The NR3B Subunit Does Not Alter the Anesthetic Sensitivities of Recombinant *N*-Methyl-D-Aspartate Receptors

Tomohiro Yamakura, MD, PhD*, Ahmed R. Askalany, MD*, Andrey B. Petrenko, MD, PhD*, Tatsuro Kohno, MD, PhD*, Hiroshi Baba, MD, PhD*, and Kenji Sakimura, PhD†

*Division of Anesthesiology, Niigata University Graduate School of Medical and †Dental Sciences; Department of Cellular Neurobiology, Brain Research Institute, Niigata University, Niigata, Japan

The *N*-methyl-D-aspartate (NMDA) receptor NR3B subunit co-assembles with NR1 and NR2 subunits to form a receptor complex with distinct channel properties. In the present study, we investigated the effects of co-expression of the NR3B subunit on the anesthetic sensitivities of NMDA receptors for NR1/NR2 channels expressed in *Xenopus* oocytes. Although the NR3B subunit prominently reduced the current amplitude of NR1/NR2A-B channels, the sensitivities of NR1/NR2A-B channels to Mg^{2+}, ketamine, isoflurane, nitrous oxide, and ethanol were not altered by co-expression of the NR3B subunit. These results suggest that the anesthetic sensitivities of NMDA receptors do not depend on the presence or absence of the NR3 subunit. Mutations of two amino acid residues in the NR3B subunit at positions homologous to the N and N + 1 sites in the NR1 and NR2 subunits, which constitute the blocking sites for Mg^{2+} and ketamine, did not affect the sensitivities of NR1/NR2B/NR3B channels to Mg^{2+}, ketamine and isoflurane. Thus, the amino acid residues at the N and N + 1 sites in NR3 subunits are unlikely to be involved in the formation of channel blocking sites in NR1/NR2/NR3 channels.

(Anesth Analg 2005;100:1687–92)

Several studies on the mechanisms of general anesthesia suggest that ligand-gated ion channels are an important target of general anesthetics. These channels include the inhibitory γ-aminobutyric acid (GABA) and glycine receptors and the excitatory nicotinic acetylcholine, serotonin, and glutamate receptors. Among the ligand-gated ion channels, the GABA type A receptor is considered to be a prime target of volatile and IV anesthetics (1,2), whereas the *N*-methyl-D-aspartate (NMDA) receptor, one of the glutamate receptor subtypes, is considered to be an important target of dissociative and gaseous anesthetics (3–5).

The NMDA receptor is composed of at least two families of subunits, the NR1 (mouse ζ) and NR2 (mouse ε) subfamilies. The functional properties of NR1/NR2 heteromeric NMDA receptors are determined by the constituting NR2 subunit species (NR2A-D) (6). In addition to the main classes of NMDA receptor subunits, NR3 subunits (NR3A and NR3B) have been shown to modulate the function of NMDA receptors. When co-expressed with NR1 and NR2 subunits in heterologous cells, NR3 subunits reduce the agonist-induced whole-cell currents, channel conductance and Ca^{2+}-permeability of NMDA receptors (7–13). The NR3A subunit is ubiquitously expressed during development, with only low levels present in adult animals (7,8). In contrast, the NR3B subunit mRNA shows very restricted expression in somatic motoneurons of the brainstem and spinal cord at almost constant levels during development (10,12).

A recent study demonstrated that spinal NMDA receptors contribute to the immobilizing action of isoflurane (14). Given the motoneuron-specific localization of the NR3B subunit in the spinal cord, which is the primary site mediating immobilization (15), the NR3B subunit may play a role in determining the immobility produced by general anesthetics.

In the adult spinal cord, NR2A and NR2B subunit mRNAs are the predominant transcripts, whereas the expressions of NR2C and NR2D subunit mRNAs are insignificant (16). Furthermore, we previously demonstrated the presence of the NR2A and NR2B subunits at the protein level in the adult mouse spinal cord (17).

In the present study, we used a *Xenopus* oocyte expression system to determine whether co-expression of

Supported, in part, by a grant-in-aid (13557130) from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan.

Accepted for publication November 18, 2004.

Address correspondence and reprint requests to Tomohiro Yamakura, MD, PhD, Division of Anesthesiology, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951–8510, Japan. Address e-mail to yamakura@med.niigata-u.ac.jp.

