Modulation of N-Methyl-D-aspartate Receptors by Donepezil in Rat Cortical Neurons

Shigeki Moriguchi, Xilong Zhao, William Marszalec, Jay Z. Yeh, and Toshio Narahashi

Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, Illinois

Received April 13, 2005; accepted June 8, 2005

ABSTRACT

Nicotinic acetylcholine receptors and N-methyl-D-aspartate (NMDA) receptors are known to be down-regulated in the brain of patients with Alzheimer’s disease. It was previously shown that the nootropic drugs nefiracetam and galantamine potentiate the activity of both nicotinic and NMDA receptors. We hypothesized that donepezil, a nootropic with a potent anticholinesterase activity, might also affect the NMDA system. NMDA-induced currents were recorded from rat cortical neurons in primary culture using the whole-cell patch-clamp technique at a holding potential of −70 mV in Mg2+-free solutions. In multipolar neurons, NMDA currents were decreased by bath and U-tube applications of 1 to 10 μM donepezil but were increased by 30 to 100 μM donepezil. Donepezil suppression occurred in a manner independent of NMDA concentrations ranging from 3 to 1000 μM. The donepezil suppression of NMDA currents was prevented by inhibition of protein kinase C (PKC) but unaffected by protein kinase A (PKA) and G proteins. In bipolar neurons, however, NMDA currents were potently augmented by bath and U-tube applications of 0.01 to 100 μM donepezil. Donepezil potentiation occurred at high NMDA concentrations that evoked the saturating responses and in a manner independent of NMDA concentrations ranging from 3 to 1000 μM. The potentiation of NMDA currents by donepezil was decreased by inhibition of PKC and abolished by modulation of G proteins but not by PKA inhibition. It was concluded that donepezil at low therapeutic concentrations (0.01−1 μM) potentiated the activity of the NMDA system and that this action together with cholinesterase inhibition would contribute to the improvement of learning, memory, and cognition in patients with Alzheimer’s disease.

Alzheimer’s disease is a progressive neurodegenerative disorder of cognitive function. It is well known that Alzheimer’s disease is associated with down-regulation of the cholinergic system in the brain (Giacobini, 2000). Thus, stimulation of the cholinergic system may improve the cognition, learning, and memory of patients with Alzheimer’s disease. This can be accomplished by inhibiting cholinesterases. In fact, four anticholinesterases—inacrine, donepezil, rivastigmine, and galantamine—are approved for the treatment of patients with Alzheimer’s disease in the United States. It is clearly recognized that any of these four anticholinesterases do not cure Alzheimer’s disease, and they are far from ideal even for improving the patient’s conditions. Tactine is the first of the four approved for clinical use, but it suffers from some hepatotoxicity and short half-life. Donepezil and rivastigmine currently occupy 45 and 14% of the market, respectively, and galantamine is the newest cholinergic agent approved in 2001 (Jann et al., 2002). These drugs, being anticholinesterases, cause some side effects, such as nausea, diarrhea, and vomiting. However, their efficacy in improving cognition, learning, and memory does not seem to relate to their potency in inhibiting cholinesterase.

It is also known that the glutamatergic system is down-regulated in the brain of patients with Alzheimer’s disease (Fonnun et al., 1995). Glutamate is the major excitatory amino acid neurotransmitter in the central nervous system and is implicated in complex neuronal functions, such as learning/memory and synaptic plasticity (Collingridge and Singer, 1990; Monyer et al., 1992). Three glutamate ionotropic receptor subtypes are known; i.e., N-methyl-D-aspartate (NMDA), kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtype (Ozawa et al., 1998; Dingledine et al., 1999). In patients with Alzheimer’s disease, glutamate-containing neurons are decreased (Greenamyre et al., 1987; Palmer and Gershon, 1990; Francis et al., 1993; Palmer, 1996). Ischemic brain injury increases the concentration of ambient glutamate, which could cause prolonged activation of the NMDA receptor, leading to cell death. Drugs capable of modulating this tonic activity of NMDA receptors, such as memantine, have become available for the clinical

ABBREVIATIONS: NMDA, N-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; nAChR, nicotinic acetylcholine receptor(s); PKC, protein kinase C; PKA, protein kinase A; H89, N-[2-(4-bromocinnamyl-amino)ethyl]-5-isoquinoline.
management of patients with various types of dementia (Parsons et al., 1999; Danyys and Parsons, 2003).

