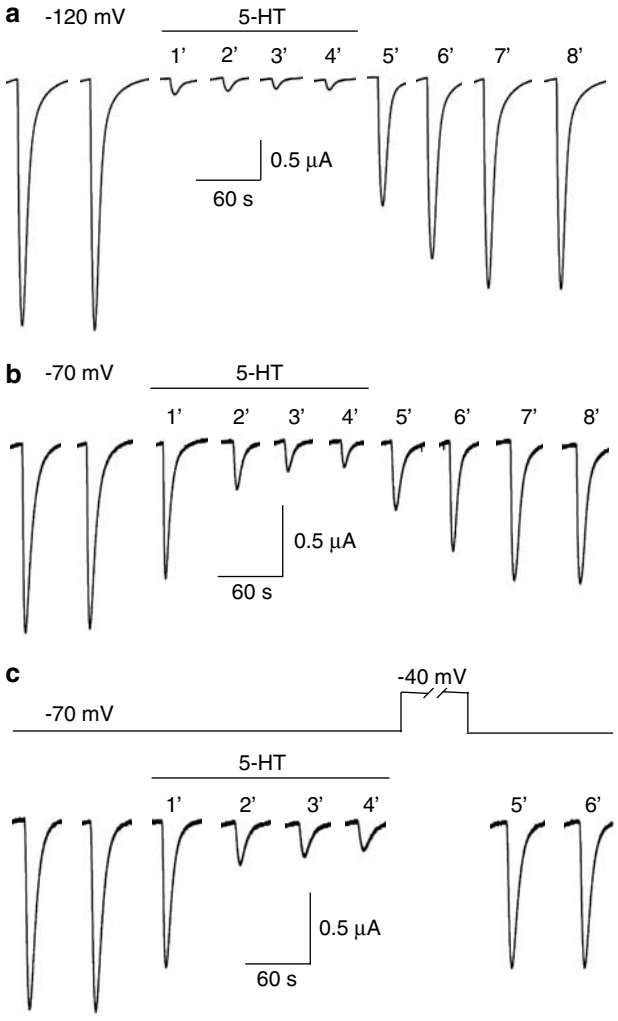

Figure 4 Use-dependent block of NMDA receptor-mediated currents by 5-HT. (a, b) Onset and recovery from the use-dependent block of glutamate-evoked currents by 0.2 mM 5-HT in oocytes voltage clamped at either −120 mV (panel a) or −70 mV (panel b). Glutamate (100 μM) was applied for 10 s every min and 5-HT was continuously present as indicated by the bar. The onset and recovery intervals are indicated above each test response. (c) The effect of depolarization on the rate of recovery from the use-dependent block in the presence of 0.2 mM 5-HT. Glutamate (100 μM) was applied during the recovery period and the oocyte was held at −40 mV for 1 min.

Table 1 Voltage dependence of block of recombinant NR1a + 2A, NR1a + 2B and NR1a + 2C NMDA receptor channels by extracellular 5-HT and Mg^{2+}

	Subunits	δ	$K_{0.5}$ (*0* mV) (mM)	n
5-HT^+	NR1a + 2A	1.3 ± 0.1	4.2 ± 0.9	4
5-HT^+	NR1a + 2B	1.4 ± 0.2	3.0 ± 0.6	4
Mg^{2+}	NR1a + 2B	1.2 ± 0.2	8.7 ± 0.4*	4
5-HT^+	NR1a + 2C	0.9 ± 0.1	1.7 ± 0.5	4

Woodhull (1973) parameters: voltage dependence of block, δ, and voltage-independent affinity of the blocker, $K_{0.5}$ (0 mV), were derived from equations (3) and (4) (see 'Data analysis' in Methods). It is assumed that at physiological pH 5-HT is in its protonated form (5-HT^+).
*Significantly different, $P < 0.01$.