DOI: 10.1213/01.ANE.0000152324.30272.49

0003-2999/05

the NR3B subunit altered the sensitivities of NR1/NR2A-B NMDA receptors to dissociative (ketamine), volatile (isoflurane), and gaseous (nitrous oxide) anesthetics and alcohol (ethanol).

Methods

This study was approved by the Committee for Guidelines on Animal Experimentation of Niigata University. The spinal cord was removed from 8-wk-old C57BL/6 mice. Mouse total RNA was isolated using the Isogen reagent (Nippon Gene, Tokyo, Japan). First-strand cDNAs were generated using an Advantage RT-for-PCR Kit (Clontech, Palo Alto, CA). To isolate the mouse NR3B subunit cDNA by reverse transcriptase-polymerase chain reaction (RT-PCR), the following primers were used: NR3B forward, 5'-GGGCCATGGAGTGTGTGCAGACG-3'; and NR3B reverse, 5'-CCCTCTAGATCAGCTCTCGGCGGGCGCCG-3'. The obtained cDNA was subcloned into the pSP35T vector (18) to yield pSPGRNR3B. To construct an NR3B-G629N/R630N mutant subunit, the amino acid residues glycine 629 and arginine 630 were replaced with asparagine residues using a QuikChange mutagenesis kit (Stratagene, La Jolla, CA). All constructs were verified by DNA sequencing.

Subunit-specific mRNAs were synthesized *in vitro* with SP6 RNA polymerase (MEGAscript; Ambion, Austin, TX) in the presence of the cap dinucleotide ^{7}mGpppG. The NR1, NR2A, and NR2B subunit-specific mRNAs were synthesized using pSPGRζ1, pSPGRϵ1, and pSPGRϵ2, respectively (19). The NR3B and NR3B-G629N/R630N subunit-specific mRNAs were synthesized using *Eco*RI-cleaved pSPGRNR3B and pSPGRNR3B-G629N/R630N as templates, respectively. *Xenopus laevis* oocytes were injected with the NR1 and NR2 subunit-specific mRNAs with or without the NR3B subunit-specific mRNA. The total amounts and molar ratios of the injected NR1, NR2, and NR3B subunit mRNAs per oocyte were adjusted so that a considerable reduction in the current amplitude of NR1/NR2 channels was observed when the NR3B subunit was co-expressed: ~3.8 ng in a 1:1:12.5 molar ratio for the NR1/NR2A/NR3B channel, ~3.3 ng in a 1:1:6 molar ratio for the NR1/NR2B/NR3B channel, and ~1.8 ng in a 1:1:2.5 molar ratio for the NR1/NR2B/NR3B-G629N/R630N channel, unless otherwise specified. Under these conditions, comparable current amplitudes (100–400 nA) were observed for each combination of NR subunits. For the expression of NR1/NR2A and NR1/NR2B channels, the total amounts of NR1 and NR2 subunit mRNAs injected per oocyte were decreased to obtain current amplitudes that were comparable to that of NR1/NR2/NR3B channels (~0.2 ng in a 1:1 molar ratio).

After incubation at ~19°C for 1–2 d, whole-cell currents evoked by bath-application of agonists for ~15 s were recorded at −70 mV membrane potential using a conventional 2-micropipette voltage clamp. The current amplitudes of NR1/NR2 channels were measured in Ba^{2+}-Ringer's solution to minimize the effects of secondarily-activated Ca^{2+}-dependent Cl^{-} currents. The effect of anesthetics on the current responses to 10 μM L-glutamate plus 10 μM glycine was examined during continuous perfusion of the anesthetics. Preapplication of the anesthetics in the absence of agonists produced no current response. The agonists were applied successively (two or three times) during the perfusion of ketamine to fully establish the inhibition as described previously (19). The Ba^{2+}-Ringer's solution contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM $BaCl_2$, and 10 mM HEPES-NaOH (pH 7.4). The anesthetic solutions were prepared as described previously (20). The anesthetic concentrations in the text represent the final bath concentrations quantified by gas chromatography as described previously (20). For nitrous oxide, the concentration tested (~0.6 atmosphere) was the maximal concentration that could be achieved in our perfusion system. Data were obtained from 3–11 oocytes taken from at least 2 different frogs. All experiments were conducted at room temperature (23°C).

