Short Communication

Effect of Donepezil on Group II mGlu Receptor Agonist- or Antagonist-Induced Amnesia on Passive Avoidance in Mice

Tomoaki Sato,[1] Koh-ichi Tanaka,[1] Yoshiko Ohnishi,[1] Masahiro Irifune[2] and Takashige Nishikawa[1]

[1]*Department of Applied Pharmacology, Kagoshima University Graduate School of Medical & Dental Sciences, Sakuragaoka, Kagoshima 890-8544, Japan;* [2]*Department of Anesthesiology, Hiroshima University Dental Hospital, Kasumi, Minami-ku, Hiroshima, 734-8533, Japan*

SUMMARY

We examined the effect of the acetylcholinesterase (AChE) inhibitor, donepezil hydrocloride (DONP), on group II metabotropic glutamate (mGlu) receptor agonist- or antagonist-induced amnesia in the step-through passive avoidance task in male mice. DCG-IV, a group II mGlu receptor agonist, at dose of 50 ng and LY341495, a group II mGlu receptor antagonist, at dose of 300 ng, significantly attenuated the latency on the step-through task. The subcutaneous injection of DONP at dose of 1 mg/kg 1 hour before passive avoidance performance ameliorated the amnesia induced by DCG-IV and LY341495, whereas donepezil alone did not affect task latency. The results suggest that activation of group II mGlu receptors and disinhibition of the cAMP/PKA signaling pathway (caused by group II mGlu receptor antagonist) have a negative action on step-through passive avoidance memory performance, and that group II mGlu receptors and ACh interact to modulate learning and memory function.

Reprint requests to: Tomoaki Sato, Department of Applied Pharmacology, Kagoshima University Graduate School of Medical & Dental Sciences, Sakuragaoka, Kagoshima 890-8544, Japan; e-mail: tomsato@dentb.hal.kagoshima-u.ac.jp

KEYWORDS

learning, memory, metabotropic glutamate receptors, acetylcholinesterase, step-through, donepezil

INTRODUCTION

Widely accepted is that the glutamatergic and cholinergic systems are actively involved in the processes of learning and memory. In fact, Li et al. (1997) demonstrated that the NMDA antagonists MK-801 and CGS-19755 significantly augmented scopolamine-induced impairment of memory performance. In addition, Pavlovsky et al. (2003) revealed that the AChE inhibitor pyridostigmine enhanced glutamatergic transmission in hippocampal CA1 neurons.

Previous behavioral studies on metabotropic glutamate receptors by Bianchin and coworkers (1994) showed that ACPD ((1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid), a selective agonist for group II mGluRs, enhances memory of the avoidance task. In addition, Riedel et al. (2002) suggested that group II mGluRs modulate neural networks involved in conditioning formation. Although these recent studies have described a relation between group II mGluRs and learning and memory, further studies are necessary to

define more clearly the nature of this relation. In previous studies, we suggested that cAMP/protein kinase A (PKA) signaling-pathway modulation through group II mGlu receptors coupled with G*i* protein disrupted learning and memory performance in step-through passive avoidance (Sato et al., 2003). The relation between ACh and group II mGlu receptors has been studied by electrophysiologic methods (Pisani, et al., 2002), but behavioral studies involving learning and memory performance have not yet been conducted. Therefore, the present study was designed to clarify whether mGlu2/3 receptor agonist- or antagonist-induced amnesia in a step-through passive avoidance task would be ameliorated by the AChE inhibitor, donepezil.

EXPERIMENTAL

Male ddY mice (Kyudou, Ltd., Kumamoto, Japan), 5 to 6 wk old, were housed with free access to standard food (Clea Japan Inc.) and water in an air-conditioned room at a temperature of 24 ± 1 °C, 50 ± 10% humidity, and a constant 12-h light/dark cycle (lights on between 7:00 and 19:00). Behavioral experiments were carried out between 9:00 and 17:00. All procedures were approved by the Committee of Animal Experimentation, Dental School, Kagoshima University.

The following drugs were used throughout the experiments: (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV) and (2S)-2-amino-2-[(1S,2S)-2-carboxy cycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495) were purchased from Tocris Cookson Ltd. (Bristol, UK). Donepezil hydrochloride (DONP) was obtained from Nippon Chemiphar Co. Ltd. (Saitama, Japan). LY341495 was dissolved in dimethylsulfoxide (DMSO) and then diluted with saline to a final concentration of 0.05% (v/v) DMSO. DCG-IV was dissolved in 0.9% saline and the solution was prepared at the same final DMSO concentration. A DMSO (0.05%) vehicle was used in control mice. The vehicle, DCG-IV (50 ng/mouse) or LY341495 (300 ng/mouse) was intracisternally injected according to the method of Ueda et al. (1979). The injections (10 μl each) were completed 10 minutes before the acquisition trial, according to the method of Matuoka et al. (1995). DONP was dissolved in 0.9% saline and injected (0.1–1 mg/kg) subcutaneously 60 minutes before the acquisition trial.