We previously reported that the nootropic drug nefiracetam was capable of potentiating the activity of the α4β2 neuronal nicotinic acetylcholine receptor (nAChR) (Zhao et al., 2001) and augmenting NMDA-activated currents in rat cortical neurons (Moriguchi et al., 2003a,b). The latter action appeared to be exerted via nefiracetam interactions with the glycine binding site on the NMDA receptor. We have recently found that galantamine, which is known to inhibit cholinesterases and potentiate the activity of α7 and α4β2 nAChR (Schrattenholz et al., 1996; Maelicke and Albuquerque, 2000; Maelicke et al., 2001; Samochocki et al., 2003), potentiates NMDA-evoked currents in rat cortical neurons (Moriguchi et al., 2004b). This action appeared to be exerted via the protein kinase C (PKC) system. Thus, these drugs may improve cognitive function by modulating the activity of NMDA receptors and nAChR in the brain of patients with Alzheimer’s disease. Wang et al. (1999) showed that donepezil inhibited binding of [3H]dizocilpine to synaptic membrane of rat cerebrospinal fluid. However, the IC50 value was as high as 135 μM, which might not be pharmacologically relevant.

In the present study, we show for the first time that donepezil, which is known to be a potent anticholinesterase, also modulates the activity of the NMDA receptor in rat cortical neurons in primary culture. There were at least two types of neurons in the culture: multipolar and bipolar neurons. NMDA currents recorded from bipolar neurons were potently and efficaciously augmented by donepezil, whereas those recorded from multipolar neurons were slightly inhibited by moderate concentrations of donepezil and augmented by high concentrations. Thus, the improvement of the conditions of patients with Alzheimer’s disease by donepezil does not seem to be caused solely by the cholinesterase inhibition; modulation of the NMDA receptor activity appears to play an important role as well. Preliminary results were reported briefly (Moriguchi et al., 2004a; Narahashi et al., 2004).

Materials and Methods

Cell Preparations. Rat cortical neurons were isolated and cultured by a procedure slightly modified from that described elsewhere (Marszalec and Narahashi, 1993). In brief, rat embryos were removed from 17-day pregnant Sprague-Dawley rats 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, 7.0 mM glucose, 0.25% (v/v) trypton (Type XI, Sigma-Aldrich, St. Louis, MO), pH 7.4, and with 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 and with osmolarity 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 with confluent glia that had been plated 2 to 4 weeks earlier. The cortical neuron/glia cultures were maintained in a humidified atmosphere of 90% air/10% CO2 at 34°C. Cells cultured for 3 to 7 weeks were used for the experiments.

Solutions for Current Recording. The Mg2+-free external solution for whole-cell recording of currents contained 150 mM NaCl, 5 mM KCl, 2.5 mM CaCl2, 30 mM glycine, 5.5 mM HEPES acid, 4.5 mM HEPES sodium, and 10 mM d-glucose. Tetrodotoxin (100 nM) was added to eliminate the voltage-gated sodium channel currents. Atropine sulfate (20 nM) was added to block the muscarinic acetylcho-

Results

Differential Actions of Donepezil on NMDA Currents in Multipolar and Bipolar Neurons

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 multipolar
neurons were suppressed by low concentrations of donepezil (1–10 μM) and potentiated by high concentrations of donepezil (30 and 100 μM). However, NMDA currents of bipolar neurons were potentiated by donepezil at concentrations ranging from 0.01 to 100 μM.

Figure 1A shows an example of an experiment using a multipolar neuron. The NMDA current was evoked by applying 30 μM NMDA for 250 ms at an interval of 1 min via a U-tube without adding Mg²⁺ to the external solution. Bath and U-tube applications of 10 μM donepezil suppressed the NMDA-induced current, and the effect was completely reversible after washing with donepezil-free solutions. The time course of changes in NMDA current amplitude during and after bath and U-tube applications of 10 μM donepezil is illustrated in Fig. 1B. The current amplitude was decreased by 10 μM donepezil to 78.7 ± 4.7% of the control (P < 0.01; n = 4). In contrast, bath and U-tube applications of 100 μM donepezil greatly potentiated the NMDA-induced current, and the effect was completely reversible after washing with donepezil-free solutions (Fig. 1C). The time course of changes in NMDA current amplitude during and after bath and U-tube applications of 100 μM donepezil is illustrated in Fig. 1D. The current was potentiated by 100 μM donepezil to 256.5 ± 4.6% of the control (P < 0.01; n = 4).

