Mechanisms of Action of Cognitive Enhancers on Neuroreceptors

Toshio Narahashi,∗a Shigeki Moriguchi,a,b Xilong Zhao,a William Marszalec,a and Jay Zeus Yeha

a Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School; 303 E. Chicago Avenue, Chicago, IL 60611, U.S.A.: and b Department of Pharmacology, Tohoku University Graduate School of Pharmaceutical Sciences; Aramaki-Aoba, Aoba-ku, Sendai 980—8578, Japan.

Received July 20, 2004

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 the glycine-binding site of the receptor. Galantamine had a moderate potentiating effect on the \( \alpha_2\beta_2 \) receptor and potentiated NMDA currents with the maximum effect at 1 \( \mu \)M. 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 \( \beta \)-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 nefiracetam2) and galantamine3—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 \( \mu \)M.3—5) Nefiracetam is extremely potent in potentiating ACh-induced currents in the \( \alpha_4\beta_2 \)-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 \( \beta \)-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-
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 models10,11 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 1 μM, and the effect was exerted via G1/Gi 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 G1/Gi and protein kinase A (PKA), but potentiated by higher concentrations (1—10 μM) via protein kinase C (PKC).17) The α7 AChRs expressed in oocytes were potentiated by ≥100 nM nefiracetam, and the α4β2 nAChRs were potentiated by ≥1 μM 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.19) Our recent studies have clearly shown that nefiracetam 0.1—1 nm potentiates ACh-induced currents in the α4β2-like receptors in rat cortical neurons20) 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 α4β2-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](image)

![Fig. 2. Potentiation of α-BuTX-Insensitive, α4β2-Like ACh Currents by Nefiracetam 1 nm (A, B) and 10 μM (D) Nefiracetam in Rat Cortical Neurons in Primary Culture](image)
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 α4β2-like ACh currents, the α7-like ACh currents were not potentiated by nefiracetam but slightly suppressed.23)

It is interesting to note that nefiracetam potentiation was observed even at ACh concentrations that caused saturating responses (Fig. 3).21) The result is similar to ethanol potentiation of the α4β2-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.

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 α4β2-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 Gi/Go 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 α4β2-like ACh currents via Gs 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 Gs membrane-delimited pathway or to a cAMP-depen-
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 \(\mu\)m) but not in bipolar neurons (diameter 15—30 \(\mu\)m). Therefore, all experiments were performed using multipolar neurons. Similar to nefiracetam potentiation of \(\alpha 4/\beta 2\)-like nAChRs, a bell-shaped dose–response relationship was obtained (Fig. 5A), and nefiracetam potentiated the saturating currents induced by high concentrations (300—100 \(\mu\)M) 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 Ne- 
fricetam Potentiation of NMDAR Currents The PKA 
inhibitor H-89 slightly decreased NMDA currents, yet nefa- 
ricetam 10 nM could still potentiate the currents. On the 
other hand, the PKC inhibitor chelerythrine, which sup- 
pressed NMDA currents, completely abolished nefiracetam 
potentiation. Pretreatment with pertussis toxin or chole- ra 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, 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 α4β2-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 α7-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 concentra- 
tions of NMDA and merely shifted the NMDA dose–re-

sponse curve in the direction of lower concentrations of 
NMDA resulting in a decrease in the EC50 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-
CIKN 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 chelery-
thrine, 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,α proteins 
or G, proteins.28)

4. DONEPEZIL

As described earlier, donepezil is the most popular Alzheimer’s disease drug in the US market. Because of its potent anticholinesterase action with an IC50 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 α4β2 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 sup- 
pressed by 1—10 μM donepezil to 90—75% of the control value, yet greatly potentiated by 30—100 μM donepezil to 
145—250% of the control value. All of these effects were re-

versible 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 rea-
sons for the differential actions of donepezil on multipolar 
and bipolar neurons remain unclear, although one possibility would be different combinations of NMDA receptor sub-
types.

Acknowledgments This work was supported in part by 
Daiichi Pharmaceutical Co. and Janssen Pharmaceutica. We thank Julia Irizarry for secretarial assistance.

REFERENCES