Learning and memory ability was assessed by the step-through-type passive avoidance test using methods modified from Matuoka et al. (1995). In short, a two-compartment step-through-type passive avoidance apparatus was used. The box was divided into bright (9 × 9 × 36 cm) and dark compartments (26 × 26 × 36 cm) by a guillotine door. In each trial, a mouse was placed in the illuminated compartment for a 30-sec habituation period, and then a guillotine door was raised to allow entry into the dark chamber. On the pre-exposure session (data not shown), the step-through latency (the length of time spent in the bright compartment after a habituation period) was measured. Mice that stepped through to the grids of the dark compartment were allowed to remain there for 30 sec without electrical stimulation and were then returned to their home cage. After the measurement of the pre-exposure latency, the acquisition trial was conducted, 10 min after intracisternal drug injection. When the hind legs of the mice entered into the dark chamber, the guillotine door was closed and electrical foot shock (20 V, duration 80 ms, alternating current) was delivered through the grid floor for a total of 3 sec. The time that elapsed before entry into the dark compartment (latency) was recorded. Retention trials were then performed at 24 h after the acquisition trial and the latency was measured for up to 300 sec.

Statistical analysis was performed by one-way analysis of variance (ANOVA) with the Bonferroni/Dunn post-hoc test. Statistically significant differences between groups are indicated by $p < 0.05$.

RESULTS AND DISCUSSION

The effects of several doses of DONP, 50 ng DCG-IV (an mGlu 2/3 receptor agonist), and 300 ng LY341495 (an mGlu 2/3 receptor antagonist) on each trail of the step-through performance task are shown in Fig. 1. One-way ANOVA revealed a significant difference among the six treatment groups on the retention trial ($F(5, 39) = 13.50$, $p < 0.01$).

Additionally, post hoc tests showed that the intracisternal injection of DCG-IV ($p < 0.01$) or LY341495 ($p < 0.01$) significantly suppressed the latency of the task compared with that of the vehicle group on the retention trial but not that of the acquisition trial. In addition, no significant difference was found between the DONP and vehicle groups in either trail.

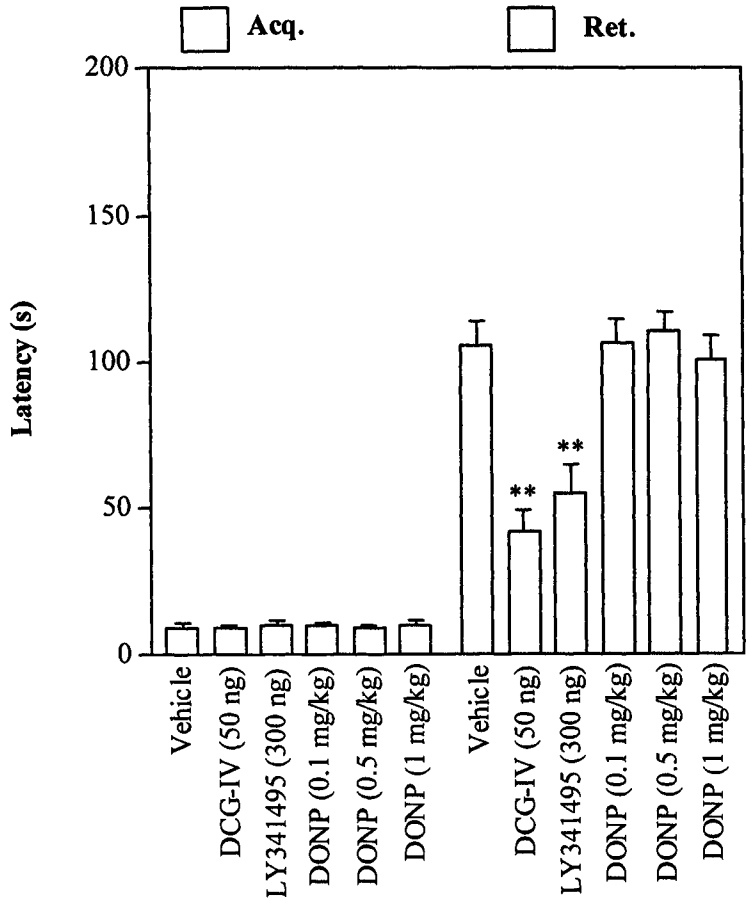

Fig. 1: Effects of DONP, DCG-IV and LY341495 on memory performance in the step-through passive avoidance task in mice. All doses of DONP were subcutaneously administered 1 h before and either vehicle, DCG-IV, or LY341495 were injected intracisternally 10 min before the acquisition trial. Values are mean ± SEM for 7 to 10 animals per group. **$p < 0.01$ vs. vehicle (one-way ANOVA followed by post-hoc test) on the retention trial. Acq. = acquisition, Ret. = retention, DONP = donepezil hydrochloride

