Full Paper

Corymine Potentiates NMDA-Induced Currents in *Xenopus* Oocytes Expressing NR1a/NR2B Glutamate Receptors

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Abstract. Previous studies demonstrated that corymine, an indole alkaloid isolated from the leaves of *Hunter zeylanica*, dose-dependently inhibited strychnine-sensitive glycine-induced currents. However, it is unclear whether this alkaloid can modulate the function of the *N*-methyl-D-aspartate (NMDA) receptor on which glycine acts as a co-agonist via strychnine-insensitive glycine binding sites. This study aimed to evaluate the effects of corymine on NR1a/NR2B NMDA receptors expressed in *Xenopus* oocytes using the two-electrode voltage clamp technique. Corymine significantly potentiated the NMDA-induced currents recorded from oocytes on days 3 and 4 after cRNA injection but it showed no effect when the current was recorded on days 5 and 6. The potentiating effect of corymine on NMDA-induced currents was induced in the presence of a low concentration of glycine ($\leq 0.1 \mu\text{M}$). Spermine significantly enhanced the potentiating effect of corymine observed in the oocytes on days 3 and 4, while the NMDA-receptor antagonist 2-amino-5-phosphonopentane (AP5) and the NMDA-channel blocker 5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine (MK-801) reversed the effect of corymine. On the other hand, the nonspecific chloride channel blocker 4,4-di-isothiocyanato stilbene-2,2-disulfonoc acid (DIDS) had no effect on the corymine potentiation of NMDA currents. There was no good correlation between corymine- and spermine-induced potentiation of the NMDA-current response in *Xenopus* oocytes. These results suggest that corymine potentiates the NMDA-induced currents by interacting with a site different from the spermine binding site.

Keywords: corymine, potentiating effect, NR1a/NR2B NMDA receptor, *Xenopus* oocyte

Introduction

Corymine is an indole alkaloid isolated from the leaves of *Hunteria zeylanica*, a plant native to Thailand. We previously demonstrated that this alkaloid *per se* did not induce convulsion but potentiated the convulsions induced by strychnine, a competitive glycine-receptor antagonist, and picrotoxin, a non-competitive γ -aminobutyric acid (GABA) receptor antagonist, in mice (1).

Moreover, in previous electrophysiological studies using a *Xenopus* oocyte receptor expression model, we have found that corymine inhibited preferentially strychnine-sensitive glycine-receptor function rather than GABA-receptor function, suggesting that the blockade of strychnine-sensitive glycine receptors mainly contributes to the convulsion potentiating effect of this alkaloid (2, 3).

Glycine acts not only as an inhibitory neurotransmitter for the strychnine-sensitive glycine receptor in the spinal cord but also as a co-agonist for the strychnine-

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insensitive glycine binding site of the NMDA receptor. Evidence indicates that intrathecal administration of glycine enhances, rather than inhibits, strychnine- and NMDA-induced convulsions by acting on the strychnine-insensitive glycine binding site of NMDA receptors (4). Thus, it is likely that the seizure-potentiating effect of corymine in the animal model may involve activation of the NMDA receptor.

The NMDA receptor plays important roles in the pathologies of the central nervous system (CNS) and is a target for several therapeutic agents, including NMDA agonists, antagonists, and modulators (5–9). Three NMDA-receptor subunit families (NR1a-h, NR2A-D, and NR3A-B) have been identified (10, 11). Native NMDA receptors in the mammalian CNS are thought to consist of tetrameric, heterooligomeric assemblies of NR1 subunits, which possesses the glycine binding site and mandates channel activity, and NR2 subunits, which possesses glutamate binding site and modulates functional and pharmacological properties of the NMDA-gated channel (12–15). Evidence indicates that different subunit combinations (or “subtypes”) have distinct biophysical and pharmacological characteristics (16–19), yielding potential for the development of NMDA-receptor-subtype-selective therapeutic agents (16). Of the NMDA-receptor subunits, the NR2B subunit is particularly interesting because it is involved in several CNS pathologies including acute and chronic pain, stroke, head trauma, drug-induced dyskinesias, and dementia in Alzheimer’s disease and Parkinson’s disease (20–23).

