Mechanism of Action of Galantamine on N-Methyl-D-Aspartate Receptors in Rat Cortical Neurons

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ABSTRACT

Galantamine, a new Alzheimer’s drug approved in the United States, is known to inhibit acetylcholinesterase and potentiate acetylcholine-induced currents in brain neurons. However, because both cholinergic and N-methyl-D-aspartate (NMDA) systems are down-regulated in the brain of Alzheimer’s patients, we studied the effects of galantamine on NMDA receptors. NMDA-induced whole-cell currents were recorded from the rat multipolar cortical neurons in primary culture. NMDA currents recorded in Mg²⁺-free media without addition of glycine were reversibly potentiated by bath and U-tube applications of galantamine at 10 to 10,000 nM, showing a bell-shaped dose-response relationship. However, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate currents were not affected by galantamine. The maximum potentiation of NMDA currents to ~130% of the control was obtained at 1 μM galantamine. The potentiation was due to a shift of the NMDA dose-response curve in the direction of lower NMDA concentrations. Glycine at 1 to 3000 nM enhanced NMDA currents, and potentiation by 1 μM galantamine and 1 to 300 nM glycine was additive. The glycine site antagonist 7-chlorokynurenic acid did not prevent the galantamine action. These results suggested that galantamine did not interact with the glycine binding site. Experiments with various concentrations of Mg²⁺ indicated that galantamine did not affect the Mg²⁺ blocking site of the NMDA receptor. PKC was involved in galantamine potentiation of NMDA currents, but protein kinase A, Gα/Gβ proteins, and Gα proteins were not involved. Potentiation of the activity of NMDA receptors is deemed partially responsible for the improvement of cognition, learning, and memory in Alzheimer’s patients.

Because the neuronal nicotinic acetylcholine receptors (nACHRs) are known to be down-regulated in the brain of Alzheimer’s patients (Vidal and Changeux, 1996; Woodruff-Pak and Hinchliffe, 1997), one of the strategies for improving the cognitive activity of the patients would be to stimulate nACHRs. This can be accomplished by inhibiting acetylcholinesterase. In fact, four of five Alzheimer’s drugs that have been approved by the Food and Drug Administration for clinical use in the United States, tacrine, donepezil, rivastigmine, and galantamine, are anticholinesterases.

Clinical tests with Alzheimer’s patients and animal experiments have indicated the effectiveness of galantamine to improve cognition (Kroll et al., 1999; Barnes et al., 2000; Raskind et al., 2000; Tariot et al., 2000). Galantamine has been shown to act as an agonist generating single-channel currents in α-bungarotoxin-insensitive, αβ2-type nACHRs of cultured rat hippocampal neurons (Pereira et al., 1993) and in the chicken αβ2 receptors expressed in fibroblast (M10) cells (Pereira et al., 1994). However, galantamine failed to generate a detectable whole-cell current in M10 cells expressing the αβ2 receptors (Pereira et al., 1994). It potentiated ACh-induced whole-cell current in PC12 cells (α3-type AChRs), in hippocampal α7-type AChRs (Schrattenholz et al., 1996; Maelicke and Albuquerque, 2000), in αβ2 AChRs expressed in human embryonic kidney cells (Samochocki et al., 2003), and in α7-type AChRs in α7/5-HT₃ chimera (Maelicke et al., 2001). Galantamine was also found to potentiate nicotine-evoked increases in intracellular Ca²⁺ and [³H]noradrenaline release in SH-SY5Y cells (Dajas-Bailador et al., 2003). The potentiating action of galantamine on nicotinic receptors facilitated synaptic transmission in the brain (Santos et al., 2002).

In addition to nACHRs, glutamate receptors are also impaired in the brain of Alzheimer’s patients (Palmer, 2002). Changes in the glutamatergic system are known to cause cognitive impairments. For instance, the loss of perikarya of piramidal cells and of the midtemporal gyrus was found to correlate with the severity of dementia (Palmer and Gershon, 2002).
1990). In Alzheimer’s patients, glutamate-containing neurons are decreased (Greenamyre et al., 1987; Palmer and Gershon, 1990; Francis et al., 1993; Palmer, 1996). Memantine is a low-affinity noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor and has recently been approved by the Food and Drug Administration for treating moderate-to-severe Alzheimer’s disease patients (Tariot et al., 2004).