In contrast to the dual effect on multipolar neuron NMDA receptors, donepezil potently increased NMDA currents in bipolar neurons (Fig. 2, A and C). Bath and U-tube applications of 100 nM and 10 μM donepezil greatly potentiated the NMDA-induced current, and the effect was completely reversible after washing with donepezil-free solutions. The time courses of changes in NMDA current amplitude during and after bath and U-tube applications of 100 nM and 10 μM donepezil are illustrated in Fig. 2, B and D, respectively. The current amplitude was potentiated by donepezil to 160.8 ± 3.2% (n = 4) and 197.8 ± 3.1% (n = 4) of the control by 100 nM and 10 μM donepezil, respectively.

Dose-Response Relationships for Donepezil Actions

Dose-response relationships for donepezil effects on multipolar and bipolar neurons are illustrated in Fig. 3. The minimal and maximal concentrations of donepezil to inhibit NMDA currents in multipolar cells were 1 μM and 10 μM, at which the currents were decreased to 86.7 ± 2.6% (P < 0.05) and 78.7 ± 4.7% (P < 0.01) of the control, respectively. The IC₅₀ was estimated to be 1.3 ± 0.3 μM. At concentrations greater than 10 μM, donepezil potentiated the NMDA current. By contrast, the minimal effective concentration of donepezil to potentiate NMDA currents in bipolar cells was 10 nM, at which the currents were potentiated to 146.1 ± 2.1% of the control (P < 0.01). At 10 μM, donepezil greatly potentiated the currents to 194.2 ± 6.2% of the control (P < 0.01). The EC₅₀ for donepezil potentiation of NMDA currents in bipolar neurons was estimated to be 10.2 ± 0.5 nM.

At least three possible mechanisms are conceivable for donepezil suppression of NMDA currents in multipolar neurons. One is a shift of the NMDA dose-response relationship in the direction of higher concentrations of NMDA. A second possibility is that the maximal NMDA response is suppressed. A third situation is a combination of these two mechanisms. To differentiate these mechanisms, the effect of donepezil on the NMDA dose-response relationship in multipolar neurons was examined as shown in Fig. 4A. In the absence of donepezil, the NMDA dose-response curve gave an EC₅₀ of 32.8 ± 0.4 μM with a Hill coefficient of 0.51 ± 0.07 (n = 4). After a 10-min bath perfusion of 10 μM donepezil, the maximal current induced by 1000 μM NMDA was reduced to 79.6 ± 1.9% of the control maximum. However, the EC₅₀ remained almost unchanged at 31.9 ± 0.3 μM with a Hill

**Fig. 1.** Donepezil suppresses NMDA-induced currents at a moderate concentration and potentiates the currents at a high concentration in multipolar neurons (30–60 μm in diameter). 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. A, currents recorded before (a), during (b), and after (c) bath and U-tube applications of 10 μM donepezil. B, time course of changes (mean ± S.E.M.; n = 4) in peak current amplitude before, during, and after bath and U-tube applications of 10 μM donepezil. C, currents recorded before (a), during (b), and after (c) bath and U-tube applications of 100 μM donepezil. D, time course of changes (mean ± S.E.M.; n = 4) in peak current amplitude before, during, and after bath and U-tube applications of 100 μM donepezil.
coefficient of 0.37 ± 0.06 (n = 4). Thus, the second mechanism is applicable to donepezil suppression of NMDA currents in multipolar neurons. A corollary of this result is that 10 μM donepezil suppresses NMDA currents uniformly regardless of the NMDA concentration (Fig. 4B).

For donepezil potentiation of NMDA currents in bipolar neurons, there also are three possible explanations. The NMDA dose-response curve may be shifted in the direction of lower concentrations of NMDA. Second, the maximal response is elevated by donepezil. Third, a mixture of these two is possible. The control NMDA dose-response curve gave an EC₅₀ of 34.1 ± 0.5 μM with a Hill coefficient of 0.51 ± 0.09 (n = 4) (Fig. 5A). After a 10-min bath perfusion of 10 μM donepezil, the maximal current was increased to 155.5 ± 3.9% of the control, and the EC₅₀ was estimated to be 31.6 ± 0.7 μM with a Hill coefficient of 0.73 ± 0.05 (n = 4) (Fig. 5A). Thus, the second explanation is also applicable to donepezil potentiation of NMDA currents in bipolar neurons. As expected from these results, the degree of potentiation of NMDA currents is almost independent of NMDA concentration, although there is a tendency for the potentiation to increase with increasing concentration of NMDA (Fig. 5B).