To further clarify the mechanism underlying the seizure-potentiating effect of corymine, in this study we investigated using a receptor expression model of *Xenopus* oocyte whether corymine can modulate the function of the NR1a/NR2B NMDA receptor.

Materials and Methods

NR1a/NR2B RNA preparation

cDNA clones of NR1a and NR2B were kindly provided by Dr. K. Igarashi (Faculty of Pharmaceutical Sciences, Chiba University). The circular cDNAs of NR1a and NR2B were linearized with NotI and EcoRI, respectively. The linearized NR1a and NR2B cDNAs were then transcribed into cRNAs in vitro with T7 and T3 RNA polymerase, respectively, using a mMessage mMachine transcription kit (Ambion, Austin, TX, USA). NR1a and NR2B cRNAs were diluted with nuclease-free water to approximately 0.5 $\mu\text{g}/\mu\text{l}$ of each and were mixed at a ratio of 1:4 before injection into *Xenopus* oocytes.

Oocyte injection

Xenopus laevis (*Xenopus* Express, Cape, South Africa) were anesthetized in ice-water, and a lobe of the ovary was dissected and placed in sterile modified Barth’s solution (MBS: 88 mM NaCl, 1 mM KCl, 0.41 mM CaCl_2 , 0.33 mM $\text{Ca}(\text{NO}_3)_2$, 0.82 mM MgSO_4 , 2.4 mM NaHCO_3 , 7.5 mM Tris-(hydroxymethyl)amino-methane, pH 7.6). Oocytes were then isolated manually and defolliculated by incubation in 1.5 mg/ml collagenase (type IA; Sigma, St. Louis, MO, USA) at 19°C for 1 h in calcium-free MBS solution. cRNA mixture (27.6 nl) was injected into oocytes, stage V-VI, with a microinjector (Drummond, Broomall, PA, USA). For expression, the oocytes were incubated in MBS containing 2.5 units/ml penicillin and 2.5 $\mu\text{g}/\text{ml}$ streptomycin at 18°C.

Electrophysiological recording

Responses to NMDA were recorded using a two-electrode voltage-clamp amplifier (GeneClamp 500B; Axon Instrument, Foster City, CA, USA) at a holding potential of -60 mV unless noted otherwise. Electrodes were filled with 3 M KCl and had resistances of 0.5–5 ΩM . Oocytes were positioned in a 50- μl chamber and continuously perfused with Mg^{2+} -free MBS solution at 1 ml/min at room temperature. The drugs were applied until a plateau or peak of the response was observed. Data were recorded and digitized for analysis (MacLab 200; ADInstruments, Castle Hill, NSW, Australia). The washout period for recovery was 3–5 min, depending on the concentration of drugs applied. Most data was expressed as the mean $\pm$ S.E.M. For statistical analysis, the Sigma Stat[®] (ver3.0) program was used. Data were subjected to the paired *t*-test when effects were compared between before and after drug application in the same oocytes or the unpaired *t*-test when the experiments were performed in different oocytes. ED_{50} values and correlation coefficients for curve fitting were determined using Prism[®] (ver.2.0) program.

Compounds

Corymine used in this study was isolated from the leaves of *Hunteria zeylanica* as previously described (2). NMDA, glycine, MK-801, DIDS, and spermine were from Sigma; and AP5 was from Tocris (Ellisville, MO, USA). In the experiment, corymine was dissolved in dimethylsulfoxide (DMSO). The final concentrations of DMSO used in this study was $\leq 0.1\%$. DMSO at this concentration did not induce membrane current or affect NMDA-induced currents in control experiments (24).