The effect of indolealkylamines structurally related to 5-HT, including tryptophan, 5-hydroxytryptophan, tryptamine, 5-methyltryptamine, 7-methyltryptamine, 5-MeOT and melatonin (see Figure 5a) applied at a concentration of 0.3 mM, were investigated on the NR1a + 2B NMDA receptor activated by 100 μM glutamate. 5-MeOT and 5-methyltryptamine were the most potent inhibitors almost completely blocking glutamate-evoked currents at both −120 and −70 mV. Tryptamine, 7-methyltryptamine and 5-HT were less potent, producing 95.9 ± 1.5% at −120 mV and 67.2 ± 3.6% inhibition at −70 mV ($n = 6$), 84.9 ± 3.6% at −120 mV and 58.9 ± 2.6% inhibition at −70 mV ($n = 5$) and 85.2 ± 2.2% at −120 mV and 50.1 ± 2.8% inhibition at −70 mV ($n = 8$), respectively. Tryptophan and melatonin produced only slight inhibition of

14.9±1.8% at −120 mV, 9.6±0.4% at −70 mV ($n=4$) and 13.2±0.8% at −120 mV, 10.7±1.0% inhibition at −70 mV ($n=4$), respectively (Figure 5b), whereas 5-hydroxytryptophan (not shown) had no effect on NMDA receptor-mediated currents ($n=4$).

Analogous to 5-HT, the most potent indolealkylamines, 5-MeOT and 5-methyltryptamine, produced a concentration- and voltage-dependent inhibition of glutamate currents (not shown) with IC_{50}'s of 3.6±0.4 μM at −120 mV and 18±1.8 μM at −70 mV for 5-MeOT and 3.5±0.1 μM at −120 mV, 29±3.0 μM at −70 mV for 5-methyltryptamine. Comparison of the IC_{50}'s obtained for inhibition of NR1a + 2B receptors by 5-HT and related indolealkylamines is presented in Table 2.

Discussion

NMDA-gated ionotropic receptors form multimeric channels permeable to K^+, Na^+ and Ca^{2+}. These receptor channels are highly regulated due to a number of binding sites, many of which can interact with the endogenous substances, including excitatory amino acids such as co-agonist glycine and D-serine, byproducts of cell metabolism such as kynurenic acid, ATP, and divalent cations such as Zn^{2+}, Mg^{2+} and Ca^{2+} (Vyklicky, 1993; Danysz & Parsons, 1998; Ortinau *et al*., 2003; Kloda *et al*., 2004). Numerous drugs such as phencyclidine, amantadine, memantine and ketamine have been found to block open NMDA receptor channels *via* a trapping mechanism (Huettner & Bean, 1988; Lerma *et al*., 1991; MacDonald *et al*., 1991; Blanpied *et al*., 1997; Dilmore & Johnson, 1998). Furthermore, several indolealkylamines and derivatives of tryptamine inhibit the specific binding of the NMDA receptor open channel blocker [^{3}H]MK-801 in rat hippocampal membranes and whole-brain homogenates (Berger, 2000; Worthen *et al*., 2001). The rank order of potency for inhibition (IC_{50}) by tryptamine homologues was 5-methyltryptamine (12 μM) > 1-methyltryptamine (111 μM) > (±)5-F-α-methyltryptamine (123 μM) > 2-methyl-5-OH-tryptamine (150 μM) > 6-methyltryptamine (218 μM) > 7-methyltryptamine (650 μM), suggesting that the group at the 5-position may be an important structural motif that governs the potency of these compounds (Berger, 2000). 5-HT has also been reported to modulate other ligand-gated ion channels, including nicotinic ACh, ATP and $GABA_A$ receptors *in vivo* (Kawahara *et al*., 1994; Koizumi *et al*., 1995; Garcia-Colunga & Miledi, 1999; Fucile *et al*., 2002).