Drugs that modulate the NMDA receptor-mediated neural transmission by acting at the glycine site are potential therapeutic agents to treat memory deficits associated with aging and Alzheimer’s disease. Both the partial glycine site agonist d-cycloserine and the glycine prodrug milacemide facilitate memory in animal paradigms (Hanndelmann et al., 1989; Baxter et al., 1994) and have been tested as cognitive enhancers in patients with Alzheimer’s disease (Schwartz et al., 1991, 1996; Dysken et al., 1992).

Because NMDA receptors seem to play a crucial role in learning and memory, our working hypothesis is that one of the mechanisms of action of galantamine is to modulate the NMDA receptor functions. Thus, the cognitive function of patients with Alzheimer’s disease or with other forms of dementia who have reduced NMDA receptors may be improved by increasing the activity of NMDA receptors. We have indeed found in the present study that galantamine augments NMDA-evoked currents in rat cortical neurons. This action seems to be exerted via the PKC system.

Materials and Methods

Cell Preparations. Rat cortical neurons were isolated and cultured by a procedure slightly modified from that described previously (Marszałek and Naraishi, 1993). In brief, rat embryos were removed from a 17-day pregnant Sprague-Dawley rat under halothane anesthesia. Small wedges of frontal cortex were excised and subsequently incubated in phosphate buffer solution for 20 min at 37°C. This solution contained 154 mM NaCl, 1.05 mM KH₂PO₄, 3.0 mM Na₂HPO₄•7 H₂O, 0.25% (w/v) trypsin (type XI; Sigma-Aldrich, St. Louis, MO), pH 7.4, and with an osmolality of 287 mOsm. The digested tissue was then mechanically triturated by repeated passages through a Pasteur pipette, and the dissociated cells were suspended in neurobasal medium with B-27 supplement (Invitrogen, Carlsbad, CA) and 2 mM glutamine. The cells were added to 35-mm culture wells at a concentration of 100,000 cells/ml. Each well contained five 12-mm poly-L-lysine-coated coverslips overlaid with confluent glial that had been plated 2 to 4 weeks earlier. The cortical neuron/glial cultures were maintained in a humidified atmosphere of 90% air and 10% CO₂ at 34°C. Cells cultured for 3 to 7 weeks were used for experiments.

Solutions for Current Recording. The external solution for recording of the whole-cell currents contained 150 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂, 5.5 mM HEPES acid, 4.5 mM HEPES sodium, 10 mM d-glucose, pH 7.3, and the osmolality was adjusted to 300 mOsm with d-glucose. Tetrodotoxin (100 μM) was added to eliminate the voltage-gated sodium channel currents. ATP-gluconate (20 mM) was added to block the muscarine AChR currents. The internal pipette solution contained 140 mM potassium gluconate, 2 mM MgCl₂, 1 mM CaCl₂, 10 mM HEPES acid, 10 mM EGTA, 2 mM ATP-Mg²⁺, and 0.2 mM GTP-Na⁺. The pH was adjusted to 7.3 with KOH and the osmolality was adjusted to 300 mOsm by adding d-glucose.

Whole-Cell Current Recording. Ionic currents were recorded using the whole-cell patch-clamp technique at room temperature (21–22°C). Pipette electrodes were made from 1.5-mm (outer diameter) borosilicate glass capillary tubes with a resistance of 2 to 3 MΩ when filled with the standard internal solution. The membrane potential was clamped at −70 mV unless otherwise stated. We allowed 5 to 10 min after membrane rupture for the cell interior to adequately equilibrate with the pipette solution. Currents through the electrode were recorded with an Axopatch-1C amplifier (Axon Instruments Inc., Union City, CA), filtered at 2 kHz, and sampled at 10 kHz in a PC-based data acquisition system that also provided preliminary data analysis. Results are expressed as mean ± S.E.M., and n represents the number of the cells examined.