Results

Pharmacological properties of the NMDA receptors expressed in oocytes

We first characterized pharmacological properties of the NMDA receptor expressed in *Xenopus* oocytes. When NMDA containing 10 μ M glycine was bath-applied to oocytes injected with NR1a and NR2B cRNAs, inward currents were elicited at holding potentials of -100 to -20 mV and outward currents at holding potentials of $+20$ to $+100$ mV (Fig. 1A). A concentration-response curve for NMDA was made at a holding potential of -60 mV. The ED_{50} value was 54 ± 12.2 μ M (Fig. 1B). In further experiments, we used 30 μ M NMDA as a standard concentration (unless specified elsewhere).

The current response elicited by 30 μ M NMDA plus 10 μ M glycine was dose-dependently and significantly reduced by the competitive antagonist AP5 (Fig. 1C) and the noncompetitive antagonist MK-801 (Fig. 1D).

Spermine (100 μ M), a NMDA modulator, significantly enhanced the NMDA response at the glycine concentrations of 0.1 and 1 μ M but not 10 μ M (Fig. 1E).

Potentiating effect of corymine on the NMDA-induced currents depends on glycine concentration

To investigate the effect of corymine on the expressed NMDA receptors, 30 μ M NMDA plus various concentrations of glycine (0.01 – 10 μ M) was applied to oocytes in the presence and absence of 100 μ M corymine. Corymine significantly produced a marked potentiation of NMDA-induced currents at low concentrations of glycine (0.01 – 0.1 μ M) but it had no potentiating effect at high concentrations (1 – 10 μ M) of glycine (Fig. 2: A and B). The potentiating effect of corymine disappeared immediately after washing-out of the alkaloid by Mg^{2+} -free MBS (Fig. 2A). The potentiating effect of corymine did not occur in any of the oocytes that responded to NMDA. Percentages of oocytes that showed corymine-induced potentiation of NMDA currents were 100 (4 of

Fig. 1. Pharmacological properties of the NMDA receptor expressed in *Xenopus* oocytes injected with NR1a and NR2B cRNAs. A) Tracings and I-V relationship show reverse potential of NMDA-induced currents at around 0 mV in an oocyte applied with 30 μ M NMDA plus 10 μ M glycine at various clamp potentials. B) A dose-response curve of NMDA relative to the maximal response elicited by 300 μ M NMDA. C) Dose-dependent inhibition of 30 μ M NMDA (plus 10 μ M glycine)-induced currents by AP5. Each datum represents the mean $\pm$ S.E.M. from 2–3 oocytes. D) Dose-dependent inhibition of 30 μ M NMDA (plus 10 μ M glycine)-induced currents by MK-801. Each data represents the mean $\pm$ S.E.M. from 3 oocytes. E) Potentiation of 30 μ M NMDA-induced currents at various glycine concentrations by 100 μ M spermine. Each data represents the mean $\pm$ S.E.M. from 4–10 oocytes. * P <0.05 and ** P <0.01, compared with control NMDA response.

Fig. 2. Potentiating effect of corymine on the NMDA-induced currents depends on glycine concentration. Oocytes injected with NR1a and NR2B cRNAs were applied with 30 μ M NMDA plus various concentrations of glycine (0.01 – 10 μ M) in the presence and absence of 100 μ M corymine. A) Typical NMDA-induced current responses in oocytes to which corymine was applied in the presence of different concentrations of glycine. B) Data are expressed as percentages of the control NMDA response. Each column shows the mean $\pm$ S.E.M. and each open circle shows data from individual oocytes. The ratio of the number of oocytes that exhibited corymine potentiation of NMDA response to the number of oocytes tested is indicated in each parenthesis. C) Dose-dependent potentiation of the 30 μ M NMDA plus 0.01 μ M glycine by corymine from oocytes of day 4 after cRNA injection. Data are expressed as the mean $\pm$ S.E.M. of percentages of the control NMDA response from 4 oocytes. * P <0.05 and ** P <0.01, significant difference from the control.