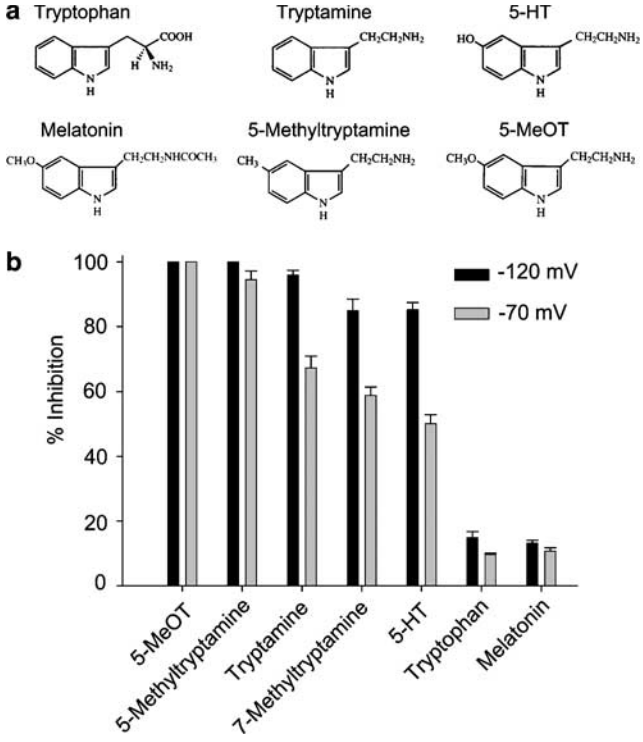

Figure 5 Differential block of glutamate-evoked currents through NR1a + 2B NMDA receptors by 5-HT and related indolealkylamines. (a) Chemical structure of the indolealkylamines used in the present study. (b) Bar graph of the inhibition of glutamate (100 μM)-evoked current amplitude by 0.3 mM of each compound. Oocytes were voltage clamped at either −120 or −70 mV, $n \geqslant 5$ oocytes for each group.

A major finding of the present study is that the neurotransmitter 5-HT and related indolealkylamines can directly block glutamate-evoked currents through open recombinant NMDA receptor channels in a voltage-dependent manner. Our data show that 5-HT acts as a noncompetitive antagonist of the NMDA receptor, whereby the degree of block of NMDA receptor-mediated currents by 5-HT was independent of the glutamate/glycine concentration. The voltage and use dependence of the 5-HT block of NMDA receptor-mediated currents is consistent with open-channel blockade. The block by 5-HT and related indolealkylamines increased markedly with membrane hyperpolarization, whereby 40 mV depolarization shifted the concentration–response curve to the right and increased the IC_{50} approximately 10-fold. In oocytes voltage clamped at −70 mV, 5-HT inhibition was use-dependent, whereas hyperpolarization to −120 mV resulted in instantaneous blockade but a similar rate of recovery as observed at −70 mV. Brief depolarization during the recovery period produced instantaneous recovery from block by 5-HT, which is most likely due to the rapid dissociation of 5-HT from the binding site upon depolarization. In contrast to extracellular Mg^{2+}, the block of NMDA receptor channels by 5-HT is both voltage- and use-dependent. Mg^{2+} block of NMDA receptors exhibits a very rapid onset and recovery from block (Ascher *et al*., 1988; Vargas-Caballero & Robinson, 2002; Kampa *et al*., 2004), whereas the time course of 5-HT block is slower and

Table 2 Inhibition of recombinant NR1a + 2B NMDA receptors by 5-HT and related indolealkylamines

Indolealkylamine	IC_{50} (μM) *−120* mV	H	IC_{50} (μM) *−70* mV	H	n
5-HT	49±2.9	1.0±0.1	286±15	1.3±0.1	8
7-Methyltryptamine	46±6.1	0.9±0.3	167±7.8	0.9±0.3	5
Tryptamine	32±2.5	1.3±0.2	138±3.6	1.1±0.3	6
5-Methyltryptamine	3.5±0.1	1.1±0.1	29±3.0	1.6±0.2	5
5-MeOT	3.6±0.4	1.0±0.1	18±1.8	1.2±0.1	6

strongly voltage-dependent. Taken together, these findings suggest that 5-HT may block NMDA receptor channels during periods of chronic activation. However, transient synaptically induced depolarization may allow 5-HT and related compounds to leave the channel pore without interfering with physiological NMDA receptor-mediated signaling.