Drug Applications. Two methods for drug application were used: one was perfusion through the bath and the other was via a U-tube controlled by a computer-operated magnetic valve system (Marszałek and Naraishi, 1993). In the fast U-tube application system, when one of the valves was open, the drug solution was allowed to bypass the chamber. When it was closed, the drug solution was ejected through the hole of the U-tube, which was located close to the cell. At the same time, another valve controlling the suction tube was opened, allowing a laminated flow of the test solution across the recording cell such that the external solution surrounding the cell could be completely changed with the drug solution within 30 to 40 ms. In some cases, test drugs were also added to the external solution and continuously perfused to the recording chamber via a glass syringe and Teflon tube.

Chemicals. NMDA (Sigma-Aldrich), AMPA (Sigma/RBI, Natick, MA), kainate (Sigma/RBI), glycine (Sigma/RBI), and 7-chlorokynurenic acid (7-CKN) (Sigma/RBI) were first dissolved in distilled water to make stock solutions. The GABA₅ receptor inhibitor pertussis toxin, the G protein stimulator cholera toxin, the voltage-gated sodium channel blocker tetrodotoxin, the muscarine AChR blocker atropine sulfate, and the PKC inhibitor chelerythrine chloride were purchased from Sigma-Aldrich. The PKA inhibitor H-89 was obtained from Calbiochem-Novabiochem (La Jolla, CA). Galantamine was a gift from Janssen Pharmaceutica (Titusville, NJ) and was first dissolved in distilled water to make stock solutions. The stock solutions were stored at 4°C and diluted to prepare test solutions with the standard external solution shortly before the experiments.

Data Analyses. Current records were initially analyzed by the Clamp-Fit module of the PClamp6 program to assess the whole-cell peak current amplitude and decay kinetics. Data were expressed as the mean ± S.E.M. unless otherwise stated. The concentration-response data were subsequently compiled for graphical analysis in SigmaPlot 8.0. Analysis of variance and Student’s t tests were performed to assess the significance of observed differences, and the p values less than 0.05 were considered statistically significant.

Results

Potentiation of NMDA-Induced Currents by Galantamine. Rat cortical neurons in long-term primary culture comprised at least three types of cells: pyramidal neurons, multipolar neurons, and bipolar neurons. NMDA currents of pyramidal and multipolar neurons were potentiated by galantamine, whereas those of bipolar neurons were not affected at all. Experiments were performed without adding Mg²⁺ and glycine to the external solution unless otherwise stated. Bath and U-tube applications of galantamine at a concentration of 1 μM to multipolar neurons (30–60 μm in diameter) caused reversible potentiation of peak currents evoked by 30 μM NMDA (Fig. 1A). Potentiation of peak currents was maintained during a 10-min perfusion of 1 μM galantamine, and the effect was completely reversible after washing with galantamine-free solution (Fig. 1B). The peak current amplitude was significantly increased by 1 μM galantamine to 134 ± 8.60% (p < 0.01; n = 4). Currents induced by 10 μM glutamate in bipolar neurons were also increased.

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by 1 μM galantamine to 135% of the control. However, no galantamine potentiation of NMDA currents was observed in bipolar neurons (15–30 μm in diameter) (data not shown).

Dose-response relationships for galantamine potentiation of NMDA currents in multipolar neurons are shown in Fig. 1, C and D. The minimal effective concentration of galantamine to potentiate NMDA currents was 10 nM at which only about 5% current potentiation over and above the control was observed. Galantamine at 1 μM potentiated the current by about 30%, yet a further increase in the concentration to 10 μM caused a decrease in efficacy. This resulted in a bell-shaped dose-response relationship with a maximal efficacy at 1 μM galantamine (Fig. 1D). The bell-shaped dose-response curve was also observed in nefiracetam potentiation of the α4β2 AChR currents (Zhao et al., 2001) and the NMDA receptor currents (Moriguchi et al., 2003a), and animal behavioral responses to nefiracetam (Nabeshima et al., 1994).

