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RESEARCH PAPER

Mutations within the selectivity filter of the NMDA receptor-channel influence voltage dependent block by 5-hydroxytryptamine

A Kloda and DJ Adams

School of Biomedical Sciences, University of Queensland, Brisbane, Queensland, Australia

Background and purpose: Voltage-dependent block by Mg^{2+} is a cardinal feature of NMDA receptors which acts as a coincidence detector to prevent the receptor from over-activation. Inhibition of NMDA receptor currents by 5-hydroxytryptamine (5-HT) indicated that 5-HT, similar to Mg^{2+}, binds within the membrane electric field. In the present study, we assessed whether point mutations of critical asparagine residues located within the selectivity filter of NR1 and NR2A subunits of NMDA receptor-channel affect voltage-dependent block by 5-HT.
Experimental approach: The mode of action of 5-HT and Mg^{2+} on wild-type and mutated NMDA receptor-channels expressed in *Xenopus* oocytes was investigated using the two-electrode voltage clamp recording technique.
Key results: The mutation within the NR1 subunit NR1(N0S or N0Q) strongly reduced the voltage dependent block by 5-HT and increased the IC_{50}. The corresponding mutations within the NR2 subunits NR2A(N0Q or N + 1Q) reduced the block by 5-HT to a lesser extent. This is in contrast to the block produced by external Mg^{2+} where a substitution at the NR2A(N0) and NR2A(N + 1) sites but not at the NR1(N0) site significantly reduced Mg^{2+} block.
Conclusion and implications: The block of NMDA receptor-channels by 5-HT depends on the NR1-subunit asparagine residue and to a lesser extent on the NR2A-subunit asparagine residues. These data suggest that the interaction of 5-HT with functionally important residues in a narrow constriction of the pore of the NMDA receptor-channel provides a significant barrier to ionic fluxes through the open channel due to energetic factors governed by chemical properties of the binding site and the electric field.
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Keywords: serotonin; NMDA receptors; glutamate; single point mutations; *Xenopus* oocytes; voltage-dependent block

Abbreviations: HEPES, 4-2-(hydroxyethyl)piperazine-1-ethanesulfonic acid; 5-HT, 5-hydroxytryptamine; IC_{50}, half-maximal inhibitory concentration; NMDA, *N*-methyl-D-aspartate

Introduction

The NMDA receptor is an oligomeric cation channel, which mediates important physiological processes such as long-term potentiation, synaptic plasticity and neuro-degeneration via conditional Ca^{2+} signalling (Bliss and Collingridge, 1993; Castellano *et al*., 2001; Cull-Candy *et al*., 2001). The ionic influx through the open channel pore coincides with the presynaptic release of glutamate and postsynaptic membrane depolarization, which relieves voltage-dependent Mg^{2+} block (Mayer *et al*., 1984; Nowak *et al*., 1984). Impairment of the Mg^{2+} block of NMDA receptors can lead to excessive Ca^{2+} influx into neurons and the generation of nitric oxide and/or reactive oxygen species as well as activation of many downstream targets including Ca^{2+}-dependent enzymes and protein kinase signalling pathways. These biochemical events may be associated with neuronal excitoxicity, which underlies many neuro-degenerative disorders, neuro-inflammation as well as cognitive deficits associated with normal aging (Rosi *et al*., 2004; Waxman and Lynch, 2005).

The functional NMDA receptor-channel is most likely a tetramer composed of two NR1 and two NR2 subunits (Laube *et al*., 1998). The transmembrane topology of NMDA receptors predicts three transmembrane domains (M1, M3 and M4) and a cytoplasmic re-entrant loop (M2), which lines the channel pore (Kuner *et al*., 1996; Dingledine *et al*., 1999). The M2 segment of both subunits harbours an asparagine residue at a position homologous to the Q/R site of AMPA receptors. The RNA editing of the Q/R site controls Ca^{2+} and Mg^{2+} permeability, which affects single-channel