The EC_{50} (agonist concentration for half-maximal response) values for agonists of the NR1/NR2 and NR1/NR2/NR3B channels were calculated according to the equation $F_{ago} = 1/[1 + (EC_{50}/A)^n]$, where F_{ago} represents the fractional response, A is the concentration of the agonist, and n is the Hill coefficient. The IC_{50} (inhibitor concentration for half-control response) and Hill coefficient values for ketamine for the NR1/NR2 and NR1/NR2/NR3B channels were calculated according to the equation $F_{ket} = 1/[1 + (K/IC_{50})^n]$, where F_{ket} represents the fractional response, K is the concentration of ketamine, and n is the Hill coefficient. The results obtained were analyzed statistically by one-way analysis of variance or Student's t-tests. P values < 0.05 were considered significant. Data were represented as the mean ± SD.

Results

L-glutamate (up to 100 μM) plus glycine (up to 100 μM) or glycine alone evoked only minimal or no current response from oocytes injected with NR1 and NR3B subunit mRNAs. However, when the NR3B subunit was co-expressed with the NR1 and NR2 subunits, it markedly reduced the current amplitude of NR1/NR2 channels, as reported previously (10). The extent of the reduction in the current amplitude of NR1/NR2B channels was dependent on the amount of co-injected NR3B subunit mRNA provided that the amounts of the NR1 and NR2B subunit mRNAs were fixed (Fig. 1A, analysis of variance, $P < 0.0001$). Therefore, to examine the effects of co-expression of the NR3B subunit in the following experiments, we co-injected an amount of NR3B subunit mRNA that was