At least two potential mechanisms of galantamine potentiation of NMDA currents are conceivable. One is an increase in the affinity of the receptor for NMDA, which would reflect in a shift of the NMDA dose-response curve in the direction of lower concentrations of NMDA. The other possibility is an increase in current amplitude even at high concentrations of NMDA that produce a saturating response without changing the NMDA affinity for the binding site. Dose-response relationships for NMDA to induce currents before and during bath and U-tube applications of 1 μM galantamine are illustrated in Fig. 2A. The peak current amplitudes normalized to the maximum control current induced by 1000 μM NMDA were fitted by a sigmoid curve with an EC_{50} value of 36.7 ± 0.2 μM and a Hill coefficient of 0.60 ± 0.09 (Fig. 2A, E; n = 4). After a 10-min bath and U-tube applications of 1 μM galantamine, the maximal current amplitude induced by 1000 μM NMDA was 104.8 ± 6.8% of the control maximum, an EC_{50} was 26.2 ± 0.4 μM, and a Hill coefficient was 0.64 ± 0.07 (Fig. 2A, F; n = 4). Thus, the dose-response curve was shifted significantly (p < 0.05) in the direction of lower NMDA concentrations by galantamine application without changing the amplitude of maximum current, indicating that galantamine increased the receptor affinity for NMDA. This resulted in a decrease in the percentage of galantamine potentiation of NMDA currents with an increase in NMDA concentration (Fig. 2B). The situation is in sharp contrast with nefiracetam potentiation of the α4β2 AChR currents.

Fig. 1. Galantamine potentiation of NMDA currents in rat cortical neurons in primary culture. Currents were evoked at a holding potential of ~70 mV by 250-ms applications of 30 μM NMDA via a U-tube system at an interval of 1 min. No Mg^{2+} and glycine were added to the external solution. A, currents recorded from a multipolar neuron before (a), during (b), and after (c) bath and U-tube application of 1 μM galantamine. B, time course of changes in peak current amplitude before, during, and after bath and U-tube application of 1 μM galantamine in the same multipolar neuron as that of A. C, potentiation of NMDA-induced currents in multipolar neurons by various concentrations of galantamine. Data are given as the mean ± S.D. (n = 4) in a value relative to the control. D, bell-shaped dose-response relationship for galantamine potentiation of NMDA currents in multipolar neurons (mean ± S.E.M.; n = 4).
(Zhao et al., 2001) and NMDA receptor currents (Moriguchi et al., 2003a), in which currents were increased beyond the saturating response produced by high concentrations of ligands.

**Does Galantamine Interact with the Glycine Binding Site of NMDA Receptors?** Our previous study showed that nefracetam interacted with the glycine binding site of the NMDA receptors (Moriguchi et al., 2003a). The interactions between glycine and galantamine were studied using various concentrations of glycine on the NMDA currents induced by 30 or 300 μM NMDA (Fig. 3). Figure 3A shows that glycine, when applied via the bath and U-tube, potentiated NMDA currents in a concentration-dependent manner until the current amplitude reached a maximum at 3000 nM glycine. Similar glycine potentiation was also observed in the presence of 1 μM galantamine in the bath and U-tube (Fig. 3A). Galantamine potentiation was 29% in nominal glycine-free solution and those in the presence of 10, 30, 100, and 300 nM glycine were 30, 37, 31, and 26%, respectively. Thus, galantamine and glycine act additively in potentiating NMDA currents except at very high concentrations of glycine (1000 and 3000 nM). When the NMDA currents were induced by 300 μM NMDA, glycine still potentiated the NMDA current (Fig. 3B), but galantamine failed to further enhance the current. Thus, it seems that galantamine does not interact with the glycine binding site of the NMDA receptor.

