Review

Mechanisms of Action of Cognitive Enhancers on Neuroreceptors

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No strategies for curing Alzheimer's disease have been developed yet as we do not know the exact cause of the disease. The only therapy that is available for patients is symptomatic treatment. Since Alzheimer's disease is associated with downregulation of the cholinergic system in the brain, its stimulation is expected to improve the patients' cognition, learning, and memory. Four anticholinesterases have been approved in the U.S.A. for the treatment of Alzheimer's disease patients. However, because of the inhibition of cholinesterases, these drugs have side effects and their effectiveness does not last long. Thus new approaches are needed. One approach is to stimulate directly nicotinic acetylcholine (nACh) receptors in the brain, and another is to stimulate NMDA receptors which are also known to be downregulated in Alzheimer's patients. Nefiracetam has been shown to potentiate ACh currents in the $\alpha4\beta2$ receptor of rat cortical neurons with a bell-shaped dose–response relationship and the maximum effect at 1 nM. This effect was exerted *via* G_s proteins. The $\alpha7$ receptor was almost unaffected by nefiracetam. Nefiracetam also potentiated NMDA currents with the maximum effect at 10 nM *via* interaction with the glycine-binding site of the receptor. Galantamine had a moderate potentiating effect on the $\alpha4\beta2$ receptor and potentiated NMDA currents with the maximum effect at 1 μM. However, galantamine did not interact with the glycine-binding site. Donepezil, a potent anticholinesterase, also potentiated NMDA currents at 1—10000 nM. In conclusion, these three drugs potentiate the activity not only of the cholinergic system but also of the NMDA system, thereby stimulating the downregulated nACh receptors and NMDA receptors to improve patients' learning, cognition, and memory.

Key words Alzheimer's disease; nefiracetam; galantamine; donepezil; acetylcholine receptor; NMDA receptor

1. INTRODUCTION

Accumulation of β-amyloid in the brain is a hallmark of Alzheimer's disease. However, no strategies for curing the disease have been developed yet as we do not know the exact cause of the disease. The only therapy that is available for patients is symptomatic treatment. Since Alzheimer's disease is associated with downregulation of the cholinergic system in the brain, stimulation of the cholinergic system may improve patients' cognition, learning, and memory. This approach has proven successful, albeit to a limited extent, and four anticholinesterases have been approved in the U.S.A. for the treatment of Alzheimer's disease patients. These are tacrine (Cognex), donepezil (Aricept), rivastigmine (Exelon), and galantamine (Reminyl) (Fig. 1).

It is recognized that none of these four anticholinesterases cures Alzheimer's disease, and they are far from ideal even for improving patients' conditions. Tacrine is the first of the four approved for clinical use, but it has the disadvantages of hepatotoxicity and short half-life. Donepezil and rivastigmine currently have 45% and 14% of the US market share, respectively, and galantamine is the newest Alzheimer's drug approved in 2001.[1] 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 be related to their anticholinesterase activity.

Under the circumstances, alternative approaches are urgently needed. One of these approaches is to potentiate directly the activity of neuronal nicotinic acetylcholine receptors (nAChRs) in the brain. It has been demonstrated that nefiracetam[2] and galantamine[3—6] potentiate ACh-induced currents in nAChRs. Although galantamine inhibits cholinesterase, its potency is low with an IC_{50} of 600—800 nM as compared with the IC_{50} values of donepezil (6.7—26 nM) and rivastigmine (4.3 nM). The optimal concentration of galantamine to potentiate ACh-induced currents maximally is 0.1—1 μM.[3—5] Nefiracetam is extremely potent in potentiating ACh-induced currents in the $\alpha4\beta2$-like AChRs in rat cortical neurons at concentrations as low as 0.1—1.0 nM efficaciously (to 200% of control).[2] Thus, direct potentiation of nAChR activity is a promising approach.

Reductions in NMDA receptors are also found in Alzheimer's disease patients, possibly contributing to memory deficits.[7] One hypothesis for the development of Alzheimer's disease is that neurotoxic β-amyloid peptides cause a deleterious influx of calcium ions into neurons, which in turn triggers intracellular events that eventually cause cell death.