Correspondence: Dr DJ Adams, School of Biomedical Sciences, University of Queensland, Brisbane, Queensland 4072, Australia.
E-mail: dadams@uq.edu.au
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conductance and alters the current–voltage (*I–V*) relation of AMPA receptors (Hume *et al*., 1991; Verdoorn *et al*., 1991; Burnashev *et al*., 1992a). The site homologous to the Q/R site located on the NR2A subunit of NMDA receptors at position 595, the N0 site, and the adjacent non-homologous asparagine residue at position 596, N + 1 site, strongly affect Mg^{2+} permeability and channel rectification owing to block by extracellular Mg^{2+}. In contrast, the asparagine residue at a position homologous to the Q/R site, N598 or the (N0) site on the NR1 subunit has little effect (Kupper *et al*., 1998; Wollmuth *et al*., 1998). Interestingly, NR1(N0), NR2(N0) and NR2(N + 1) asparagine residues are located at the tip of the M2 re-entrant loop and thus form the narrowest constriction or the selectivity filter of the channel pore (Wollmuth *et al*., 1996) (Figure 1).

The co-release of 5-hydroxytryptamine (5-HT) and glutamate by single raphe neurons (Johnson, 1994) suggests that 5-HT may also be an important physiological modulator of NMDA receptors at central synapses. It is possible that during synaptic transmission, the concentrations of 5-HT in the synaptic cleft can reach levels (high micromolar) sufficient to modulate ongoing synaptic activity. The inhibition of recombinant NMDA receptor-channels by indolealkylamines and monoamines including 5-HT has been reported recently (Masuko *et al*., 2004; Kloda and Adams, 2005). The block of NMDA receptors composed of NR1 and NR2 subunits by 5-HT was shown to be strongly voltage-dependent (Kloda and Adams, 2005) and several monoamines were suggested to interact directly with the narrowest region of the channel pore (Masuko *et al*., 2004). In the present study, the effects of NR1(N0S and N0Q), NR2A(N0Q) and NR2A(N + 1Q) mutations, which differentially influence Mg^{2+} block, were assessed on the voltage sensitivity of the block by extracellular 5-HT using the *Xenopus* oocyte expression system and the two-electrode voltage clamp recording technique. Our results indicate that unlike Mg^{2+} block, the binding of 5-HT involves interaction with the Q/R site on the NR1 subunit and to a lesser extent with sites at a similar position on NR2A subunit located within the selectivity filter of NMDA receptors.

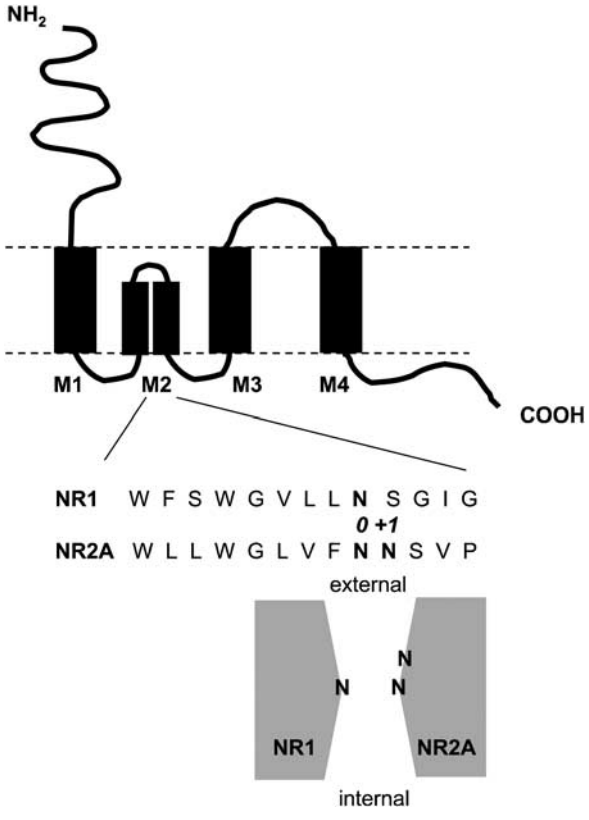

Figure 1 Membrane topology of NMDA receptors with structural details including the amino-acid sequence of the M2 region encompassing the selectivity filter. The mutagenized N0 and N + 1 site asparagines are in bold and numbered correspondingly. A schematic representation of the NMDA receptor pore region (below) shows the position of asparagines (N0) and (N + 1) at the narrow constriction formed by NR1 and NR2A subunits.