The voltage sensitivity of the block indicates that 5-HT, similar to Mg^{2+}, binds within the membrane electric field. The block of glutamate-activated currents by 5-HT followed a simple model (Woodhull, 1973) over the range of physiological membrane potentials (−120 to −60 mV), but deviated from linearity at more depolarized membrane potentials. Although subunit specific sensitivity to the Mg^{2+} block has been reported for NMDA receptors composed of NR1a + 2A and NR1a + 2B subunits which showed higher voltage dependence ($\delta > 1$) compared to NR1a + 2C receptor channels ($\delta \sim 0.7$), all share a similar affinity for Mg^{2+} at 0 mV ($K_{0.5} \sim 5$ mM) (Kuner & Schoepfer, 1996; Wollmuth *et al*., 1998). Our results show that NR1a + 2B NMDA receptors exhibit a significantly higher $K_{0.5}$ (0 mV) for Mg^{2+} compared to that of 5-HT, although both blockers share a similar voltage dependence (see Table 1). Thus, the voltage-dependent and competitive interaction of 5-HT and other indolealkylamines would be expected to block the channel pore more potently compared to Mg^{2+} at depolarized membrane potentials.

Related indolealkylamines inhibited glutamate-evoked currents with different potencies and the potency sequence, 5-MeOT = 5-methyltryptamine > tryptamine > 7-methyltryptamine > 5-HT ≫ tryptophan = melatonin, suggesting that the inhibition is stringent with regard to the side chain of the molecule and indicates the importance of the 5-position to indolealkylamine block of the NMDA receptor channel. The block of glutamate-induced currents by 5-HT and 5-MeOT in the presence of external Mg^{2+} suggests a competition between Mg^{2+} and indolealkylamines for a binding site(s) in the channel pore. Given that 5-MeOT and 5-methyltryptamine were > 10 times more potent than 5-HT, further studies of the mode of indolealkylamine–receptor interaction may guide the design of novel potent inhibitors of the NMDA receptor. Indeed, NMDA receptor antagonists are promising leads for the development of therapeutic agents against acute or chronic glutamate-mediated excitoxicity such as ischemia, dementia, epilepsy, trauma and treatment of pain (Danysz & Parsons, 1998). Taken together, voltage-dependent drugs based on 5-HT analogues may be more effective in protecting against the glutamate-mediated neurotoxic effects of NMDA receptor activation than physiological concentrations of Mg^{2+}, and without the psychotropic side effects often observed with blockers such as MK-801 and phenylcyclidine (Parsons *et al*., 1993; Danysz & Parsons, 1998).

In conclusion, the present study shows that 5-HT and related indolealkylamines can directly block currents through NMDA receptor channels in a voltage- and use-dependent manner, consistent with the open channel block. The binding site for 5-HT is located deep within the membrane electric field and competitive interactions of 5-HT and 5-MeOT with Mg^{2+} suggest that the indolealkylamine and Mg^{2+}-binding sites are in close proximity. Our data support the view that inhibition of NMDA receptors by 5-HT may contribute to CNS synaptic physiology, particularly in the spinal cord and hippocampus, which are well known to receive serotogenic innervation, with potent effects on somatosensory transmission, motorneuron activity and LTP (Murase *et al*., 1990; Staubli & Otaky, 1994; Chesnoy-Marchais & Barthe, 1996; Siegel *et al*., 1998). 5-HT and related compounds may modify the action of these pathways in a Mg^{2+}-dependent manner and thus may provide a basis for drug treatment of pain, seizures and glutamate-mediated excitoxic neurodegeneration.

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