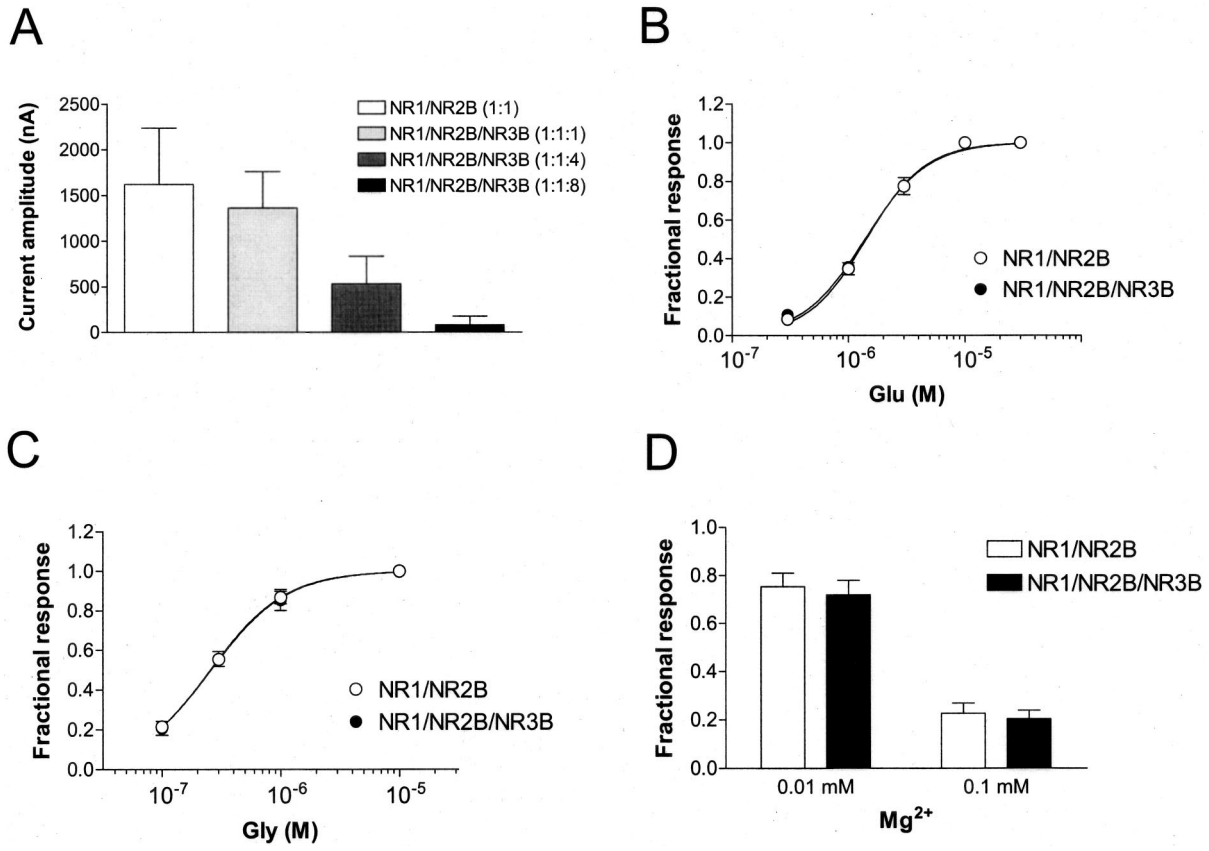

Figure 1. Effects of co-expression of the NR3B subunit on the properties of NR1/NR2B channels. A, Current amplitude of NR1/NR2B/NR3B channels as a function of the amount of co-injected NR3B subunit mRNA. Under the condition that the amounts of the injected NR1 and NR2B subunit mRNAs were fixed (~0.8 ng per oocyte), the mRNAs were injected at the indicated molar ratios. Each set of data represents the mean ± SD of measurements of the current responses to 10 μM L-glutamate plus 10 μM glycine for 6–11 oocytes. B, Effect of the NR3B subunit on the dose-response relationship of NR1/NR2B channels for L-glutamate. The EC_{50} values (μM) of the NR1/NR2B and NR1/NR2B/NR3B channels for L-glutamate in the presence of 10 μM glycine are 1.6 ± 0.2 and 1.6 ± 0.1, respectively, and the Hill coefficient values are 1.8 ± 0.2 and 1.8 ± 0.1, respectively (n = 5–6). Error bars not visible are smaller than the symbols. C, Effect of the NR3B subunit on the dose-response relationship of NR1/NR2B channels for glycine. The EC_{50} values (μM) of the NR1/NR2B and NR1/NR2B/NR3B channels for glycine in the presence of 10 μM L-glutamate are 0.23 ± 0.10 and 0.18 ± 0.13, respectively, and the Hill coefficient values are 1.3 ± 0.4 and 1.2 ± 0.7, respectively (n = 5). D, Effect of the NR3B subunit on the sensitivity of NR1/NR2B channels to Mg^{2+}. The effects of 0.01 and 0.1 mM Mg^{2+} on the NR1/NR2B and NR1/NR2B/NR3B channels were measured in 5–10 oocytes.

large enough to decrease the current amplitude of NR1/NR2 channels.

We examined whether co-expression of the NR3B subunit altered the dose-response relationships of NR1/NR2B channels for agonists. The EC_{50} value (μM) of NR1/NR2B/NR3B channels for L-glutamate in the presence of 10 μM glycine was not significantly different from that of NR1/NR2B channels (Fig. 1B). Similarly, the EC_{50} value (μM) of NR1/NR2B/NR3B channels for glycine in the presence of 10 μM L-glutamate was not significantly different from that of NR1/NR2B channels (Fig. 1C). We next examined whether the NR3B subunit affected the sensitivity to Mg^{2+} blocking of NMDA receptors. The extents of inhibition by 0.01 and 0.1 mM Mg^{2+} were not significantly different between the NR1/NR2B and NR1/NR2B/NR3B channels (Fig. 1D).

We then examined whether the anesthetic sensitivities of NMDA receptors were affected by co-expression of the NR3B subunit. The dissociative anesthetic ketamine, which is a channel blocker for NMDA receptors, blocked the NR1/NR2B and NR1/NR2B/NR3B channels to similar degrees (Fig. 2A). The anesthetic concentrations of ketamine (~10 μM) (21) prominently inhibited the NR1/NR2B and NR1/NR2B/NR3B channels. The IC_{50} value (μM) of NR1/NR2B/NR3B channels for ketamine was not significantly different from that of NR1/NR2B channels. The volatile anesthetic isoflurane inhibited both the NR1/NR2B and NR1/NR2B/NR3B channels. The extents of inhibition by twofold and fivefold the minimum alveolar concentration (MAC) of isoflurane (0.5 and 1.3 mM, respectively) were not significantly different between the NR1/NR2B and NR1/NR2B/NR3B channels (Fig. 2B). Similarly, the extents of inhibition by the