Methods

Preparation of RNA

Clones of the rat wild-type and mutated NMDA receptors, NR1(N0S), NR1(N0Q), NR2A(N0Q) and NR2A(N + 1Q), used in this study were a gift of Dr L Wollmuth (SUNY Stony Brook, NY, USA). In this study, we adopted the mutant channel nomenclature used by Wollmuth *et al*. (1998). Plasmid DNA of NR1 and NR2A and their mutated forms were linearized with *Mlu*I restriction enzyme. Linear templates were used for *in vitro* synthesis of 5′-capped mRNA with SP6 polymerase using the mMessage mMachine Transcription Kit (Ambion, Austin, TX, USA).

Expression in Xenopus *oocytes*

Mature *Xenopus laevis* female frogs were anaesthetized 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 type I dissolved in ND96 media containing (in mM) 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 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. NR1 and NR2A RNA transcripts were mixed in a molar ratio of 1:3 and oocytes were microinjected with 50 nl of the final RNA mixture (0.01–1 μg μl^{-1}) into the oocyte cytoplasm. Oocytes were incubated in ND96 media at 18°C for 2–5 days before electrophysiological recordings.

Electrophysiology

Oocytes were placed in the recording chamber (0.1 ml volume) and continuously perfused with Ca^{2+}- and Mg^{2+}-free solution containing (in mM) 115 NaCl, 2.5 KCl, 0.36 $BaCl_2$ and 10 HEPES (pH 7.3), unless otherwise stated. In control experiments, $MgCl_2$ (0.3 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, USA), filtered at 200 Hz and digitized using a Digidata 1200A 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 −120 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 was performed as described previously (Kloda and Adams, 2005). The data are presented as the mean ± s.e.m. (n = number of cells) and were analysed statistically using Student's *t*-test with the level of significance taken as $P<0.05$.

Results

The effects of NR1(N0) (where asparagine was substituted by either serine or glutamine) as well as corresponding NR2A(N0) and (N + 1) mutations (where asparagine residues were substituted by glutamine) on the block by 5-HT of recombinant NMDA receptors expressed in *Xenopus* oocytes were examined in Ca^{2+}-free solution but in a bath solution containing a low concentration of Ba^{2+} to minimize contamination by Ca^{2+}-dependent Cl^- currents (Boton *et al.*, 1989; Leonard and Kelso, 1990) and prevent Ca^{2+} block of the mutant channels (Sakurada *et al.*, 1993; Sharma and Stevens, 1996). NMDA receptors assembled from the NR2A wild-type subunit and either the NR1 wild-type or the NR1 subunit where asparagine was substituted at position (N0) were differentially blocked by 0.3 mM 5-HT (Figure 2a and b and Table 1). Serine or glutamine substitutions on the NR1 subunit produced similar results and the data presented are with the NR1(N0S) mutant.

The normalized *I–V* curves shown in Figure 2 suggest a different voltage dependence of block between wild-type NMDA receptor-channels compared to channels harbouring mutations in the NR1 subunit at position (N0) whereas the NR2A(N + 1) and the NR2A(N0) mutations had a smaller effect on the block by 5-HT. Furthermore, the block by 5-HT appears to be different from that produced by external Mg^{2+}. The *I–V* relation for the wild-type NMDA receptors recorded in the presence of 0.3 mM Mg^{2+} exhibits more pronounced rectification at negative potentials compared to that obtained in the presence of 0.3 mM 5-HT. In contrast to the block by Mg^{2+}, where the rectification is abolished in NR2A(N0) and NR2A(N + 1) mutants, the *I–V* relation obtained in the presence of 5-HT exhibits rectification in channels harbouring mutations on the NR2A subunit. However, the NR1(N0) mutant, which rectifies in the presence of Mg^{2+}, does not exhibit rectification at negative potentials in the presence of 5-HT (for further references on Mg^{2+} block, see also Burnashev *et al.*, 1992b; Kupper *et al.*, 1998; Wollmuth *et al.*, 1998). In the double-mutant NR1(N0) + NR2(N + 1), the voltage-dependent block by 0.3 mM 5-HT was completely abolished.