The activation of NMDA receptors opens the cation channels that are permeable to sodium and calcium ions. An increase in intracellular calcium would initiate a cascade of events leading to enhancement of synaptic activity. The activity-dependent synaptic enhancement is called long-term potentiation (LTP) and considered to be a model for learning and memory.[8] However, excess Ca^{2+} influx could occur when the NMDA receptors are repeatedly activated by endogenous glutamate associated with acute central nervous system injuries such as stroke and trauma, triggering a cascade of intracellular events eventually causing cell dysfunction and death. Thus, there is a trade-off between too much receptor function and not enough receptor function, because reductions in NMDA receptors may worsen memory deficit in Alzheimer's disease patients and because too much stimu-

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lation of the receptors may cause excitotoxicity.[9)]

Drugs that modulate 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 models[10,11)] and have been tested as cognitive enhancers in both healthy subjects and patients with Alzheimer's disease.[12—14)]

Our working hypothesis is that one of the mechanisms by which nootropic drugs improve cognitive function is to modulate the nACh and/or NMDA receptor functions. Nootropic drugs improve cognitive function by increasing the activity of nACh and/or NMDA receptors in patients with Alzheimer's disease and patients with other forms of dementia who have reduced nACh and NMDA receptors; in post-stroke patients who have excess glutamate release, nootropic drugs with a partial agonist action reduce the excess activation of NMDA receptors.

2. NEFIRACETAM

The mechanisms of action of nefiracetam (Fig. 1) on neuroreceptors and ion channels have been studied for the past 10 years. *L*-type and *N*-type calcium channel currents of neuroblastoma-glioma hybrid cells (NG108-15) were potentiated by nefiracetam at doses $\geq 1\,\mu$M, and the effect was exerted *via* G_i/G_o proteins.[15,16)]

Torpedo and brain nicotinic AChRs have been found to be sensitive to nefiracetam. *Torpedo* AChRs expressed in *Xenopus* oocytes were suppressed by low concentrations (0.01—0.1 μM) of nefiracetam *via* G_i/G_o and protein kinase A (PKA), but potentiated by higher concentrations (1—10 μM) *via* protein kinase C (PKC).[17)] The $\alpha 7$ AChRs expressed in oocytes were potentiated by ≥ 100 nM nefiracetam, and the $\alpha 4\beta 2$ nAChRs were potentiated by ≥ 1 nM nefiracetam, both *via* PKC, but not *via* PKA.[18)] Nefiracetam 1 μM caused LTP-like facilitation in hippocampal slices *via* nAChRs and PKC, but not *via* NMDA receptors.[19)] Field excitatory postsynaptic potentials were potentiated by 1 μM nefiracetam, yet NMDA-evoked currents were suppressed by 1 μM nefiracetam suggesting that NMDA receptors are not responsible for synaptic facilitation.[20)] Our recent studies have clearly shown that nefiracetam 0.1—1 nM potentiates ACh-induced currents in the $\alpha 4\beta 2$-like receptors in rat cortical neurons[2)] and that it also potentiates NMDA-induced currents at ≥ 1 nM.[21)]

2.1. Nefiracetam Potentiates nACh Receptor Activity

Nefiracetam was highly potent and efficacious in augmenting $\alpha 4\beta 2$-like ACh currents (Figs. 2A, B) in rat cortical neurons in primary culture.[2)] The threshold concentration was 0.1 nM. At a higher concentration of 10 μM, nefiracetam initially potentiated the current to 400% of the control value, but the current later declined to 200% of the control value (Fig. 2D).