To further corroborate the aforementioned notion, galantamine potentiation of NMDA currents was tested in the presence of 1 μM 7-ClKN, a specific inhibitor of the glycine binding site of the NMDA receptor. 7-ClKN drastically suppressed NMDA currents, yet 1 μM galantamine was able to potentiate the currents to 149 ± 6.0% of the control (p < 0.0002; n = 4) in the presence of 7-ClKN (Fig. 4). In keeping with this result, the dose-response curve of the 7-ClKN inhibition of NMDA currents was not affected by the presence of 1 μM galantamine (Fig. 5). The IC50 values estimated from the dose-response relationships are not significantly different (p > 0.05), being 298 ± 26.5 and 316.6 ± 24.6 nM in the absence and presence of galantamine, respectively. Thus, it
was concluded that galantamine did not interact with the glycine binding site of the NMDA receptor.

**Does Galantamine Interact with the Mg²⁺ Binding Site of NMDA Receptors?**

Mg²⁺ ions are known to block the NMDA receptor in a voltage-dependent manner, especially at large negative membrane potentials. Partial block occurs at the normal resting potential. Thus, our hypothesis is that galantamine potentiates NMDA currents by eliminating Mg²⁺ block. This hypothesis was tested by the following experiments.

When the membrane potential was held at −70 mV, Mg²⁺ inhibited NMDA currents in a concentration-dependent manner, almost completely blocking the currents at 1000 μM (Fig. 6A). Galantamine at 1 μM potentiated NMDA currents, and Mg²⁺ concentrations ranging from 1 to 1000 μM also suppressed the currents (Fig. 6B). The dose-response curves for Mg²⁺ block without and with 1 μM galantamine are plotted in Fig. 6C. The IC₅₀ for Mg²⁺ to block the NMDA current was 121 ± 9.6 μM in the absence of galantamine. Galantamine increased the NMDA current by 15, 16, 17, 15.4, and 28% in the presence of 0, 1, 10, 100, and 1000 μM Mg²⁺, respectively. An addition of 16% to the NMDA currents predicted from the dose-response relationship in the absence of galantamine could fit the data obtained in the presence of galantamine with the exception of a very high concentrations of Mg²⁺ at 1000 μM.

The weaker suppression of NMDA currents by 1 mM Mg²⁺ in the presence of galantamine was further investigated in the experiments shown in Fig. 7. In addition to blocking the channel, external millimolar Mg²⁺ ions have also been shown to potentiate NMDA currents under certain conditions (Paolelli et al., 1995; Wang and MacDonald, 1995). To assess the effect of galantamine on the Mg²⁺ potentiation of the NMDA current, the current was recorded at +40 mV in the presence of 1 mM Mg²⁺ and 30 nM glycine. Mg²⁺ at 1 mM alone did not change the NMDA current (data not shown), but increased 13.2 ± 2.3% (n = 5) over and beyond the control level in the presence of 1 μM galantamine. With this correction, the galantamine-induced increase of the NMDA current in the presence of 1 mM Mg²⁺ at −70 mV became 15%, which is in line with the increases in the absence of Mg²⁺ ions or in lower Mg²⁺ concentrations. Thus,

Fig. 4. 7-ClKN, a glycine site blocker, does not prevent galantamine potentiation of NMDA currents in a multipolar neuron. 7-ClKN was applied to both bath and U-tube. Glycine was added to the external solution at a concentration of 1 μM. A, 7-ClKN 1 μM suppressed currents induced by 30 μM NMDA (b), yet galantamine at 1 μM still potentiated the current in the presence of 7-ClKN (c). Washout caused a complete recovery of current (d). B, time course of changes in NMDA current amplitude in the presence of 1 μM 7-ClKN, 1 μM 7-ClKN plus 1 μM galantamine, and during washout. a to d correspond to those in A.

Fig. 5. Galantamine at 1 μM does not shift the dose-response relationship for 7-ClKN inhibition of currents induced by 30 μM NMDA in multipolar neurons. IC₅₀ was 298 ± 26.5 nM in control (n = 4) and 316.6 ± 24.6 nM in galantamine (n = 4). 7-ClKN and galantamine were applied to the bath and stet U-tube. Glycine was added to the external solution at a concentration of 1 μM. Data are given as the mean ± S.E.M.