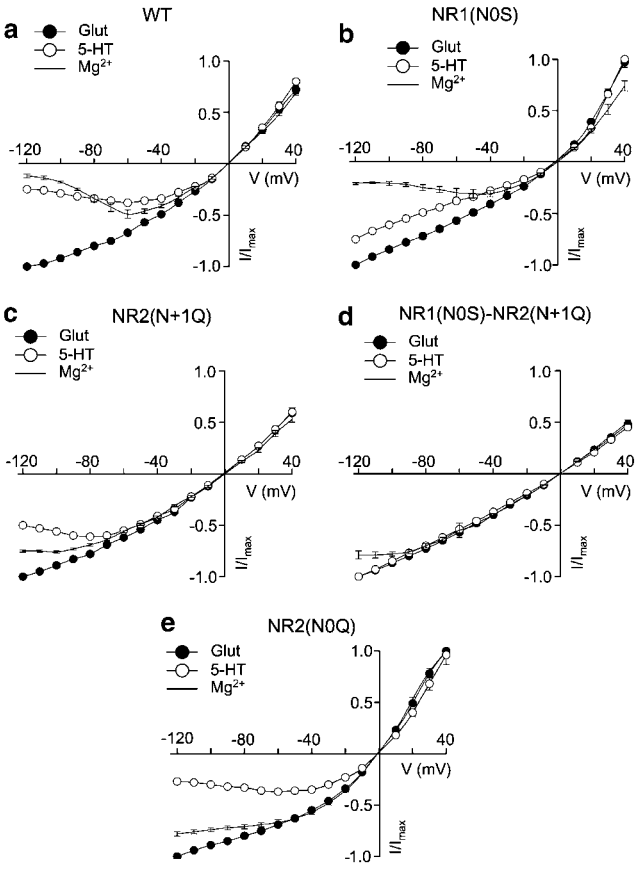

Figure 2 Voltage-dependent block of wild-type and mutated NMDA receptor channels by extracellular 5-HT. Normalized *I–V* relations of glutamate-activated NMDA receptors in the absence (closed symbols) and presence of 0.3 mM 5-HT (open symbols) obtained for (**a**) wild-type NR1-NR2A subunit combinations, (**b**) a mutant comprising NR1(N0S) and NR2A subunits, (**c**) a mutant comprising NR1 and NR2A(N + 1Q) subunits, (**d**) a double mutant composed of NR1(N0S) and NR2A(N + 1Q) subunits and (**e**) a mutant channel comprising NR1 and NR2(N0Q) subunits. Control experiments in the presence of 0.3 mM Mg^{2+} are superimposed as a line without symbols. Currents were obtained in response to voltage ramps during steady-state responses to glutamate (100 μM) + glycine (10 μM) before and after bath application of the blocker ($n=5$).

Table 1 Voltage dependence and potency of extracellular 5-HT block of the NR1 + NR2A wild-type and mutant receptors

Subunits	δ	$K_{0.5}$ (*0 mV*) (*mM*)	IC_{50} (μM)	H	n
WT	0.8 ± 0.1	2.9 ± 0.4	56 ± 15	0.9 ± 0.2	5
NR1(N0S)	0.2 ± 0.01**	1.8 ± 0.1*	1500 ± 103**	0.9 ± 0.0.1	5
NR2(N0Q)	0.5 ± 0.1	1.2 ± 0.1*	103 ± 18	0.8 ± 0.3	5
NR2(N + 1Q)	0.8 ± 0.1	12.9 ± 0.9**	230 ± 12**	1.0 ± 0.1	5

The 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), was estimated according to the Boltzmann equation:

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

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 respective thermodynamic meanings.