4), 56 (22 of 39), 54 (7 of 13), and 17 (8 of 46) in the presence of 0.01, 0.1, 1, and 10 μ M glycine, respectively (Fig. 2B). Corymine significantly potentiated the NMDA responses at 100 μ M but not at 10 μ M (Fig. 2C).

Potentiating effect of corymine on the NMDA-induced currents depends on incubation period of oocytes after cRNA injection

To clarify factors that are involved in the variation of corymine potentiation of the NMDA response, we elucidated the effects of corymine on the NMDA-induced currents elicited in oocytes on days 2 to 6 after cRNA injection. When applied with 30 μ M NMDA plus 0.1 μ M glycine, corymine markedly and significantly potentiated the NMDA response in oocytes recorded on day 3 after cRNA injection. The magnitude of corymine potentiation was gradually decreased after day 3, but the effect of corymine was still significant when examined on day 4 (Fig. 3A). Percentages of oocytes that showed corymine-induced potentiation of NMDA currents were 0 (0 of 3), 100 (5 of 5), 71 (12 of 17), 40 (4 of 10), and 20 (1 of 5) at days 2, 3, 4, 5, and 6 after cRNA injection, respectively. When 10 μ M glycine was used instead of 0.1 μ M, the potentiating effect of corymine was rarely observed during the experiment period of 6 days after cRNA injection (data not shown). Corymine (1 – 100

μ M) hardly potentiated NMDA responses in oocytes recorded on day 6 after cRNA injection, while spermine, a NMDA modulator, exhibited a marked and significant potentiation of the NMDA response in all oocytes tested (Fig. 3B). Percentages of oocytes that showed corymine-induced potentiation of NMDA currents were 0 (0 of 5), 0 (0 of 5), 20 (1 of 5), and 100 (5 of 5) for corymine at 1, 10, 100 μ M, and spermine, respectively.

Potentiating effect of corymine on the NMDA-induced currents was enhanced by spermine and antagonized by AP5 and MK-801

To study a possible mechanism by which corymine potentiates the NMDA currents, the effects of spermine and other antagonists (AP5, MK-801, and DIDS) on corymine potentiation were examined. After the NMDA response was potentiated by 100 μ M corymine, either spermine (100 μ M), AP5 (10 μ M), MK-801 (100 μ M), or DIDS (100 μ M) was co-applied and the changes of NMDA response were observed. Simultaneous application of corymine and spermine to oocytes caused a significant increase of the NMDA responses when compared to the response caused by corymine alone (Fig. 4: A and B). In contrast, the effect of corymine was significantly decreased by co-application of AP5 or MK-801, but not by DIDS. A linear regression analysis

Fig. 3. Potentiating effect of corymine on the NMDA-induced currents depends on the incubation period of oocytes after injection of NR1a and NR2B cRNAs. A) Potentiating effect of corymine was found from days 2 to 6 after injection of NR1a and NR2B cRNA. NMDA (30 μM) plus glycine (0.1 μM) was applied to oocytes in the presence and absence of 100 μM corymine. Data are expressed as the mean percentage of the control response ± S.E.M. from 3 – 17 oocytes. * $P < 0.05$ and ** $P < 0.01$, compared with control response; # $P < 0.05$ and ## $P < 0.01$, compared with the data obtained at day 3 after cRNA injection. B) Effect of corymine (1 – 100 μM) and spermine (100 μM) on NMDA response recorded in the presence of 0.1 μM glycine on day 6 after cRNA injection. Data are expressed as the mean percentage of control NMDA response ± S.E.M. from 5 oocytes. * $P < 0.05$ compared with control response, # $P < 0.05$ compared with 100 μM corymine. The ratio of the number of oocytes that exhibited corymine potentiation of the NMDA response to the number of oocytes tested is indicated in parentheses.

revealed a correlation coefficient (r^2) of 0.5251 between the magnitudes of the potentiating effects of corymine and spermine (Fig. 4C).