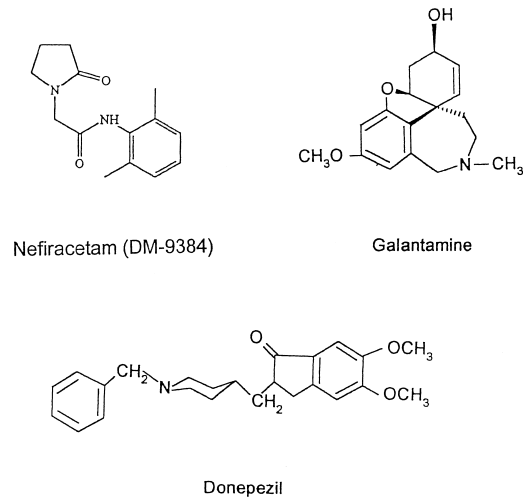

Fig. 1. Structures of Nefiracetam, Galantamine, and Donepezil

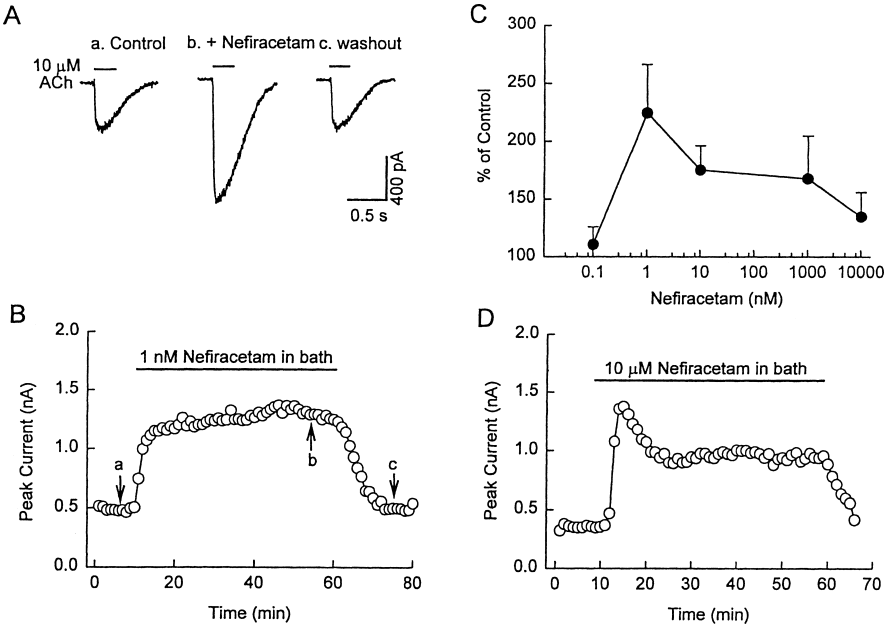

Fig. 2. Potentiation of α-BuTX-Insensitive, $\alpha 4\beta 2$-Like ACh Currents by Nefiracetam 1 nM (A, B) and 10 μM (D) Nefiracetam in Rat Cortical Neurons in Primary Culture

The bell-shaped dose–response relationship is shown in C.[2)]

A bell-shaped dose–response relationship for nefiracetam potentiation of ACh responses (Fig. 2C) was also observed in various *in vitro* and animal behavioral experiments with nootropic drugs.[22] Contrary to the $\alpha4\beta2$-like ACh currents, the $\alpha7$-like ACh currents were not potentiated by nefiracetam but slightly suppressed.[2]

It is interesting to note that nefiracetam potentiation was observed even at ACh concentrations that caused saturating responses (Fig. 3).[2] The result is similar to ethanol potentiation of the $\alpha4\beta2$-like ACh currents.[23] This raises the question of whether nefiracetam potentiation at high ACh concentrations is due to an increase in the total receptors available for activation by rapid exocytosis of the receptors or changes in single-channel properties. The latter may include: 1) an increase in single-channel conductance; 2) an increase in open probability; 3) a prolongation of open time; and 4) a combination of any of the three. Preliminary single-channel experiments indicated that an increase in channel open probability was one important factor.