IC_{50} values were determined at −120 mV. H is the Hill coefficient and n is the number of cells used. Statistically different from the corresponding values for wild-type subunits (*t*-test): *$P<0.05$, **$P<0.01$.

The block by 5-HT was further evaluated by determining the percentage of block in the presence of 0.3 mM 5-HT at −120 and −60 mV (Figure 3a). Wild-type NMDA receptor-channels assembled from NR1 + NR2A subunits were strongly inhibited at −120 mV, with the current amplitude reduced to about 25% of control and to about 50% of control at −60 mV. In the NR1 + NR2A(N0Q) mutated channels, the corresponding responses were almost identical at either voltage (Figure 3a). In the NR1 + NR2A(N + 1Q) mutated channels, there was less inhibition than in the wild-type channels, although the inhibition was still voltage dependent. In contrast, the current amplitude of mutated NMDA receptors assembled from a combination of NR1(N0S) + NR2A subunits, obtained in the presence of 5-HT, was only inhibited by about 25% and showed no voltage dependence. The mutated NMDA receptor-channels remained unblocked at positive potentials similar to the wild-type NMDA receptors.

The voltage dependence of 5-HT block of the wild-type and mutated NMDA receptor-channels containing substitutions located at NR1(N0S) or NR2(N0Q) and NR2A(N + 1Q) was further analysed according to the Woodhull (1973) model (Figure 3b and Table 1). A plot of the ratio of glutamate-evoked current amplitude obtained in the absence and presence of 0.3 mM 5-HT as a function of voltage revealed an e-fold change in current amplitude per 33 ± 2.8 mV ($n = 5$) for the NR1 + NR2A wild type. A similar e-fold change per 31 ± 2.7 mV was obtained for the NR2A(N + 1Q) mutated channel, but a slightly higher e-fold change per 50 ± 2.8 mV was obtained for NR2A(N0Q) mutated channel. The e-fold change increased significantly to 137 ± 30 mV in the NR1(N0S) mutated channel.

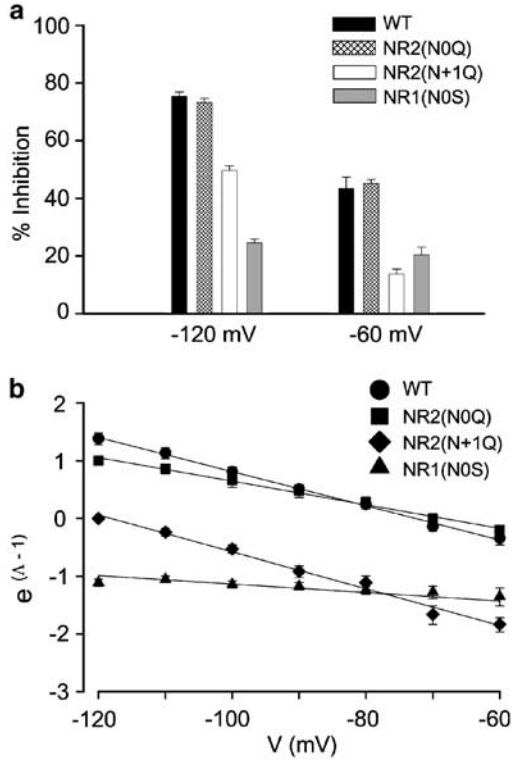

Figure 3 Voltage dependence of inhibition of glutamate-activated currents by 5-HT for the wild-type and mutated NMDA receptor channels. (**a**) Bar graph summarizing the pattern of 5-HT block of the wild-type, NR1(N0S), NR2A(N0Q) and NR2A(N + 1Q) NMDA receptor mutants at −120 and −60 mV estimated as a % of the control current ($n = 5$). (**b**) The ratio of current amplitudes obtained in the absence and presence of 0.3 mM 5-HT (Λ) was determined at different membrane potentials. Λ−1 is plotted as a function of membrane potential.