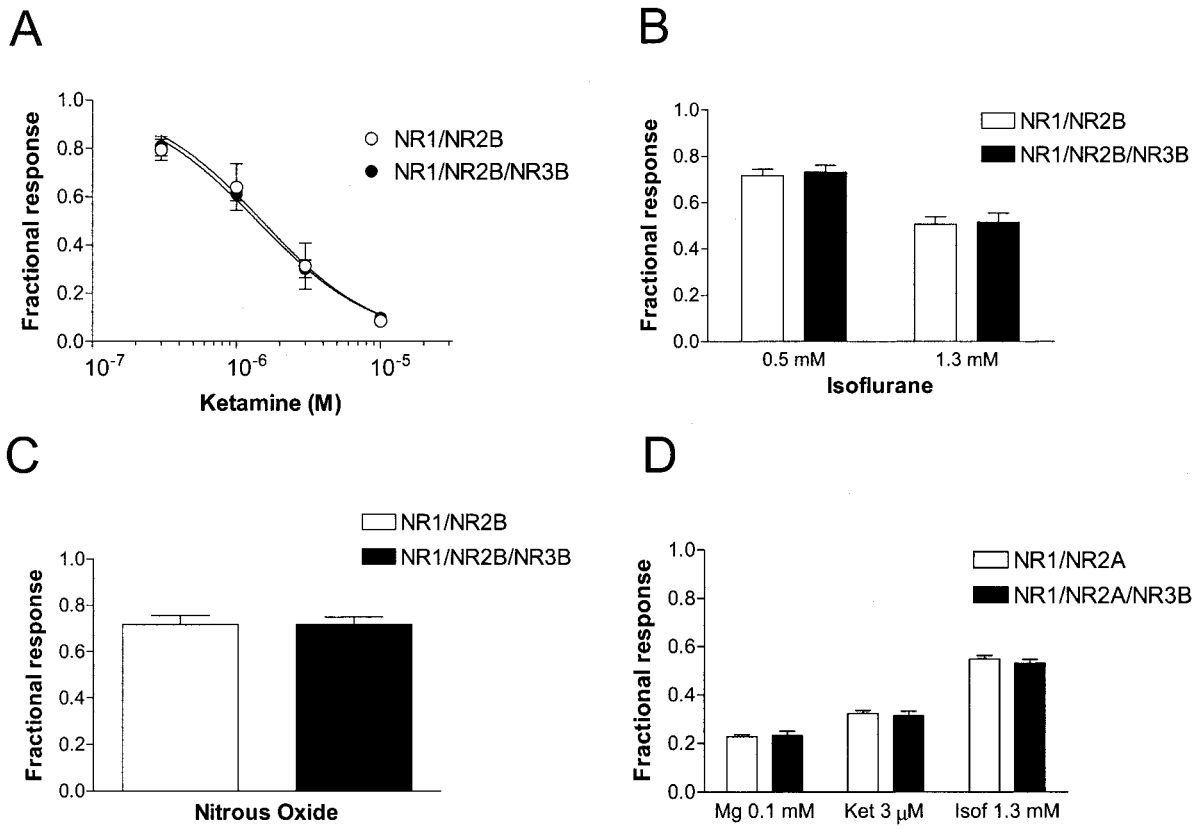

Figure 2. Effects of co-expression of the NR3B subunit on the anesthetic sensitivities of NR1/NR2 channels. A, Effect of the NR3B subunit on the dose-inhibition relationship of NR1/NR2B channels for ketamine. The IC_{50} values (μM) of the NR1/NR2B and NR1/NR2B/NR3B channels for ketamine are 2.2 ± 0.9 and 1.8 ± 0. 3, respectively, and the Hill coefficient values are 1.7 ± 0.3 and 1.3 ± 0.4, respectively (n = 4–7). B, Effect of the NR3B subunit on the sensitivity of NR1/NR2B channels to isoflurane. The effects of 0.5 and 1.3 mM isoflurane (2 and 5 MAC, respectively) on the NR1/NR2B and NR1/NR2B/NR3B channels were measured in 5 oocytes. C, Effect of the NR3B subunit on the sensitivity of NR1/NR2B channels to nitrous oxide. The effects of nitrous oxide (~0.6 atmosphere) on the NR1/NR2B and NR1/NR2B/NR3B channels were measured in 9 oocytes. D, Effect of the NR3B subunit on the inhibition of NR1/NR2A channels by blockers. The effects of 0.1 mM Mg^{2+} (Mg 0.1 mM), 3 μM ketamine (Ket 3 μM), and 1.3 mM isoflurane (Isof 1.3 mM) on the NR1/NR2A and NR1/NR2A/NR3B channels were measured in 3–7 oocytes.

gaseous anesthetic nitrous oxide (~0.6 atmosphere) was not significantly different between the NR1/NR2B and NR1/NR2B/NR3B channels (Fig. 2C). The extent of inhibition (%) of NR1/NR2B channels by 200 mM ethanol was not significantly different from that of NR1/NR2B/NR3B channels (59 ± 4 [n = 5] and 59 ± 2 [n = 4], respectively).