Role of Protein Kinases and G Proteins in Donepezil Modulation of Multipolar Neurons

PKC Inhibition Prevents Donepezil Suppression but PKA Inhibition Does Not. The activity of NMDA receptors is known to be modulated by protein kinases (Ben-Ari et al., 1992; Chen and Huang, 1992; Leonard and Hell, 1997; Logan et al., 1999). To see whether the donepezil inhibition of NMDA receptors is affected by PKA or PKC, specific inhibitors of these kinases were used in the absence of Mg²⁺ in the external solutions. Chelerythrine is a membrane-permeable specific inhibitor of PKC. Results of the experiments with chelerythrine are shown in Fig. 6, A and B. Bath perfusion of 3 μM chelerythrine slightly suppressed NMDA currents to 92.8 ± 4.3% of the control (n = 4), but the suppression is not statistically significant (P > 0.05). Addition of 10 μM donepezil to the bathing solution did not further suppress NMDA currents (91.4 ± 7.1% of the control; P > 0.05).

By contrast, the membrane-permeable PKA inhibitor H89
did not prevent donepezil suppression of NMDA-induced currents (Fig. 6, C and D). H89 by itself at 1 μM slightly but insignificantly suppressed the current to 98.5 ± 0.8% of the control (n = 4; P > 0.05). Addition of 10 μM donepezil decreased the currents to 80.0 ± 5.4% of the control (P < 0.05). Washing for 10 min with donepezil- and H89-free solutions restored the currents to 96.6 ± 3.6% of the control. Thus, it was concluded that donepezil suppression of NMDA currents required active PKC but not PKA in multipolar neurons. Potentiation of NMDA currents in multipolar neurons by high concentrations (30–100 μM) of donepezil was not studied in connection with protein kinases because these concentrations were deemed pharmacologically irrelevant.

**G Proteins Are Not Involved in Donepezil Suppression.** To determine the role of G proteins in the donepezil suppression of NMDA-induced currents in multipolar neurons,
neurons pretreated with pertussis toxin or cholera toxin were tested for their responses to donepezil. Pretreatment with 200 ng/ml pertussis toxin, a Gi/Go inhibitor, for 24 to 26 h did not prevent 10 μM donepezil from suppressing the NMDA currents (86.6 ± 4.4% of the control; \( n = 4; \ P < 0.01 \)) (Fig. 7, A and B). Washing for 10 min with donepezil-free solutions caused recovery of currents (97.3 ± 3.1% of the control). Therefore, the Gi/Go proteins were not involved in donepezil suppression of NMDA currents in multipolar neurons.

After pretreatment with 500 ng/ml cholera toxin, a Gs protein stimulator, for 24 to 26 h, 10 μM donepezil still suppressed the NMDA-evoked currents (85.2 ± 6.2% of the control; \( n = 4; \ P < 0.05 \)) (Fig. 7, C and D). Washing for 10 min with donepezil-free solutions caused recovery of currents (96.6 ± 2.7% of the control). Therefore, Gs proteins were not involved in donepezil depression of NMDA currents in multipolar neurons.

Role of Protein Kinases and G Proteins in Donepezil Modulation of Bipolar Neurons

PKC Inhibition Decreases Donepezil Potentiation but PKA Inhibition Does Not. We also examined whether PKC or PKA is related to donepezil potentiation of NMDA currents in bipolar neurons. Bath perfusion of 3 μM chelerythrine slightly suppressed the currents induced by 30 μM NMDA to 91.1 ± 2.1% of the control (\( n = 4; \ P < 0.01 \)). Addition of 1 μM donepezil caused an increase in the currents to 140.3 ± 7.9% of the control (Fig. 8, A and B). The amount of increase is less than the increase in NMDA currents by 1 μM donepezil (184%; Fig. 3) without chelerythrine. Washing for 10 min with donepezil- and chelerythrine-free solutions restored the currents to 100.8 ± 0.9% of the control (Fig. 8, A and B).

H89 at 1 μM slightly suppressed the currents to 94.1 ± 1.3% of the control (\( n = 4; \ P < 0.01 \)), but addition of 10 μM donepezil caused an increase in the currents to 171.4 ± 16.4% of the control (Fig. 8, C and D), which is almost the same as the current increase in the control without H89 (184%; Fig. 3). Washing for 10 min with donepezil- and H89-free solutions restored the currents to 98.5 ± 1.4% of the control (Fig. 8, C and D). Thus, it was concluded that donepezil potentiation of NMDA currents in bipolar neurons partially required active PKC but not PKA.