The voltage dependence of the block (Table 1) was attenuated approximately fourfold for NR1(N0S) receptor-channels compared to the wild type and was lower for NR2(N0Q) channels, but remained the same for NR2(N + 1Q) mutant. Furthermore, the voltage-independent affinity for 5-HT ($K_{0.5}$ (0 mV); Table 1) of the NR1(N0S) and NR2(N0Q) receptor-channels was lower than that of the wild type. Interestingly, the NR2(N + 1Q) mutant showed a significantly higher voltage-independent affinity for 5-HT (Table 1).

To further assess the effect of mutations within the selectivity filter on 5-HT block of NMDA receptor-mediated currents, the half-maximal inhibitory concentration (IC_{50}) was determined for the wild-type and mutant channels at −120 mV (Figure 4 and Table 1). The IC_{50} values obtained for 5-HT inhibition of the wild type (NR1 + NR2A) and NR1 + NR2A(N0Q) were similar, but were increased for the NR2A(N + 1Q) mutant. The NR1(N0S) mutant displayed a much higher IC_{50} compared to the wild-type channel and those harbouring mutations on the NR2A subunits.

Discussion and conclusions

In the present study, we used mutational analysis to identify the structural elements of the NMDA receptor-channel that mediate the block by 5-HT. A major finding is that 5-HT inhibits currents through open glutamate-activated NMDA receptor-channels composed of NR1 and NR2A subunits by interacting with functionally important sites within the M2 domain which form the selectivity filter. Our data demonstrate that mutations of NR1(N0), NR2A(N0) and NR2A(N + 1) asparagines that control ion permeation and open channel blockade (Burnashev *et al*., 1992b; Mori *et al*., 1992; Sharma and Stevens, 1996; Kupper *et al*., 1996, 1998; Wollmuth *et al*., 1996, 1998) differentially affect the block of NMDA receptors by extracellular 5-HT. Substitution of asparagine at the NR1(N0) site with either serine or glutamine attenuated the magnitude of the block by 5-HT and abolished the voltage dependence of block. The corresponding mutations within the NR2 subunit at the NR2A(N + 1) and NR2A(N0) sites (where asparagine residues were substituted by glutamine) disrupted the block by 5-HT to a lesser extent compared to its homologous NR1(N0) site. This is opposite to the block produced by external Mg^{2+} where a substitution of the NR2A(N0) and NR2A(N + 1) site asparagine but not the NR1(N0) site significantly reduces the block (Kupper *et al*., 1998; Wollmuth *et al*., 1998). The attenuation of 5-HT block by corresponding asparagine mutations in the NR1 and NR2A subunits suggests that, in contrast to the Mg^{2+} block, 5-HT block critically depends on the NR1(N0) residue and to a lesser extent on the NR2(N + 1) and NR2(N0) residues.