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Galantamine did not alter Mg\textsuperscript{2+} block of the NMDA receptor even in the presence of 1 mM Mg\textsuperscript{2+}.

**Role of PKA, PKC, and G Proteins in Galantamine Potentiation.** Nefiracetam potentiates the activity of the α4β2 nAChR via G\textsubscript{i} proteins, but not G\textsubscript{i}/G\textsubscript{o} proteins, PKA, or PKC (Zhao et al., 2001). To assess the roles of these G proteins and kinases in galantamine potentiation of NMDA currents, the following experiments were conducted using specific chemicals agents.

The membrane-permeable PKC-specific inhibitor chelerythrine, when applied to the bathing solution at a concentration of 3 μM, slightly suppressed NMDA currents (Fig. 8, A and B). This is in keeping with the observation that the activation of PKC potentiates NMDA responses (Chen and Huang, 1991; Ben-Ari et al., 1992; Raymond et al., 1994; Leonard and Hell, 1997; Logan et al., 1999). Bath and U-tube applications of 1 μM galantamine in the presence of chelerythrine did not potentiate NMDA currents (Fig. 8, A and B). In the presence of 3 μM PKC inhibitor chelerythrine, 1 μM galantamine did not significantly increase the NMDA currents.
current (104.0 ± 4.6% of the control; p > 0.5). Thus, PKC is involved in the galantamine potentiation of NMDA currents.

The membrane-permeable PKA-specific inhibitor H-89, when applied to the bath at a concentration of 1 μM, slightly suppressed NMDA currents (Fig. 8, C and D). Unlike PKC inhibition, 1 μM galantamine was still able to potentiate NMDA currents 117.2 ± 6.20% of the control (p < 0.04) (Fig. 8, C and D). Thus, PKA is not involved in the galantamine potentiation of NMDA currents.

Potentiation of NMDA currents by 1 μM galantamine was not prevented by 24- to 26-h preincubation of neurons with either 200 ng/ml pertussis toxin, a Gi/Go protein inhibitor, or 500 ng/ml cholera toxin, a Gs protein stimulator (Fig. 9). The currents were increased by galantamine to 123.8 ± 3.7% (p < 0.0003) of the control after pertussis toxin treatment and to 117.8 ± 3.4% (p < 0.002) after cholera toxin treatment. Thus, Gi/Go and Gs proteins are not involved in the galantamine potentiation of NMDA currents.

AMPA and Kainate Currents Are Not Affected by Galantamine. Besides the NMDA currents, rat cortical neurons in primary culture generated currents in response to U-tube application of AMPA or kainate. Our previous study showed that nefiracetam did not significantly potentiate AMPA- and kainate-induced currents (Moriguchi et al., 2003a). Currents induced by 30 μM AMPA (n = 4) or 30 μM kainate (n = 4) in multipolar neurons were not changed by bath and U-tube applications of 1 μM galantamine (Fig. 10). The lack of effect of galantamine on the AMPA-induced current, which is mediated by the AMPA receptors, and the lack of effect of galantamine on the kainate-activated current, which could be mediated by both AMPA and kainate receptors, led us to conclude that galantamine has no potentiating action on both receptors. Thus, it was concluded that among the three subtypes of glutamate receptors, the AMPA and kainate receptors were not responsive to the modulating action of galantamine, and only the NMDA receptors responded to galantamine.

Discussion

The present study demonstrated that galantamine selectively modulated the NMDA receptor activity without affecting the AMPA or kainate receptors. It enhanced the NMDA current in a dose-dependent manner with the maximal potentiation of 30% at 1 μM galantamine, and shifted the NMDA dose-response relationship toward the lower concentrations without affecting the maximal response. Galantamine raised the foot of the dose-response curve for glycine to coactivate the NMDA receptor in the presence of 30 μM NMDA without affecting the maximal response. However, galantamine did not affect the glycine dose-response curve when 300 μM NMDA was coapplied to activate the receptor nor the dose-response relationship of 7-ClKN to inhibit the glycine site. The potentiating action was abolished by the PKC inhibitor chelerythrine, but not by the PKA inhibitor H-89 or pretreatment with pertussis toxin or cholera toxin. Together, these results suggest that galantamine exerts an indirect action on the NMDA receptor and that the PKC pathway is involved in galantamine action by increasing the affinity of the receptor for NMDA.