Discussion

The present study has demonstrated that corymine can potentiate NMDA-receptor function in the presence of low glycine concentrations in *Xenopus* oocytes injected with NR1a and NR2B cRNAs. The results suggested that the potentiating effect is mediated by an interaction of this alkaloid with a site other than the spermine binding site on the NMDA receptor.

In this study, corymine failed to activate the NMDA-receptor channel in the absence of glycine (data not shown), but it showed a marked enhancement of NMDA-induced currents in the presence of low glycine concentrations (≤ 0.1 μM). Moreover, the NMDA-response-potentiating effect of corymine disappeared immediately after washing out by MBS buffer. These findings indicate that corymine acts to reversibly and positively modulate the function of the NMDA receptor and that the effect of this alkaloid depends on the presence of glycine. Glycine concentration in the synaptic cleft of glutamatergic synapses is generally estimated to be lower than the K_D value (100 – 500 nM)

for high affinity glycine binding sites on the NMDA receptor (18, 25 – 28). Taken together, the present finding raises a possibility that corymine enhances small current responses elicited by NMDA-receptor stimulation at low extracellular glycine concentrations.

The effect of the alkaloid found in this study is quite similar to the action of spermine on the NMDA-receptor-mediated response since it has been demonstrated that positive modulation of NMDA-receptor function by spermine also depends on glycine concentration (29 – 32). This similarity allows us to infer that corymine, like spermine, may potentiate the NMDA response by increasing the affinity of glycine for the glycine site on the NMDA receptor (32 – 34). However, spermine potentiation of the NMDA response was invariably observed in all the oocytes tested (data not shown), whereas corymine did not show the NMDA response in every oocyte during a 6-day period of experiments. Moreover, a combination of corymine and spermine potentiated the NMDA-receptor-mediated current response in oocytes in an additive manner, but no good correlation was found between magnitudes of corymine- and spermine-induced potentiation of the NMDA response. Together, it is unlikely that corymine and spermine share the common binding site involved in positive modulation of the NMDA-receptor function.

Fig. 4. Potentiating effect of corymine on the NMDA-induced currents in oocytes injected with NR1a and NR2B cRNAs was enhanced by spermine and antagonized by AP5 and MK-801. Currents were elicited by application of 30 μ M NMDA plus 0.1 μ M glycine in the presence and absence of test drugs (100 μ M corymine, 100 μ M spermine, 10 μ M AP5, 100 μ M MK-801, or 100 μ M DIDS). A) Typical effects of test drugs on NMDA current in the presence of 0.1 μ M glycine. B) Effects of spermine, AP5, MK-801, and DIDS on corymine-induced potentiation of NMDA currents. * $P < 0.05$ and ** $P < 0.01$. C) Correlation between the magnitudes of potentiation caused by corymine and spermine. Each point indicates the data from individual oocytes ($n = 24$).

The exact mechanism underlying corymine-induced potentiation of the NMDA-receptor response remains unclear but there is a possibility that corymine apparently potentiates the NMDA-receptor function in *Xenopus* oocytes by activating the Ca^{2+} -dependent Cl^- channel because an increase in intracellular Ca^{2+} by NMDA reportedly induces Cl^- currents via Ca^{2+} -dependent Cl^- channels that are natively expressed on the oocyte membrane (35–38). However, this possibility seems to be very small since the NMDA-receptor antagonist AP5 and the NMDA-channel blocker MK-801 completely abolished the positive modulation of the NMDA-receptor function by corymine, whereas treatment of *Xenopus* oocytes with DIDS, a non-specific chloride-channel blocker, did not affect the effect of corymine.