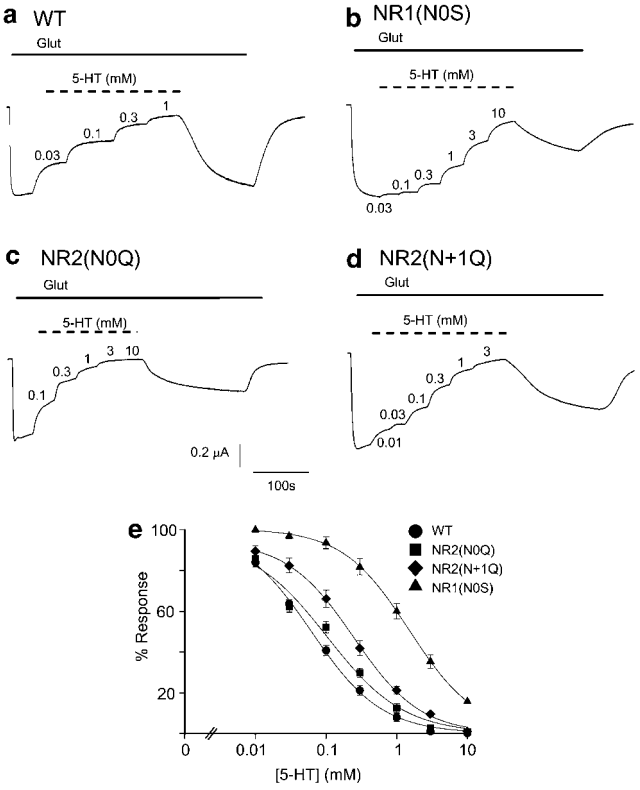

Figure 4 Concentration dependence of 5-HT blockade of wild-type and mutated NMDA receptor channels. Representative current traces (**a**–**d**) and concentration–response curves (**e**) obtained for 5-HT inhibition of glutamate-induced currents show concentration-dependent inhibition of the currents by 5-HT. Glutamate was applied as indicated by the solid horizontal lines and the dashed lines indicate the application of 5-HT. Steady-state currents induced by 100 μM glutamate were measured in the presence of various concentrations of 5-HT in oocytes expressing (**a**) NR1-NR2A wild type, (**b**) NR1(N0S)-NR2A, (**c**) NR1-NR2A(N0Q) and (**d**) NR1-NR2A(N+1Q) mutants. Current amplitude after 5-HT block was scaled to the initial maximal current. Current recovery was not complete for the mutant channels after application of high concentrations of 5-HT ($\geqslant$3 mM). Oocytes were voltage clamped at a holding potential of -120 mV; $n=5$ oocytes at each concentration tested.

The NR1(N0), NR2(N0) and NR2(N+1) asparagines of NMDA receptors are positioned at the mouth of the narrow constriction of the channel (Figure 1). At this narrow constriction or selectivity filter of the NMDA receptor-channel, the two adjacent asparagines located on the NR2 subunit (N-site) and (N+1) site, but not the asparagine of the NR1 subunit (N0) site, form a critical blocking site for extracellular Mg^{2+} (Burnashev *et al*., 1992b; Mori *et al*., 1992; Sakurada *et al*., 1993; Wollmuth *et al*., 1996, 1998). The substitutions at both the NR1(N0) and the NR2(N+1) positions were found to alter the pore size; however, the size of the pore itself does not determine the magnitude of the Mg^{2+} block (Wollmuth *et al*., 1996). Given that the block by 5-HT differed from that produced by Mg^{2+} and as 5-HT is a larger-sized cation compared to Mg^{2+}, we postulate that the narrow constriction within the pore region could be the main structural determinant of 5-HT block. A charged molecule such as 5-HT (at physiological pH, 5-HT is protonated) is less likely to interact with the pore as it increases in size. Measurements of the cross-sectional diameter of the narrow constriction were estimated to be 0.55 nm for the NR1-NR2A wild-type NMDA receptor, decreased to 0.48 nm for NR2A(N+1Q) but remained unchanged for the NR1(N0S) mutant (Wollmuth *et al*., 1996). Therefore, we hypothesize that the magnitude of the block will not change in the NR1(N0S) mutant, but will increase in the NR2A(N+1Q) mutant. However, our data do not support the assumption that differences in the magnitude of the 5-HT block between the NR1(N0S) and NR2A(N+1Q) mutants reflect a change in the size of the pore, as the block of the NMDA receptor-channels by 5-HT was attenuated regardless of whether the channel pore was reduced or remained the same. Thus, it appears that the block by 5-HT is owing to energetic factors caused by alteration of the chemical properties of side chains at the binding site itself, rather than owing to changes in the size of the pore region.

NMDA receptor-channels containing neutral substitutions of the NR2(N0) and (N+1) site but not the NR1(N0) site asparagines showed increased Mg^{2+} permeability, suggesting that the NR2 asparagines participate in the formation of a barrier for inward Mg^{2+} fluxes. Changes to this barrier contribute to disruption of the voltage-dependent block by Mg^{2+} (Burnashev *et al*., 1992b; Mori *et al*., 1992; Wollmuth *et al*., 1998). In contrast, reduction in the magnitude of 5-HT block and attenuation of its voltage dependence caused by substitutions of asparagines at the NR1(N0) site to either serine or glutamine indicates that this residue contributes to an energy barrier for 5-HT entry. The block of the NR2A(N+1Q) and NR2A(N0Q) mutants increased at negative potentials but was unchanged, over a similar voltage range, in the NR1(N0S or N0Q) mutants. This suggests that, unlike Mg^{2+} block, the NR1 subunit asparagine contributes more to the energy barrier for 5-HT entry than the NR2A subunit asparagines. This differential block may be owing to a different degree of steric hindrance introduced by the mutations or more global changes in the receptor conformation that affects the channel gate. Interestingly, 5-HT block of NMDA receptor-channels composed of double mutants NR1(N0S)+NR2(N+1Q) was abolished, indicating the importance of corresponding asparagine residues on both subunits to the block by 5-HT and a complex interaction of 5-HT at the selectivity filter.