Alzheimer’s disease is a progressive neurodegenerative disorder of cognitive function. It is known that the Alzhei-
Fig. 9. G proteins are not involved in galantamine potentiation of NMDA currents. A and B, galantamine at 1 μM potentiated currents induced by 30 μM NMDA after 24- and 26-h pretreatment with 200 ng/ml pertussis toxin (124 ± 3.7%; p < 0.0003; n = 4), indicating that Gαi proteins are not involved in galantamine potentiation. C and D, galantamine at 1 μM potentiated NMDA currents after 24- to 26-h pretreatment with 500 ng/ml cholera toxin (118 ± 3.4%; p < 0.002; n = 4), indicating that Gs proteins are not involved in galantamine potentiation (mean ± S.E.M.; n = 4).

Fig. 10. Absence of the effects of bath and U-tube perfusion of 1 μM galantamine on currents evoked by 30 μM AMPA (A and B; n = 4) and 30 μM kainate (C and D; n = 4) in multipolar neurons. Holding potential was −70 mV. Neither AMPA-induced currents nor kainate-induced currents were affected by 1 μM galantamine (mean ± S.E.M.).
imer's disease is associated with down-regulation of both nAChRs and NMDARs in the brain (Fonnun et al., 1995; Giacobini, 2000). Thus, potentiation of the activity of these systems is expected to improve learning/memory/cognition of Alzheimer's patients. Galantamine potentiation of NMDA currents described here is deemed to contribute significantly to the therapeutic effects.

Galantamine weakly inhibits acetylcholinesterase with an IC50 value of 600 to 800 nM. However, galantamine directly stimulates nAChRs also. Earlier studies showed that galantamine at 1 to 10 μM opened single AChR channels in the rat hippocampus and in the chicken α4β2 receptors expressed in mouse fibroblast (M10) cells but failed to evoke measurable whole-cell currents (Pereira et al., 1993, 1994). However, acetylcholine-induced whole-cell currents were potentiated by galantamine (0.1–1 μM) in PC12 cells (α3-type AChRs), in hippocampal α7-type AChRs (Schattenholz et al., 1996; Maelicke and Albuquerque, 2000), in α4β2 AChRs expressed in human embryonic kidney cells (Samochocki et al., 2003), and in α7/5HT3 chimera (Maelicke et al., 2001). Because galantamine potentiation was blocked by the monoclonal antibody FK1 that recognizes the α subunit of nAChRs, galantamine is thought to bind to a site distinct from the ACh binding site (Schattenholz et al., 1996) acting as an allosterically potentiating ligand. A bell-shaped dose-response relationship was obtained with the potentiation peaking at 1 μM (Schattenholz et al., 1996), but the underlying mechanism has yet to be elucidated.

We have already reported that nefiracetam potentiates NMDA currents in the nominal absence of glycine or in the presence of low concentration of glycine (Moriguchi et al., 2003b). In fact, d-cycloserine, a glycine site partial agonist, is known to improve implicit memory performance of words in Alzheimer's patients (Schwartz et al., 1996) and reversal learning of rats in the water maze (Riekkinen et al., 1998). Thus, nefiracetam and d-cycloserine, as the glycine site partial agonists, share the common mechanism of action with respect to stimulation of NMDA receptors. In contrast, galantamine has a different mechanism of action from nefiracetam in potentiating NMDA currents (Moriguchi et al., 2003a).