The reason for the cell-to-cell and day-by-day vari-

ability of corymine-induced potentiation of the NMDA response is unclear. Speculative explanation for the variability is that it may be due to a difference in the expression between NR1a and NR2B NMDA-receptor subunits with different sensitivities to corymine in *Xenopus* oocytes, that is, corymine-sensitive and -insensitive NMDA-receptor subunit compositions. The corymine-sensitive NMDA-receptor subunit complexes may be not expressed to the same extent in all cells injected with NMDA-receptor subunit cRNAs or not stable enough to show the potentiating effect of corymine during the experiment period of 5–6 days after cRNA injection. In this study, each oocyte received cRNAs of NR1a and NR2B subunits at a constant ratio of 1:4 to avoid NR1a homomer formation (39). However, we can not exclude the possibility that the NR1a or NR2B homomers or both are generated (40, 41)

since spermine reportedly potentiates the function of NMDA receptors consisting of NR1 homomer and NR1/NR2B heteromers (32–34). Thus, it can be inferred that the corymine-sensitive NMDA receptors expressed in the oocytes may be either the NR1a homomer or the NR1/NR2B heteromer.

Evidence indicates that the NR1a/NR2B NMDA receptor is involved in learning and memory (22). Moreover, psychiatric disorders such as schizophrenia appear to implicate the NMDA-receptor hypofunction (42–44). Thus, the potentiation of NMDA-receptor-mediated responses by corymine may be beneficial for the treatment of diseases involving NMDA-receptor dysfunction.

In conclusion, corymine can positively modulate the NMDA-receptor function at low glycine concentrations probably via interacting with a site different from the spermine binding site. It is likely that the positive modulatory effect on the NMDA receptor involved in corymine-induced potentiation of strychnine- and picrotoxin-induced convulsion in mice (1). However, further investigation is needed to better understand the mechanism underlying corymine potentiation of the NMDA-receptor function.