The assumption that NR1(N0) asparagine contributes to the energy barrier for the entry of 5-HT to a greater extent than the NR2A(N+1) and NR2A(N0) asparagines is further supported by differences in the voltage dependence of 5-HT block of the mutant NMDA receptor-channels estimated as a fractional electrical distance. The substitution of the asparagine residue at the NR2A(N0) site reduced the voltage dependence to $\sim$0.5 of the fractional electric distance, which corresponds to the estimated position of the narrow constriction of 0.5–0.6 of the fractional electric field (Wollmuth *et al*., 1998). This suggests that the contribution of the NR1 subunit asparagine to the energy barrier is largely owing to the voltage drop at the narrow constriction. However, the Woodhull model placed the apparent binding site for 5-HT across the transmembrane electric field ($\delta\sim1$). As the substitution of NR1(N0) asparagine generated a

voltage dependence of $\delta \sim 0.2$, factors other than the electric field must contribute to the voltage dependence of 5-HT block of the wild-type NMDA receptor-channels. There is an approximately fourfold increase in the IC_{50} for 5-HT block of the NR2A(N+1) mutant at −120 mV and approximately 30-fold increase at the NR1(N0) mutated NMDA receptors compared to control. In contrast, changes in the affinity for 5-HT estimated at 0 mV as $K_{0.5}$ (0 mV) were less dramatic for the NR1(N0) and NR2(N0) mutants but significantly higher for the NR2(N+1) mutant (Table 1), further supporting a view that the structural determinants of the binding site as well as the electric field contribute to 5-HT block.

The ability of 5-HT to block the NMDA receptor-channel pore could depend on geometrical arrangements of the side chains within the narrow constriction of the selectivity filter. It is possible that carboxyl side chains of both asparagines of NR1 and NR2 subunits located at the narrow constriction can participate in the formation of hydrogen bonding with 5-HT (Almaula *et al*., 1996). A homologous group at the 5-position of 5-HT was found to influence the potency of several structurally related indolealkylamines, which differentially block the NMDA receptor (Kloda and Adams, 2005). Thus, changing the geometrical arrangements within the selectivity filter may not only introduce a steric hindrance, but also the nature of the 5-HT interaction with the binding site may influence the degree of 5-HT block of the mutated channels. Interestingly, similar mutations of critical asparagine residues located on NR1 and NR2B subunits markedly reduced block by the NMDA receptor-channel blockers such as MK-801, memantine and TB-3-4 (N^1-N^4-N^8-tribenzylspermidine). It has been suggested that these residues interact directly with the amino groups of these blockers (Kashiwagi *et al*., 2002).

In conclusion, the present study shows that substituting the asparagine residue at NR1(N0) of the NMDA receptors disrupts the voltage dependency of the block by 5-HT. Analogous to Mg^{2+}, 5-HT binds to the site within the narrow constriction of the pore. However, unlike Mg^{2+}, which interacts with adjacent asparagines on the NR2 subunit, 5-HT interacts principally with the asparagine on the NR1 subunit positioned within the narrowest region of the channel pore (see also Masuko *et al*., 2004). It is this interaction that abolishes the voltage dependence of block by 5-HT. In conclusion, our data suggest an allosteric competition between Mg^{2+} and 5-HT for binding site(s) in the channel pore as proposed previously (Kloda and Adams, 2005).

Conflict of interest

The authors state no conflict of interest.

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