Design and Synthesis of a Novel Water-Soluble NMDA Receptor Antagonist with a 1,4,7,10-Tetraazacyclododecane Group

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Polyamines, especially spermine, inhibit N-methyl-D-aspartate (NMDA) receptors as open channel blockers. Two types of water-soluble NMDA receptor antagonist, ACCn (1) and TGCn (2), with a 1,4,7,10-tetraazacyclododecane cyclic polyamine group, were synthesized and the effects of both compounds on NMDA receptors were studied using voltage-clamp recordings of recombinant NMDA receptors expressed in Xenopus oocytes. These compounds inhibited macroscopic currents in both NR1/NR2A and NR1/NR2B receptor subtypes in oocytes voltage-clamped at −70 mV. Inhibition by the compounds of NR1/NR2A receptors were more prominent than that of NR1/NR2B receptors. The inhibitory effects of ACCn (1) on both NMDA receptors were more potent than those of TGCn (2).

Key words N-methyl-D-aspartate (NMDA) receptor; 1,4,7,10-tetraazacyclododecane; Xenopus oocytes

Glutamate receptors are classified into two major groups termed ionotropic and metabotropic glutamate receptors. The ionotropic receptors can be subdivided into three distinct types of receptors, the receptors for N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionoate (AMPA), and kainate, all of which contain glutamate-gated, cation-specific ion channels. Among ionotropic receptors, the NMDA receptor subtype has been found to play a key role in glutamate effects promoting synaptic plasticity, long-term potentiation, and neuronal cell death.1 The NMDA receptor combines to form heteromeric complexes containing NR1 and NR2 subunits. The NR1 subunit is ubiquitous and assembles with a second family of subunits termed NR2, including NR2A, NR2B, NR2C and NR2D.

Overactivation of these receptors can lead to neuronal cell death, and the receptors also play a role in seizure activity. Thus NMDA receptors are potential targets for neuroprotective agents and anticonvulsants.2,3 NMDA receptors have a complex pharmacology and are targets for antagonists acting at the glutamate and glycine coagonist sites, at a large number of modulatory sites, and at sites within the ion channel of the receptor. A number of organic polycations, including the endogenous polyamines spermine and spermidine, are antagonists at native and recombinant NMDA receptors.4 In addition, a number of polyanine-conjugated spider and wasp toxins are more potent antagonists than spermine at glutamate receptors.5 These toxins, which include the philantoxins, argiotoxins, and α-agatoxins, are characterized structurally by the presence of an aromatic amino acid head group linked through a carbonamide bond to a polyamine tail such as spermine or a pentamine or hexamine.

In this paper, we report the syntheses of two novel water-soluble compounds ACCn (1) and TGCn (2) with two 1,4,7,10-tetraazacyclododecane groups to determine whether these compounds have a role as NMDA receptor antagonists (Fig. 1).

Results and Discussion

The host ACCn (1) was synthesized as shown in Chart 1. Treatment of 4,4′-dihydroxydiphenylmethane (3) with methyl bromoacetate in N,N-dimethylformamide (DMF) in the presence of K₂CO₃ furnished 4 in 93% yield, and then hydrolysis of the methyl ester 4 with 5% KOH in methanol provided di-carboxylic acid (5, 100%), which underwent smooth esterification upon treatment with N,N-dicyclohexylcarbodiimide (DCC) and pentafluorophenol to give the pentafluorophenyl ester 6 in 92% yield. The pentafluorophenyl ester function in compound (6) was converted into carboxamide (7, 98%) after treatment of pentafluorophenol ester with 25% NH₄OH in THF, followed sequentially by reduction with BH₃ to the corresponding primary amine (8, 85%), which was converted into 10 by treatment with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-carboxymethyl-4,7,10-tris(tert-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (9)6 Finally, deprotection of 10 with concentrated HCl in THF resulted in the desired compound ACCn (1) in 91% yield.

On the other hand, TGCn (2) was synthesized as follows (Chart 2). Treatment of pentafluorophenyl ester (11)7 with 4,7,10-tris(tert-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (12)8 in the presence of triethylamine (TEA) gave 13 in 96% yield, and removal of the tert-butoxycarbonyl groups of 13 by treatment with concentrated HCl in THF synthesized the desired compound TGCn (2) in quantitative yield.