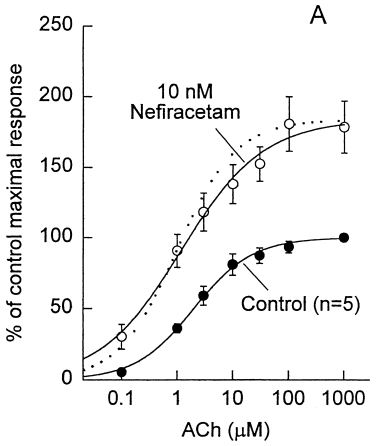

Fig. 3. ACh Dose–Response Relationships in Inducing α-BuTX-Insensitive, $\alpha4\beta2$-Like Currents before and During Exposure to 10 nM Nefiracetam in Rat Cortical Neurons

Nefiracetam potentiated the currents even with the ACh concentrations that gave the saturating response.[2]

2.2. Roles of Protein Kinases and G Proteins in Nefiracetam Potentiation To determine whether PKA, PKC, and G proteins are involved in nefiracetam potentiation of $\alpha4\beta2$-like ACh currents, specific agents were used.[2] None of the three PKA inhibitors, H-89 (1 μM external application), peptide 5—24 (200 nM internal), and KT 5720 (560 nM internal) prevented nefiracetam potentiation (Fig. 4A). Similarly, none of the three PKC inhibitors, peptide 19—36 (3 μM internal), calphostin C (0.5 μM internal), and chelerythrine (3 μM external) was effective in preventing nefiracetam potentiation (Fig. 4B). Preincubation of cells with 200 ng/ml pertussis toxin also did not prevent nefiracetam potentiation either (Fig. 4C). Thus, PKA, PKC, and G_i/G_o proteins are not involved in nefiracetam action. However, preincubation with 500 ng/ml cholera toxin completely eliminated nefiracetam potentiation of ACh currents (Fig. 4D). Therefore, nefiracetam potentiates $\alpha4\beta2$-like ACh currents *via* G_s proteins.

The results that the nefiracetam potentiation of nAChR currents is prevented by cholera toxin but not by PKA inhibitors are at variance with the cholera toxin-cAMP: PKA cascade. However, there have been many cases in which cellular processes are modulated by elevated cAMP levels *via* PKA-independent pathways.[24—27] Further experiments are needed to confirm whether nefiracetam potentiation is due to the $G_{\alpha s}$ membrane-delimited pathway or to a cAMP-depen-