G Proteins Are Involved in Donepezil Potentiation. We also examined whether G proteins were related to done-
pezil potentiation of NMDA currents in bipolar neurons. Pretreatment with 200 ng/ml pertussis toxin for 24 to 26 h prevented 100.9 ± 4.5% (n = 4; P > 0.05) (Fig. 9, A and B). Therefore, Gi/Go proteins were involved in donepezil potentiation of NMDA currents in bipolar neurons. After pretreatment with 500 ng/ml cholera toxin for 24 to 26 h, 1 μM donepezil did not potentiate the NMDA currents (101.9 ± 2.5% of the control; n = 4; P > 0.05) (Fig. 9, C and D). Therefore, Gs proteins were involved in donepezil potentiation of NMDA currents in bipolar neurons.

**Donepezil Does Not Affect AMPA and Kainate Currents**

Rat cortical neurons in primary culture generated currents in response to U-tube application of 30 μM AMPA or kainate. AMPA- or kainate-induced currents were not affected by 10 μM donepezil in both multipolar and bipolar neurons (data not shown).

**Donepezil Does Not Affect Acetylcholine-Induced Current**

Whereas donepezil inhibits cholinesterases very potently, the question of whether it directly affects nAChR was tested. Acetylcholine-induced currents in α4β2 nAChR (Aistrup et al., 1999) in the presence of 100 nM donepezil were 98.7 ± 3.1% of the control (Fig. 10, A and B; n = 4; P > 0.05) and in 10 μM donepezil were 97.1 ± 16% of the control (Fig. 10, C and D; n = 4; P > 0.05). Thus, donepezil at these concentrations has no effect on α4β2 nAChR currents.

**Discussion**

The present study showed that donepezil differentially modulated the NMDA receptors in multipolar and bipolar neurons. In multipolar neurons, donepezil exerted a biphasic action suppressing the NMDA-induced currents at moderate concentrations (1–10 μM) and potentiating the currents at high concentrations (30–100 μM). By contrast, donepezil potently augmented the NMDA-induced currents at 0.01 to 100 μM in bipolar neurons. Donepezil suppression of NMDA currents in multipolar neurons appears to be modulated by PKC but not by PKA, Gi/Go proteins, or Gs proteins. On the other hand, donepezil potentiation of NMDA currents in bipolar neurons is modulated by Gi/Go proteins and Gs proteins, partially modulated by PKC, but not modulated by PKA.

Whereas nefiracetam (Moriguchi et al., 2003a,b), galantamine (Moriguchi et al., 2004b), and donepezil (present study) all are capable of modulating the activity of NMDA receptors, their mechanisms of action appear to be different.
Table 1). Although donepezil at 30 to 100 μM potentiated the NMDA currents in multipolar neurons, this action is not included in the discussion because the concentrations are too high and are not deemed pharmacologically significant.

Nefiracetam was very potent in potentiating NMDA currents in multipolar neurons without effect on bipolar neuron NMDA currents (Moriguchi et al., 2003b). The maximum effect occurred at a concentration of 10 nM. The affinity of the receptors for NMDA was not altered by nefiracetam, yet the saturating responses caused by high concentrations of NMDA in the absence of added glycine were greatly potentiated. At high glycine concentrations (>10 μM), nefiracetam did not have any effect on NMDA currents. Gabapentin, a very effective treatment of chronic pain, has been shown to potentiate NMDA response by increasing the glycine affinity for NMDA receptor (Gu and Huang, 2001). This potentiating action required the active PKC. However, nefiracetam acts as a partial agonist at the glycine site of the NMDA receptor. The potentiation by nefiracetam also requires activation of PKC (Moriguchi et al., 2003a).

Galantamine resembles nefiracetam in that it potentiates the multipolar neuron NMDA receptor via PKC but not PKD or G proteins and that it lacks effect on bipolar neuron NMDA receptors. However, galantamine differs from nefiracetam in three aspects (Moriguchi et al., 2004b). First, the effect was much less potent, with the maximum effect occurring at 1 μM; second, the receptor affinity for NMDA is increased without change in the maximum saturating responses; and third, there is no interaction with the glycine binding site of the NMDA receptor.