The effects of ACCn (1) and TGCn (2) on NMDA receptors were studied using voltage-clamp recordings of recombinant NMDA receptors expressed in Xenopus oocytes. We measured the effects of ACCn (1) and TGCn (2) (10 μM) on responses to glutamate (10 μM, with 10 μM glycine) or GABA 10 μM at NR1/NR2A, NR1/NR2B, or GABAc (ρ-1)
receptors in oocytes voltage-clamped at −70 mV. Both ACCn (1) and TGCn (2) inhibited macroscopic currents at both NMDA receptor subtypes (Fig. 2), but not at GABAc (–1) receptors (data not shown). These results indicate that the compounds specifically block NMDA receptors. The inhibition by these compounds of NR1/NR2A receptors were more potent than those of NR1/NR2B receptors. An NMDA glutamate receptor subtype is thought to play a predominant role in triggering glutamate neurotoxicity in central nervous system, it is possible that ACCn (1), TGCn (2) or these derivatives have neuroprotective effects to glutamate neurotoxicity.

**Experimental**

Melting points were determined using the Yanagimoto melting point apparatus Yanaco MP and are uncorrected. 1H-NMR spectra were recorded on a JEOL JNM-LA400 spectrometer containing tetramethylsilane as the standard. Mass spectra (MS) were measured on a JEOL JMS-GC mate instrument. Adult female Xenopus laevis were chilled on ice, and the preparation and maintenance of oocytes were carried out as described previously. 9,10) Capped cRNAs were prepared from linearized cDNA templates using mMessage mMachine kits (Ambion, Austin, TX, U.S.A.). Oocytes were injected with NR1A and NR2 cRNAs at a ratio of 1 : 5 (0.2—4 ng of NR1A plus 1—20 ng of NR2). Macroscopic currents were recorded with a two-electrode voltage clamp using a Dual Electrode Voltage Clamp Amplifier CEZ-1250 (Nihon Koden, Tokyo, Japan). Electrodes were filled with KCl 3 M. Oocytes were continuously superfused (ca. 5 ml/min with Mg2+/free saline solution (NaCl 96 m M, KCl 2 mM, BaCl2 1.8 mm, HEPES 10 mm, pH 7.5). This solution contained BaCl2, rather than CaCl2, and, in most experiments, oocytes were injected with K+/-1,2-bis(2-aminophenoxy)ethane-N,N,N‘,N’-tetraacetic acid (BAPTA; 100 nl of 40 mM solution at pH 7.5) on the day of recording to eliminate Ca2+-activated Cl− currents.2,3) Glutamate and glycine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). BAPTA was purchased from Sigma (St. Louis, MO, U.S.A.).

4,4′-Bis(carboxymethoxy)diphenylmethane (5) A mixture of 4,4′-dihydroxydiphenylmethane (3) (1.0 g, 5 mmol), methyl bromoacetate (1.53 g, 10 mmol), and K2CO3 (1.38 g, 10 mmol) in DMF (20 ml) was refluxed for 2 h. After removal of the solvent under reduced pressure, the residue was dissolved in 100 ml of H2O. The solution was acidified to pH 1 with 10% HCl. The filtrate was extracted with EtOAc (50 ml×3), washed with brine, and dried over MgSO4. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel with EtOAc : CHCl3 (1 : 9) as an eluent to give 4 (1.6 g, 92%) as a colorless solid. An analytical sample was obtained by recrystallizing this material from EtOAc–hexane, yielding colorless needles, mp 51—52 °C. 1H-NMR (CDCl3) δ: 3.80 (6H, s); 3.85 (2H, s); 4.60 (4H, s); 6.82 (4H, d, J=8.8 Hz); 7.08 (4H, d, J=8.8 Hz). EI-MS m/z: 344 [M]+. HR-EI-MS m/z: 344.1256 [M]+ (Calcd for C19H20O6: 344.1259).

4,4′-Bis(carboxymethoxy)diphenylmethane (5) A mixture of 4 (1.19 g, 3.46 mmol) and 5 N KOH/MeOH (4 ml) in MeOH (40 ml) was refluxed for 2 h. After removal of the solvent under reduced pressure, the residue was dissolved in 100 ml of H2O. The solution was extracted with EtOAc (100 ml). The aqueous solution was acidified to pH 1 with 10% HCl.
and extracted with EtOAc (300 ml). TheEtOAc layer was washed with brine, dried over MgSO₄, and concentrated under reduced pressure to give 5 as a colorless powder (1.09 g, 100%). An analytical sample was obtained by recrystallizing this material from EtOAc–hexane, yielding colorless needles, mp 199–200 °C. ¹H-NMR (DMSO-d₆): δ: 3.79 (2H, s); 4.59 (4H, s); 6.80 (4H, d, J = 8.8 Hz); 7.10 (4H, d, J = 8.8 Hz); 12.90 (2H, s). EI-MS m/z: 316 [M⁺]. HR-FAB-MS m/z: 316.0944 [M⁺].