As shown in Fig. 2, in the passive avoidance task DONP significantly affected (A) DCG-IV- ($F[3, 24] = 3.29$, $p < 0.05$) and (B) LY341495-induced ($F[3, 24] = 3.06$, $p < 0.05$) amnesia on the retention trial. Post hoc tests showed that DONP at 1 mg/kg significantly inhibited the amnesia induced by DCG-IV at 50 ng (A) and by LY341495 at 300 ng (B) on the retention trial ($p < 0.05$, for both). No significant differences in locomotor activity were seen among the treatment groups (data not shown).

In present study, we found that DONP administration at 1 mg/kg improved either DCG-IV- or LY341495-induced impairment of passive-avoidance performance in mice. The ameliorative effect of DONP was not caused by non-cognitive effects, such as a decrease in locomotion or electrical sensitivity, because the administration DONP affected neither the locomotor activity nor the latency of the vehicle group on the acquisition trial. As mGlu2 and/or mGlu3 receptors are reportedly linked to the cAMP/PKA signaling pathway (Schoepp et al., 2001; Robbe et al., 2002), the inhibition of latency induced by DCG-IV can be caused by an inhibition of the cAMP/ PKA signaling pathway via the activation of mGlu2/3 receptors (Schoepp, et al., 2001; Sato, et al., 2003). The enhancement of glutamate release is known to be caused by ACh in hippocampal regions, such as CA1 (Pavlovsky, et al., 2003; Takeda, et al., 2003) or in the dentate gyrus (Wheal, et al., 1980). The administration of DONP at 1 mg/kg might cause an increase of ACh at synapses, and consequently, the enhancement of glutamate release could counteract the inhibition of the cAMP/PKA signaling pathway induced by DCG-IV via other receptors such as mGlu 1 and 5, or NMDA receptors. In addition, DCG-IV reportedly reduces ACh release in the striatum of rats (Pisani, et al., 2002); therefore, the administration of DCG-IV at 50 ng might have also inhibited ACh release in the present study. Together, these findings suggest that the ameliorating effect of donepezil against DCG-IV-induced amnesia is due to an increase in synaptic ACh.

The administration of LY341495 at 300 ng reduced the latency on the passive avoidance task, in agreement with the results in our previous report (Sato, et al., 2003), therefore reinforcing the notion that excessive activation of the cAMP/PKA-signaling pathway impairs learning and memory. Because LY341495 is an antagonist of mGlu2 and/or mGlu3 receptors, some think that LY341495 tends to stimulate cAMP formation (Kingston et al., 1998). In fact, our previous study also showed that excessive administration of forskolin or rolipram decreases latency on the passive avoidance task (Sato et al., 2003). Donepezil, however, inhibited the reduction of latency induced by LY341495.

The mechanism underlying the ameliorating effect of donepezil is unknown, though we can speculate on some of the possible mechanisms. First, donepezil has been reported to increase the amount of ACh by inhibiting AChE, thus resulting in increased levels of ACh, which can bind not only with postsynaptic muscarinic M1 receptors but also with presynaptic muscarinic M2 receptors. Therefore, ACh binding to M2 receptors can inhibit ACh release through inhibitory feedback. Consequently, at certain concentrations (achieved by the inhibitory feedback effect on synaptic ACh release), ACh decreases the release of glutamate in the synaptic region (Marchi et al., 1989). Thus, the decreased glutamate release can ameliorate the excess (LY341495 induced) activation of the signaling-pathway. The ameliorating effect of donepezil might therefore be mediated by the balance between M1 receptor activation and M2 receptor binding. Also possible is that muscarinic receptor activation induced by donepezil increases GABA release in intermediate synapses (Vogt & Pegehr, 2001), and the released GABA might modulate the activation of postsynaptic neurons because this neurotransmitter inhibits a higher