We also tested combinations with the NR2A subunit because this subunit has been reported to be expressed in the ventral horn in the adult mouse cervical cord (16), where the NR3B subunit is also expressed (10,12). The EC_{50} value (μM) of NR1/NR2A/NR3B channels for L-glutamate in the presence of 10 μM glycine was not significantly different from that of NR1/NR2A channels (4.2 ± 0.6 [n = 6] and 4.4 ± 0.8 [n = 5], respectively). The EC_{50} value (μM) of NR1/NR2A/NR3B channels for glycine in the presence of 10 μM L-glutamate was not significantly different from that of NR1/NR2A channels (2.4 ± 0.3 [n = 4] and 2.2 ± 0.1 [n = 5], respectively). The extent of inhibition of NR1/NR2A/NR3B channels by 0.1 mM Mg^{2+}, 3 μM ketamine and 1.3 mM (5 MAC) isoflurane was not significantly different from those of NR1/NR2A channels (Fig. 2D).

The NR1 and NR2 subunits possess conserved asparagine (N) residues at the N site in the channel-lining segment M2 (6) (Fig. 3A). The NR2 subunits also have an asparagine residue at the N + 1 site (one position downstream of the carboxyl-terminal side of the N site). Site-directed mutagenesis has revealed that the asparagine residue at the N site of the NR1 subunit and the adjacent asparagine residues at the N and N + 1 sites of the NR2 subunits contribute to the blocking sites for Mg^{2+} and dissociative anesthetics (6). To investigate whether the corresponding positions in the NR3B subunit were involved in determining anesthetic sensitivities, we examined the effects of replacing the glycine (G) and arginine (R) residues at the corresponding N and N + 1 sites of the NR3B subunit with asparagine (N) residues (producing the

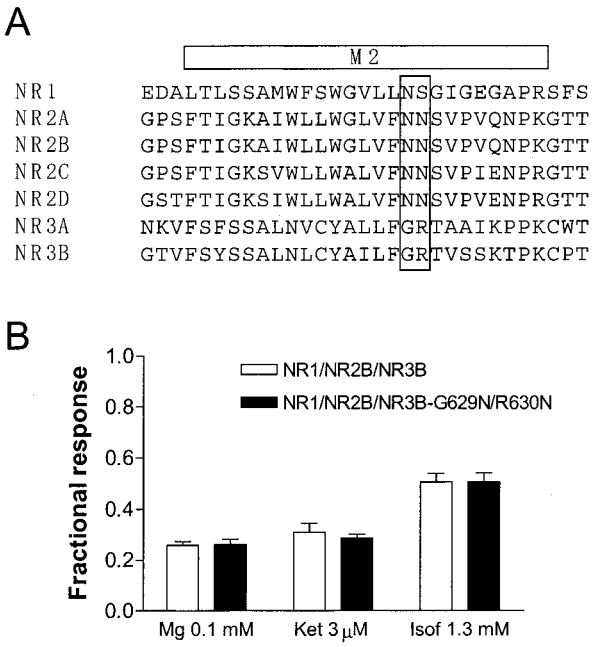

Figure 3. Effects of the NR3B-G629N/R630N mutant subunit on the sensitivities of NR1/NR2B/NR3B channels to Mg^{2+}, ketamine and isoflurane. A, Aligned amino acid sequences around segment M2 of the mouse NR1, NR2 and NR3 subunits (except for the NR3A subunit which is the rat sequence). The channel-lining segment M2 is indicated by the rectangle at the top. The amino acid residues at the N and N + 1 sites are boxed. B, Effects of 0.1 mM Mg^{2+} (Mg 0.1 mM), 3 μM ketamine (Ket 3 μM), and 1.3 mM isoflurane (Isof 1.3 mM) on NR1/NR2B/NR3B and NR1/NR2B/NR3B-G629N/R630N channels measured in 3–6 oocytes.

mutant NR3B-G629N/R630N) on the anesthetic sensitivities. Co-expression of the NR3B-G629N/R630N mutant subunit reduced the current amplitude of NR1/NR2 channels similarly to the wild-type NR3B subunit. The NR3B-G629N/R630N mutant did not significantly affect the extent of inhibition of NR1/NR2B/NR3B channels by 0.1 mM Mg^{2+}, 3 μM ketamine, and 1.3 mM (5 MAC) isoflurane (Fig. 3B).