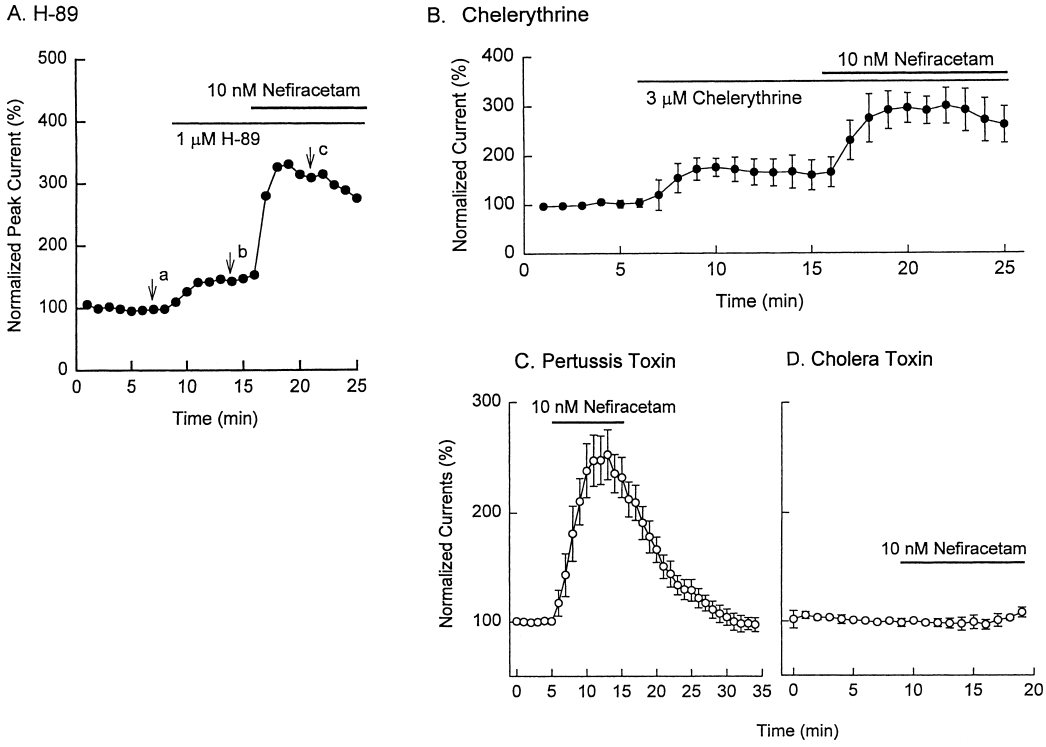

Fig. 4. Effects of Protein Kinase Inhibitors and G Protein Inhibitor and Stimulator on Nefiracetam Potentiation of $\alpha4\beta2$-Like nAChR Currents in Rat Cortical Neurons

(A) The PKA inhibitor H-89 did not prevent nefiracetam potentiation. (B) The PKC inhibitor chelerythrine did not prevent nefiracetam potentiation. (C) The G_i/G_o protein inhibitor pertussis toxin did not prevent nefiracetam potentiation. (D) The G_s protein stimulator cholera toxin prevented nefiracetam potentiation.[2]

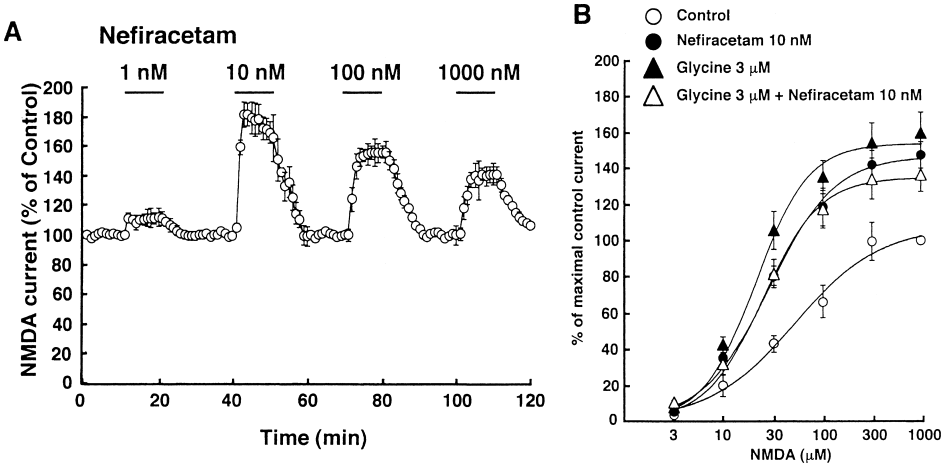

Fig. 5. (A) Nefiracetam (1—1000 nM) Potentiation of NMDA Currents Exhibiting a Bell-Shaped Dose–Response Relationship in Rat Cortical Neurons and (B) Dose–Response Curves for NMDA Currents in the Control, in 10 nM Nefiracetam, in 3 μM Glycine, and in 3 μM Glycine Plus 10 nM Nefiracetam[21)]