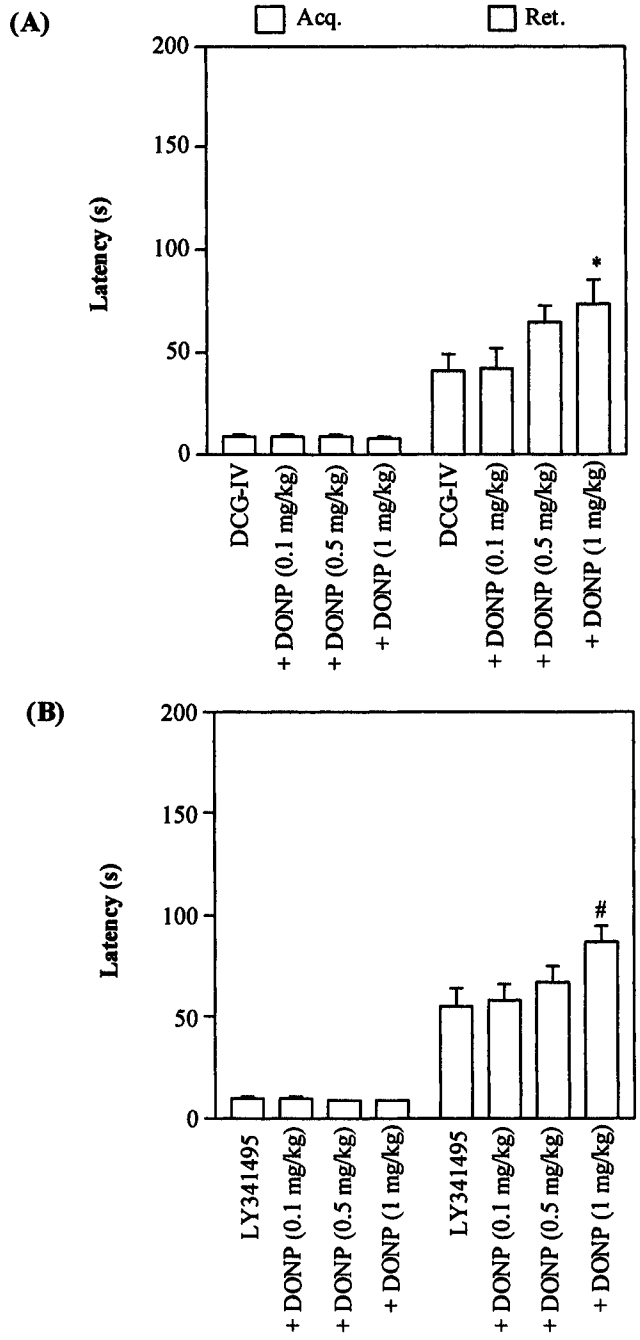

Fig. 2: Effects of DONP on the passive avoidance task in mice with DCG-IV- (A) and LY341495- (B) induced amnesia. All doses of DONP were subcutaneously administered 1 h before the acquisition trial, and DCG-IV and LY341495 were injected intracisternally 10 min prior to the acquisition trial. Values are mean ± SEM for seven animals per groups. *$p < 0.05$ vs. DCG-IV; #$p < 0.05$ vs. LY341495 (one-way ANOVA followed by post-hoc test) on the retention trial.

dimension function. As mentioned above, LY341495 can increase glutamate release by disinhibiting mGlu 2/3 receptors, and the increase in glutamate release can cause an excess activation of the cAMP/PKA signaling pathway and an impairment of learning and memory performance (Sato et al., 2003). Therefore, the GABA release induced by donepezil might inhibit the excess activation of the signaling pathway and restore optimal activity of the neurons.

Another possibility is that the ameliorating effect of donepezil might be caused by nicotinic acetylcholine receptors (nACh-R). Excess glutamate release resulting from disinhibition by mGlu 2/3 receptor antagonist can cause neuronal cell damage. In fact, Takeda et al. (2003) reported a protective effect of donepezil against glutamate neurotoxicity in rat cortical neurons, which is consistent with the results of the present study. Another possibility is that the increase in ACh at presynaptic nerve terminals modulates glutamate release via nACh-R, as Santos et al. (2002) reported that strong and tonic activation of nACh-R in glutamatergic fibers reduces glutamate release in pyramidal neurons. Therefore, in the present study, the reduction of glutamate release might ameliorate the excess signaling pathway activity induced by LY341495. A final possibility is that both the cholinergic and group II mGluR drugs act on different brain systems because the drugs were not locally infused.

In conclusion, the results presented here provide primary evidence that the AChE inhibitor donepezil can ameliorate group II mGlu receptor agonist- and antagonist-induced disruption of the step-through task performance and suggest that the modulation of glutamate release by ACh might play an important role in this process. Further detailed studies are needed to examine the role of acetylcholine in modulating glutamate release and cognitive function.

ACKNOWLEDGEMENTS

This work supported, in part, by a Grant-in-Aid for Science Research (13771099; 2001–2002) from the Ministry of Education, Science, Sports and Culture of Japan. We thank Nippon Chemiphar for the providing donepezil hydrochloride.

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