Discussion

Previous studies using the specific antibodies and the fluorescent markers showed that the expression levels of NR1 and NR2 subunit proteins were not affected by co-expression of the NR3 subunits (8,10). Furthermore, the electrophysiological studies demonstrated that the NR3 subunits altered single-channel properties and Ca^{2+}-permeability of NMDA receptors (9,11–13). These findings suggest that the NR3 subunits do not prevent the expression of NR1/NR2 channels but co-assemble with NR1 and NR2 subunits to form a receptor complex with distinct channel properties. Because the anesthetic sensitivities of recombinant ligand-gated ion channels sometimes vary considerably, even among closely related subunits/receptors (2), we examined whether co-expression of the NR3B subunit could alter the sensitivity of NMDA receptors to general anesthetics. Although the NR3B subunit prominently reduced the current amplitude of NR1/NR2 channels, the sensitivities of the channels to ketamine, isoflurane, nitrous oxide, and ethanol were not altered. The ethanol sensitivities of NR1/NR2A-D channels were reported to remain unaltered by co-expression of the NR3A subunit (22). Because the NR3A and NR3B subunits share many functional properties such as reducing the current amplitude and Ca^{2+}-permeability and affecting the cell surface expression of NMDA receptors (7–13), the effects on the anesthetic sensitivities of NMDA receptors may be conserved between the NR3A and NR3B subunits. We previously showed that the sensitivity of NMDA receptors to ketamine varies only slightly among the NR2A-D subunits (19). Furthermore, the sensitivities to volatile anesthetics (e.g., isoflurane, desflurane, sevoflurane) were reported to be similar between the NR2A and NR2B subunits (23). Thus, the anesthetic sensitivity of NMDA receptors may not be appreciably altered by their subunit composition.

NR3A or NR3B subunits were reported to co-assemble with the NR1 subunit to form unique excitatory glycine receptors in *Xenopus* oocytes (24). However, we did not observe any channel activity in response to glycine in the oocytes injected with the NR1 and NR3B subunit mRNAs. Our results are consistent with previous reports on the NR3A and NR3B subunits in various recording systems (10,12,22). Although NR3 subunits may be involved in the formation of excitatory glycine receptors under certain experimental conditions, a recent study in heterologous cells and neurons argues against this possibility (13).

The sensitivity of NR1/NR2 channels to Mg^{2+} blocking was not affected by the presence of the NR3B subunit. This result is consistent with previous reports on the NR3A and NR3B subunits (8,10). Conversely, NR3A or NR3B subunits were shown to reduce the Ca^{2+}-permeability of NMDA receptors (9,11–13). In NR1/NR2 channels, the Mg^{2+} blocking site is constituted by the adjacent asparagine residues at the N and N + 1 sites in the NR2 subunits, whereas the Ca^{2+}-permeability is determined by the asparagine residue at the N site in the NR1 subunit (25,26). Thus, the different contributions of the NR3 subunits to the Mg^{2+} blocking and Ca^{2+}-permeability may further support the notion that these two properties of NMDA receptors are regulated by distinct molecular determinants.

Although co-expression of the NR3B subunit did not alter the effects of Mg^{2+} and channel blockers on NMDA receptors, it does not necessarily follow that this subunit is not involved in the constitution of sites for channel blockers. It is possible that co-assembly of the NR3B subunit with the NR1 and NR2 subunits

results in the formation of blocking sites with sensitivity to channel blockers similar to that of NR1/NR2 channels. A previous report showed that mutation of the arginine at the N + 1 site in the NR3A subunit did not change the Mg^{2+} blocking of NR1/NR2B/NR3A channels (8). In the present study, mutation of the amino acid residues at the N and N + 1 sites in the NR3B subunit did not affect the sensitivities to Mg^{2+}, ketamine and isoflurane. Thus, the amino acid residues at the N and N + 1 sites in NR3 subunits are unlikely to be involved in the formation of the blocking sites of NMDA receptors. However, there is still a possibility that other amino acid residues located in the channel-lining regions of the NR3 subunits are involved in the formation of the channel blocking sites of NR1/NR2/NR3 channels.

The authors thank Drs. Maya Yamazaki and Manabu Abe (Department of Cellular Neurobiology, Brain Research Institute, Niigata University) for their assistance in the cDNA cloning of the NR3B subunit.