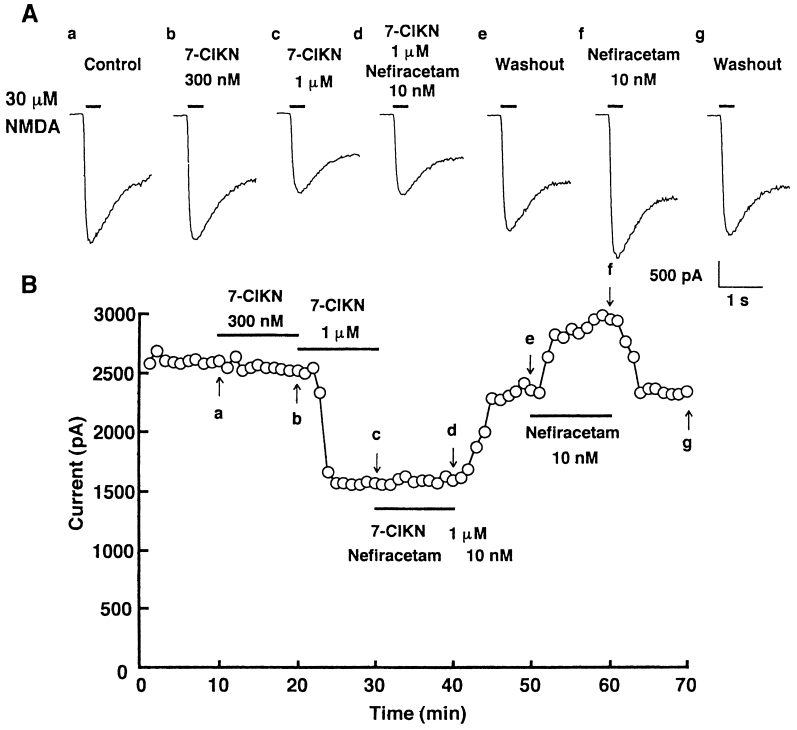

Fig. 6. 7-Chlorokynurenic Acid (7-ClKN) at 1 μM Suppresses NMDA Currents and Abolishes Nefiracetam (10 nM) Potentiation in a Rat Cortical Neuron[21)]

dent process other than the PKA pathway.

2.3. Nefiracetam Potentiation of NMDAR Currents: Interactions with Glycine It is well known that glutamate receptors play an important role in memory/learning and excitotoxicity. Thus, it is possible for nefiracetam to modulate glutamate receptor currents. The responses of NMDA receptors to nefiracetam application depended on the presence or absence of glycine added in the bath.[21)] The initial experiments were performed in Mg^{2+}-free media to avoid voltage-dependent Mg^{2+} block. In cortical neurons without the addition of glycine, nefiracetam 1—1000 nM potentiated NMDA-induced currents in multipolar neurons (diameter 30—60 μm) but not in bipolar neurons (diameter 15—30 μm). Therefore, all experiments were performed using multipolar neurons. Similar to nefiracetam potentiation of $\alpha4\beta2$-like nAChRs, a bell-shaped dose–response relationship was obtained (Fig. 5A), and nefiracetam potentiated the saturating currents induced by high concentrations (300—100 μM) of NMDA (Fig. 5B).

Nefiracetam appears to interact with the glycine-binding site of the NMDA receptor.[21)] Glycine 100—3000 nM potentiated NMDA-induced currents and abolished nefiracetam potentiation of the currents (Fig. 5B). 7-Chlorokynurenic acid (7-ClKN), a glycine site blocker, decreased NMDA currents and abolished nefiracetam potentiation of the currents (Fig. 6). One possible explanation for these results is that nefiracetam binds to the glycine site in the NMDA receptors, acting as a partial agonist.

Nefiracetam 10 nM also potentiated AMPA-evoked currents in cortical neurons, but the effect was much less effica-

cious than that on NMDA currents. It had no effect on kainate-induced currents in cortical neurons.[21)]

2.4. Roles of Protein Kinases and G Proteins in Nefiracetam Potentiation of NMDAR Currents The PKA inhibitor H-89 slightly decreased NMDA currents, yet nefiracetam 10 nM could still potentiate the currents. On the other hand, the PKC inhibitor chelerythrine, which suppressed NMDA currents, completely abolished nefiracetam potentiation. Pretreatment with pertussis toxin or cholera toxin did not prevent nefiracetam potentiation. Thus, the NMDA receptor is different from the nAChRs with respect to nefiracetam modulation: PKC plays a role in the nefiracetam modulation of the former, and G_s proteins play a role in that of the latter.

3. GALANTAMINE

3.1. Galantamine Modulation of nAChRs In confirmation of the results of previous studies,[3—6)] we have recently found that galantamine potentiated the $\alpha4\beta2$-like nAChR current at doses of 100 nM—1 μM. It was not as potent and efficacious as nefiracetam, and potentiation was limited to 15—20% of the control ACh current. We observed no effect of galantamine on the $\alpha7$-like nAChR.