Mg2+ ions at lower concentrations are known to block the NMDA channel in a voltage-dependent manner with the block augmented at negative potentials and attenuated at positive potentials (Mayer et al., 1984; Ascher and Nowak, 1988). Galantamine does not seem to interact with the Mg2+ blocking site of the NMDA receptor. Mg2+ ions at higher concentrations are known to enhance the NMDA currents, which is best seen at positive potentials. Galantamine enhanced the NMDA current measured at +40 mV in the presence of 1 mM Mg2+ ions. The latter observation might explain some of the differences between galantamine and nefiracetam on the NMDA current. A reduction in Mg2+ block was observed in the presence of nefiracetam (Moriguchi et al., 2003b) but not in the presence of galantamine.

It is well established that protein phosphorylation plays an important role in various neuroreceptors (Huganir and Greengard, 1990; Hoffman et al., 1994). Our experiments with the cortical neuron NMDA receptors indicated that PKC inhibition prevented galantamine potentiation but PKA inhibition did not. Pretreatment with the G/Gi, inhibitor pertussis toxin and the Gs stimulator cholera toxin did not prevent galantamine from potentiating NMDA currents. Thus, PKC seems to be involved in galantamine potentiation of NMDA currents.

Intracellular protein kinases are known to potentiate NMDA-mediated responses. Specifically, the activation of PKC is known to potentiate NMDA responses (Chen and Huang, 1991; Ben-Ari et al., 1992; Kelso et al., 1992; Urushihara et al., 1992; Logan et al., 1999) and to phosphorylate the receptor (Raymond et al., 1994; Leonard and Hell, 1997). PKC potentiation has been attributed to an increase in the channel open probability (Chen and Huang, 1992; Xiong et al., 1998) or a reduction in Mg2+ block (Chen and Huang, 1992). PKC activation has also been shown to reduce NMDA responses recorded from hippocampal CA1 pyramidal neurons (Markram and Segal, 1992) and in cerebellar granule cells (Snell et al., 1994). These differences in PKC modulation of NMDA responses might arise from different PKC isozymes (Nishizuka, 1988) and different subunit compositions of the NMDA receptors that exist in the brain (Snell et al., 1994).

The NMDA current was potently and efficaciously enhanced by nefiracetam via the PKC pathway (Moriguchi et al., 2003b). In the presence of nefiracetam, the Mg2+ block observed at −70 mV was almost completely eliminated. In the present study, the PKC inhibitor chelerythrine prevented the potentiating action of galantamine. Despite the involvement in the PKC pathway, the Mg2+ block was not affected by galantamine. The difference in Mg2+ block between nefiracetam and galantamine might be partly due to the degree of potentiation. Nefiracetam increased the NMDA current to nearly 300% of the control, whereas galantamine increased only by 30%. In addition, PKC activation has been shown to yield variable results on Mg2+ block, reducing Mg2+ block in some studies (Chen and Huang, 1991, 1992) but not in others (Wagner and Leonard, 1996).

Galantamine seems to work via multiple pathways to improve the memory/learning/cognition of Alzheimer's patients. The present study has demonstrated galantamine potentiation of the NMDA receptor activity. In addition to weakly inhibiting cholinesterase (Thomson et al., 1991), galantamine has been shown to allosterically potentiate the activity of nACh receptors (Pereira et al., 1993, 1994; Schattenholz et al., 1996; Maelicke and Albuquerque, 2000; Samochocki et al., 2003). In the brain of Alzheimer's patients, not only nAChR activity is down-regulated (Vidal and Changeux, 1996; Woodruff-Pak and Hinchcliffe, 1997) but also the glutamatergic system is impaired (Greenamyre et al., 1987; Palmer and Gershon, 1990; Francis et al., 1993; Palmer, 1996). Thus, galantamine stimulation of both nACh and glutamatergic systems is deemed to work in concert contributing to the improvement of the patient's conditions.

In conclusion, the Alzheimer's drug galantamine enhances the NMDA responses of rat cortical neurons in long-term primary culture. The potentiation of the NMDA receptor activity by galantamine is mediated by PKC but not by PKA or G proteins. The potentiation of the activity of NMDA receptors is an important aspect of galantamine action in improving learning/memory/cognition when the NMDA receptor activity is low in Alzheimer's patients.

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