4.4'-Bis(pentafluorophenoxycarbonyl)benzophenone (6) A mixture of 5 (2.95 g, 9.3 mmol), pentafluorophenol (3.46 g, 18.8 mmol) and DCC (3.38 g, 18.8 mmol) in THF (100 ml) was stirred at room temperature for 24 h. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with CHCl₃:Methanol:25% NH₄OH (20:8:1) under reduced pressure to afford a pale yellow oil, which was purified by column chromatography on silica gel with CHCl₃:MeOH:25% NH₄OH (100:40:4) as an eluent to give 8 (243 mg, 85%) as a colorless amorphous powder that was used in the next step without further purification. ¹H-NMR (CDCl₃) δ: 2.99 (4H, t, J = 5.6 Hz); 3.82 (2H, s); 3.98 (4H, t, J = 5.6 Hz); 6.84 (4H, d, J = 8.4 Hz); 7.07 (4H, d, J = 8.4 Hz). FAB-MS m/z: 287 [M⁺+H⁺]. HR-FAB-MS m/z: 287.1757 [M⁺+H⁺]. (Calcd for C₂₂H₁₄F₁₀O₆: 397.3059). Anal. Calcd for C₂₂H₁₄F₁₀O₆: C, 53.73; H, 2.18. Found: C, 53.73; H, 2.14.

4.4'-Bis(carbamoylmethoxy)benzophenone (7) A solution of 6 (4.0 g, 6.17 mmol) in THF (30 ml) was added 25% NH₄OH (12 ml) at room temperature. After stirring for 12 h, saturated NaHCO₃ (200 ml) was added to the reaction mixture. The precipitate was collected by filtration, washed with H₂O, EtOH, and Et₂O, and dried under vacuum to give 7 (1.9 g, 98%) as a colorless powder which was used in the next step without further purification. mp 233–234 °C. ¹H-NMR (DMSO-d₆): δ: 3.80 (2H, s); 4.36 (4H, s); 6.85 (4H, d, J = 8.8 Hz); 7.11 (4H, d, J = 8.8 Hz); 7.32 (2H, s); 7.43 (2H, s). FAB-MS m/z: 315 [M⁺+H⁺]. HR-FAB-MS m/z: 315.1346 [M⁺+H⁺]. (Calcd for C₁₅H₁₄N₂O₄: 287.2877). Anal. Calcd for C₁₅H₁₄N₂O₄: C, 54.43; H, 4.28. Found: C, 54.46; H, 4.29.

4.4'-Bis(2-aminoethoxy)benzophenone (8) A mixture of 7 (314 mg, 1 mmol) and BH₃-DMS (1.16 ml, 12 mmol) in THF (12 ml) was stirred for 24 h at 80 °C under a N₂ atmosphere, then was cooled to room temperature. Six milliliters of 0.7 m hydrochloric–MeOH solution was added, and the mixture was refluxed for 0.5 h and evaporated under reduced pressure. The residue was basified with 25% NH₄OH. The mixture was added with CHCl₃, washed with brine, and dried over Na₂SO₄. Removal of the solvent under reduced pressure afforded a pale yellow oil, which was purified by column chromatography on silica gel with CHCl₃:MeOH:25% NH₄OH (123 mg, 0.43 mmol), 9 (456 mg, 0.86 mmol) and EDC (197 mg, 1.03 mmol) in CH₂Cl₂ (10 ml) was stirred at room temperature for 24 h. The reaction mixture was diluted with CHCl₃ (10 ml), washed with 2N NaOH, and dried over Na₂SO₄. The solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel with CHCl₃:MeOH (20:8). FAB-MS m/z: 111 [M⁺+H⁺]. HR-FAB-MS m/z: 111.8190 [M⁺+H⁺]. (Calcd for C₁₁H₁₁N₂O₂: 179.2182). Anal. Calcd for C₁₁H₁₁N₂O₂: C, 71.61; H, 6.92. Found: C, 71.51; H, 6.78; N, 9.52.

Fig. 2. Effects of ACCn and TGCn on NMDA Receptors at −70 mV
(a) Representative traces showing blocking by ACCn and TGCn 10 μM at NR1/NR2A and NR1/NR2B receptors at −70 mV. (b) Effects of ACCn and TGCn 10 μM were determined at NMDA receptors at −70 mV. Data are shown as percentage of control measured in the absence of the compounds. Values are mean ± S.E.M. from four oocytes.

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References
5) Jackson H., Usherwood P. N. R., Trends Neurosci., 11, 278–283
6) Joong W. J., Sang J. S., Chang E. Y., In S. H., Jung B. S., Junghun S., 


3068—3076 (1997).


10) Masuko T., Kuno T., Kashiwagi K., Kusama T., Williams K., Igarashi 