3.2. Galantamine Modulation of NMDA Receptors Galantamine 10 nM—10 μM reversibly potentiated NMDA-induced currents in cortical neurons (Fig. 7).[28)] Similar to the potentiation of nAChR currents, a bell-shaped dose–response relationship was seen. However, galantamine was different from nefiracetam in at least two respects: 1) Galantamine did not potentiate the saturating currents evoked by high concentrations of NMDA and merely shifted the NMDA dose–response curve in the direction of lower concentrations of NMDA resulting in a decrease in the EC_{50} value for NMDA from 37 μM to 26 μM; and 2) galantamine did not interact with the glycine-binding site of the NMDA receptor, and 7-ClKN did not prevent galantamine from potentiating NMDA currents (Fig. 8).

The potentiation of the NMDA current caused by 1 μM galantamine was abolished by the PKC inhibitor chelerythrine, but not by the PKA inhibitor H-89, pertussis toxin or cholera toxin. Therefore, galantamine potentiation of NMDA currents is mediated by PKC but not by PKA, G_i/G_o proteins or G_s proteins.[28)]

4. DONEPEZIL

As described earlier, donepezil is the most popular Alzheimer's disease drug in the US market. Because of its potent anticholinersterase action with an IC_{50} value of 6.7—26 nM, donepezil has been believed to act primarily on the cholinergic system. We have recently found that donepezil also acts on the NMDA system by potentiating NMDA-induced currents in some of the cortical neurons tested.[29)]

Donepezil 100 nM or 10 μM had no effect on the currents induced by 30 μM ACh in the $\alpha4\beta2$ nACh receptor. The effects of donepezil on NMDA-induced currents differed greatly between multipolar and bipolar neurons in culture. NMDA currents in multipolar neurons were slightly suppressed by 1—10 μM donepezil to 90—75% of the control value, yet greatly potentiated by 30—100 μM donepezil to

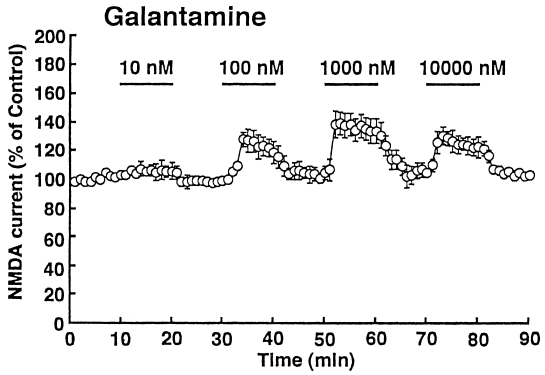

Fig. 7. Galantamine 10—10000 nM Potentiates NMDA Currents in Rat Cortical Neurons[28)]

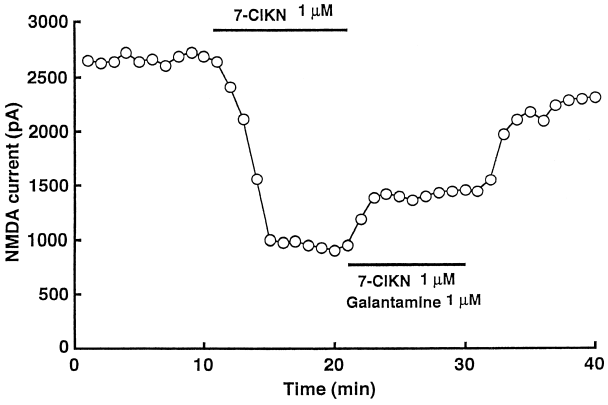

Fig. 8. 7-Chlorokynurenic Acid (7-ClKN) 1 μM Suppresses NMDA Currents, but Does not Prevent Galantamine 1 μM from Potenitating NMDA Currents in a Rat Cortical Neuron[28)]

145—250% of the control value. All of these effects were reversible after washing with donepezil-free media. In contrast, the NMDA currents of bipolar neurons were potentiated by donepezil in a concentration-dependent manner. Even at 1 nM, the currents were potentiated to 115% of the control value, and the maximum potentiation to 200% of the control value occurred at the dose of 10 μM. These effects were also reversible after washing with drug-free solutions. The reasons for the differential actions of donepezil on multipolar and bipolar neurons remain unclear, although one possibility would be different combinations of NMDA receptor subtypes.

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