Donepezil is considerably different from either nefiracetam or galantamine. First, donepezil acted on the NMDA receptors of both multipolar and bipolar neurons, albeit in the opposite directions at concentrations of 10 μM or less. The action of donepezil to enhance NMDA currents in bipolar neurons was potent: a 50% increase occurred at 10 nM, and a 100% increase occurred at 10 μM. Unlike nefiracetam and galantamine, the potentiation action of donepezil was partially blocked by the inhibitor of PKC and completely prevented by pretreatment of pertussis toxin or cholera toxin. Thus, the modulation of NMDA receptors in bipolar neurons appears to be different from that in multipolar neurons. The G protein signal pathway seems to be more important than the PKC pathway in modulating NMDA receptor activity in bipolar neurons. This may explain why nefiracetam fails to enhance NMDA receptor activity in bipolar neurons because nefiracetam acts at the PKC pathway only (Moriguchi et al., 2003a,b).

Donepezil at concentrations less than 10 μM inhibited NMDA currents in multipolar neurons. The inhibitory action of donepezil may not be caused by the pore-blocking mechanism. First, the IC50 value, 1.3 μM, estimated from the
The present whole-cell study, is much lower than 135 μM obtained by displacement of dizocilpine maleate binding (Wang et al., 1999). Second, contrary to the pore-blocking mechanism, donepezil suppresses the NMDA current in a manner independent of NMDA concentrations.

The role of PKC in donepezil inhibition of NMDA currents in multipolar neurons appears to be complex. First, the NMDA current was inhibited more by donepezil than by the PKC inhibitor, whereas the latter could prevent further inhibition by donepezil. Second, in multipolar neurons, donepezil and nefiracetam produced the opposite effects via the PKC pathway. Donepezil inhibited the NMDA current, whereas nefiracetam potentiated the NMDA current. The PKC inhibitors prevented both effects. We speculate that, although PKC-mediated phosphorylation of NMDA receptor could increase NMDA currents, the PKC activation could inhibit tonically active protein tyrosine kinase (Collett and Collingridge, 2004). Donepezil may selectively activate the PKC isoforms that inhibit protein tyrosine kinase, whereas nefiracetam may activate PKC that directly phosphorylate NMDA receptors. PKC inhibitors appear to indiscriminately block both pathways.

Donepezil is a potent anticholinesterase with an IC$_{50}$ of 6.7 to 26 nM. The present study showed that donepezil also potently and efficaciously augments NMDA-induced currents in bipolar neurons at concentrations ranging from 0.01 μM and 100 μM. Thus, at therapeutic doses, donepezil stimulates the cholinergic system via inhibition of cholinesterases and augments the activity of the NMDA system, bringing back these two transmitter systems to normal levels to improve learning, memory, and cognition of the patients.

Memantine has recently been approved for use in patients with Alzheimer's disease in the United States. This drug has a unique property in blocking the NMDA receptors (Danysz and Parsons, 2003). Because the activity of NMDA receptors is generally decreased in the brain of patients with Alzheimer's disease, the action of memantine appears to be contradictory to the aforementioned stimulation of NMDA receptors. However, after careful examinations of the data in the literature, therapeutic effects of memantine can be explained in terms of NMDA receptor block as discussed in the following paragraph.

Ischemic brain injury from cerebrovascular disease is a common cause of dementia and cognitive impairment in elderly individuals and patients with Alzheimer's disease.
During cerebral ischemia, excess release of glutamate would cause prolonged activation of NMDA receptors, leading to cell death. This tonic activation of NMDA receptors is effectively blocked by memantine. On the other hand, phasic synaptic transmission mediated by NMDA receptors is expected to be undisturbed by memantine because of memantine’s fast unblocking kinetics and voltage-dependent unblock (Chen et al., 1992; Parsons et al., 1993). Memantine block that may occur during the early phase of synaptic transmission will be quickly removed as the membrane is depolarized during the excitatory postsynaptic potential (Chen et al., 1992). Because donepezil and galantamine could potentiate the phasic NMDA receptor currents, it remains to be seen whether they enhance the tonic activation of NMDA receptors.

**Acknowledgments**

We thank Daniel M. Close for technical assistance and Julia Irizarry for secretarial assistance.
References


Schrattenholz A, Pereira EFR, Roth U, Weber KH, Albuquerque EX, and Maelicke A (1996) Agonist responses of neuronal nicotinic acetylcholine receptors are poten-

Address correspondence to: Dr. Toshio Narahashi, Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611. E-mail: narahashi@northwestern.edu