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References

- 1 Leewanich P, Tohda M, Matsumoto K, Subhadhirasakul S, Takayama H, Aimi N, et al. Behavioral studies on alkaloids extracted from the laeves of *Hunteria zeylanica*. *Biol Pharm Bull*. 1996;19:394–399.
- 2 Leewanich P, Tohda M, Matsumoto K, Subhadhirasakul S, Takayama H, Aimi N, et al. Inhibitory effects of corymine, an alkaloidal component from the laeves of *Hunteria zeylanica*, on glycine receptor expressed in *Xenopus* oocytes. *Eur J Pharmacol*. 1997;332:321–326.
- 3 Leewanich P, Tohda M, Matsumoto K, Subhadhirasakul S, Takayama H, Aimi N, et al. A possible mechanism underlying corymine inhibition of glycine-induced chloride current in *Xenopus* oocytes. *Eur J Pharmacol*. 1998;348:271–277.
- 4 Larson AA, Beitz AJ. Glycine potentiates strychnine-induced convulsions: role of NMDA receptors. *J Neurosci*. 1988;8:3822–3826.
- 5 Williams K. Ifenprodil, a novel NMDA receptor antagonist: site and mechanism of action. *Curr Drug Targets*. 2001;2:285–298.
- 6 Koch HJ, Szecey A, Haen E. NMDA-antagonism (Memantine): an alternative pharmacological therapeutic principle in Alzheimer's and vascular dementia. *Curr Pharm Des*. 2004;10:253–259.
- 7 Tamminga C. Schizophrenia and glutamatergic transmission. *Crit Rev Neurobiol*. 1998;12:21–36.
- 8 Heresco-Levy U, Ermilov M, Shimoni J, Shapira B, Silipo G, Javitt DC. Placebo-controlled trial of D-cycloserine added to conventional neuroleptics, olanzapine, or risperidone in schizophrenia. *Am J Psychiatr*. 2002;159:480–482.
- 9 Schwartz BL, Hashtroudi S, Herting RL, Schwartz P, Deutsch SI. D-cycloserine enhances implicit memory in Alzheimer patients. *Neurology*. 1996;46:420–424.
- 10 Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. *Pharmacol Rev*. 1999;51:7–61.
- 11 Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, et al. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science*. 1992;256:1217–1221.
- 12 Laube B, Kuhse J, Betz H. Evidence for a tetrameric structure of recombinant NMDA receptors. *J Neurosci*. 1998;18:2954–2961.
- 13 Hirai H, Kirsh J, Laube B, Betz H, Kuhse J. The glycine binding site of the N-methyl-D-aspartate receptor subunit NR1: identification of novel determinants of co-agonist potentiation in the extracellular M3-M4 loop region. *Proc Natl Acad Sci USA*. 1996;93:6031–6036.
- 14 Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M, et al. Molecular characterization of the family of the N-methyl-D-aspartate receptor subunits. *J Biol Chem*. 1993;268:2836–2843.
- 15 Monaghan DT, Larsen H. NR1 and NR2 subunit contributions to N-Methyl-D-aspartate receptor channel blocker pharmacology. *J Pharmacol Exp Ther*. 1997;280:614–620.
- 16 Williams K. Ifenprodil discriminates subtypes of the N-methyl-D-aspartate receptor: selectivity and mechanisms at recombinant heteromeric receptors. *Mol Pharmacol*. 1993;44:851–859.
- 17 Hollmann M, Heinemann S. Cloned glutamate receptors. *Ann Rev Neurosci*. 1994;17:31–108.
- 18 Priestley T, Laughton P, Myers J, Le Bourdelles B, Kerby J, Whiting PJ. Pharmacological properties of recombinant human N-methyl-D-aspartate receptors comprising NR1a/NR2A and NR1a/NR2B subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Mol Pharmacol*. 1995;48:841–848.
- 19 Woodward RM, Huettner JE, Guastella J, Keana JF, Weber E. In vitro pharmacology of ACEA-1021 and ACEA-1031: systemically active quinoxalinediones with high affinity and selectivity for N-methyl-D-aspartate receptor glycine sites. *Mol Pharmacol*. 1995;47:568–581.
- 20 Chazot PL. The NMDA receptor NR2B subunit: a valid therapeutic target for multiple CNS pathologies. *Curr Med Chem*. 2004;11:389–396.
- 21 Myhrer T. Adverse psychological impact, glutamatergic dysfunction, and risk factors for Alzheimer's disease. *Neurosci Biobehav Rev*. 1998;23:131–139.
- 22 Tang YP, Wang H, Feng R, Kyin M, Tsien JZ. Differential effects of enrichment on learning and memory function in NR2B transgenic mice. *Neuropharmacology*. 2001;41:779–790.
- 23 Blanchet PJ, Konitsiotis S, Whittemore ER, Zhou ZL, Woodward RM, Chase TN. Differing effects of N-methyl-D-aspartate receptor subtype selective antagonists on dyskinesias