References

1. Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature 1994;367:607–14.
2. Yamakura T, Bertaccini E, Trudell JR, Harris RA. Anesthetics and ion channels: molecular models and sites of action. Annu Rev Pharmacol Toxicol 2001;41:23–51.
3. Anis NA, Berry SC, Burton NR, Lodge D. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by *N*-methyl-aspartate. Br J Pharmacol 1983;79:565–75.
4. Jevtovic-Todorovic V, Todorovic SM, Mennerick S, et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. Nature Med 1998;4:460–3.
5. Franks NP, Dickinson R, de Sousa SLM, et al. How does xenon produce anaesthesia? Nature 1998;396:324.
6. Yamakura T, Shimoji K. Subunit- and site-specific pharmacology of the NMDA receptor channel. Prog Neurobiol 1999;59:279–98.
7. Ciabarra AM, Sullivan JM, Gahn LG, et al. Cloning and characterization of χ-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. J Neurosci 1995;15:6498–508.
8. Sucher NJ, Akbarian S, Chi CL, et al. Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. J Neurosci 1995;15:6509–20.
9. Das S, Sasaki YF, Rothe T, et al. Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. Nature 1998;393:377–81.
10. Nishi M, Hinds H, Lu HP, et al. Motoneuron-specific expression of NR3B, a novel NMDA-type glutamate receptor subunit that works in a dominant-negative manner. J Neurosci 2001;21:RC185.
11. Perez-Otano I, Schulteis CT, Contractor A, et al. Assembly with the NR1 subunit is required for surface expression of NR3A-containing NMDA receptors. J Neurosci 2001;21:1228–37.
12. Matsuda K, Kamiya Y, Matsuda S, Yuzaki M. Cloning and characterization of a novel NMDA receptor subunit NR3B: a dominant subunit that reduces calcium permeability. Mol Brain Res 2002;100:43–52.
13. Matsuda K, Fletcher M, Kamiya Y, Yuzaki M. Specific assembly with the NMDA receptor 3B subunit controls surface expression and calcium permeability of NMDA receptors. J Neurosci 2003;23:10064–73.
14. Stabernack C, Sonner JM, Laster M, et al. Spinal *N*-methyl-D-aspartate receptors may contribute to the immobilizing action of isoflurane. Anesth Analg 2003;96:102–7.
15. Sonner JM, Antognini JF, Dutton RC, et al. Inhaled anesthetics and immobility: Mechanisms, mysteries, and minimum alveolar anesthetic concentration. Anesth Analg 2003;97:718–40.
16. Watanabe M, Mishina M, Inoue Y. Distinct spatiotemporal distributions of the *N*-methyl-D-aspartate receptor channel subunit mRNAs in the mouse cervical cord. J Comp Neurol 1994;345:314–9.
17. Petrenko AB, Yamakura T, Baba H, Sakimura K. Unaltered pain-related behavior in mice lacking NMDA receptor GluRε1 subunit. Neurosci Res 2003;46:199–204.
18. Amaya E, Musci TJ, Kirschner MW. Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in Xenopus embryos. Cell 1991;66:257–70.
19. Yamakura T, Mori H, Masaki H, et al. Different sensitivities of NMDA receptor channel subtypes to non-competitive antagonists. Neuroreport 1993;4:687–90.
20. Yamakura T, Harris RA. Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels: comparison with isoflurane and ethanol. Anesthesiology 2000;93:1095–101.
21. Krasowski MD, Harrison NL. General anaesthetic actions on ligand-gated ion channels. Cell Mol Life Sci 1999;55:1278–303.
22. Smothers CT, Woodward JJ. Effect of the NR3 subunit on ethanol inhibition of recombinant NMDA receptors. Brain Res 2003;987:117–21.
23. Hollmann MW, Liu HT, Hoenemann CW, et al. Modulation of NMDA receptor function by ketamine and magnesium. Part II: interactions with volatile anesthetics. Anesth Analg 2001;92:1182–91.
24. Chatterton JE, Awobuluyi M, Premkumar LS, et al. Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. Nature 2002;415:793–8.
25. Wollmuth LP, Kuner T, Sakmann B. Adjacent asparagines in the NR2-subunit of the NMDA receptor channel control the voltage-dependent block by extracellular Mg^{2+}. J Physiol (Lond) 1998;506:13–32.
26. Burnashev N, Schoepfer R, Monyer H, et al. Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. Science 1992;257:1415–9.