- in levodopa-treated 1-methyl-4-phenyl-tetrahydropyridine monkeys. *J Pharmacol Exp Ther*. 1999;290:1034–1040.
- 24 Blank T, Nijholt I, Teichert U, Kugler H, Behrsing H, Fienberg A, et al. The phosphoprotein DARPP-32 mediates cAMP-dependent potentiation of striatal N-methyl-D-aspartate responses. *Proc Natl Acad Sci USA*. 1997;94:14859–14864.
 - 25 Wilcox KS, Fitzsimonds RM, Johnson B, Dichter MA. Glycine regulation of synaptic NMDA receptors in hippocampal neurons. *J Neurophysiol*. 1996;76:3415–3424.
 - 26 Supplisson S, Bergman C. Control of NMDA receptor activation by a glycine transporter co-expressed in *Xenopus* oocytes. *J Neurosci*. 1997;17:4580–4590.
 - 27 Buller AL, Larson HC, Schneider BE, Beaton JA, Morrisett RA, Monaghan DT. The molecular basis of NMDA receptor subtypes: native receptor diversity is predicted by subunit composition. *J Neurosci*. 1994;14:5471–5484.
 - 28 Kew JN, Richards JG, Mutel V, Kemp JA. Development changes in NMDA receptor glycine affinity and ifenprodil sensitivity reveal three distinct populations of NMDA receptors in individual rat cortical neurons. *J Neurosci*. 1998;18:1935–1943.
 - 29 Masuko T, Kuno T, Kashiwagi K, Kusama T, Williams K, Igarashi K. Stimulatory and inhibitory properties of aminoglycoside antibiotics at N-methyl-D-aspartate receptors. *J Pharmacol Exp Ther*. 1999;290:1026–1033.
 - 30 Durand GM, Bennett MV, Zukin RS. Splice variants of the N-methyl-D-aspartate receptor NR1 identify domains involved in regulation by polyamines and protein kinase C. *Proc Natl Acad Sci USA*. 1993;90:6731–6735.
 - 31 Durand GM, Gregor P, Zheng X, Bennett MV, Uhl GR, Zukin RS. Cloning of an apparent splice variant of the rat N-methyl-D-aspartate receptor NMDAR1 with altered sensitivity to polyamines and activators of protein kinase C. *Proc Natl Acad Sci USA*. 1992;89:9359–9363.
 - 32 Benveniste M, Mayer ML. Multiple effect of spermine on N-methyl-d-aspartic acid receptor responses of rat cultured hippocampal neurons. *J Physiol (Lond)*. 1993;464:131–163.
 - 33 McGurk FJ, Bennett VLM, Zukin SR. Polyamines potentiate response of N-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. *Neurobiology*. 1990;87:9971–9974.
 - 34 Zhang L, Zheng X, Paupard MC, Wang AP, Santchi L, Friedman LK, et al. Spermine potentiation of recombinant N-methyl-D-aspartate receptors is affected by subunit composition. *Proc Natl Acad Sci USA*. 1994;91:10883–10887.
 - 35 Leonard JP, Kelso SR. Apparent desensitization of NMDA responses in *Xenopus* oocytes involves calcium-dependent chloride current. *Neuron*. 1990;4:53–60.
 - 36 Miledi R, Parker I. Chloride current induced by injection of calcium into *Xenopus* oocytes. *J Physiol (Lond)*. 1984;357:173–183.
 - 37 Weber WM, Liebold KM, Reifarth FW, Uhr U, Clauss W. Influence of extracellular Ca^{2+} on endogenous Cl^{-} channels in *Xenopus* oocytes. *Pfluegers Archiv*. 1995;429:820–824.
 - 38 Weber WM, Liebold KM, Reifarth FW, Uhr U, Clauss W. The Ca^{2+} -induced leak current in *Xenopus* oocytes is indeed mediated through a Cl^{-} channel. *J Memb Biol*. 1995;148:263–275.
 - 39 Kleckner NW, Glazewski JC, Chen CC, Moscrip TD. Subtype-selective antagonism of N-methyl-D-aspartate receptors by felbamate: insights into the mechanism of action. *J Pharmacol Exp Ther*. 1999;289:886–894.
 - 40 McIlhinney RA, Philipps E, Le Bourdelles B, Grimwood S, Wafford K, Sandhu S, et al. Assembly of N-methyl-D-aspartate (NMDA) receptors. *Biochem Soc Trans*. 2003;31:865–868.
 - 41 Garcia-Gallo M, Renart J, Diaz-Guerra M. The NR1 subunit of the N-methyl-D-aspartate receptor can be efficiently expressed alone in the cell surface of mammalian cells and is required for the transport of the NR2A subunit. *Biochem J*. 2001;356:539–547.
 - 42 Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry*. 1995;52:998–1007.
 - 43 Javitt DC, Zylberman I, Zukin SR, Heresco-Levy U, Lindenmayer JP. Amelioration of negative symptoms in schizophrenia by glycine. *Am J Psychiatry*. 1994;151:1234–1236.
 - 44 Mohn AR, Gainetdinov RR, Caron MG, Koller BH. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell*. 1999;98:427–436.