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Dehydroevodiamine attenuates β-amyloid peptide-induced amnesia in mice

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Abstract

Dehydroevodiamine has been reported to have anticholinesterase activity and an anti-amnesic effect. This study examined the effects of dehydroevodiamine on scopolamine- and β-amyloid peptide-(25–35)-induced amnesia in mice, using a step-through passive avoidance test. Similarly to the cholinesterase inhibitor, physostigmine (0.03–0.3 mg/kg, i.p.), dehydroevodiamine (0.75–12.0 mg/kg, i.p.) administered 30 min before the training trial, immediately after the training trial, and 30 min before the retention test significantly improved scopolamine- and β-amyloid peptide-(25–35)-induced amnesia. In β-amyloid peptide-(25–35)-induced amnesia, the rank order of anti-amnesic potency in these three administration schedules for dehydroevodiamine was different from that for physostigmine. Furthermore, dehydroevodiamine was more potent to improve β-amyloid peptide-(25–35)-induced amnesia than scopolamine-induced amnesia when administered before the training trial. These results suggested that dehydroevodiamine may have an action other than that of an anticholinesterase and may be a novel and effective ligand for improvement of β-amyloid type amnesia.

Keywords: Dehydroevodiamine; β-Amyloid peptide; β-Amyloid peptide-(25–35); Amnesia; Step-through passive avoidance; Physostigmine

1. Introduction

It is well known that the cognitive deficits of patients with Alzheimer's disease are closely associated with dysfunction of central cholinergic neurotransmission (Whitehouse et al., 1981; Mountjoy et al., 1984). Therefore, one major current pharmacological therapy for Alzheimer's disease is to increase the level of the central cholinergic neurotransmitter, acetylcholine, with cholinesterase inhibitors (Knopman, 1998). Six separate cholinesterase inhibitors, including tacrine, have been shown to exhibit modest efficacy in well-designed clinical trials. However, use of many of these agents is restricted due to gastrointestinal side effects, short biological half-life, or narrow therapeutic range. Therefore, the cholinesterase inhibitors with fewest side effects and easiest dosing will have the greatest chance of acceptance (Knopman, 1998).

Dehydroevodiamine, one of the quinazoline alkaloids isolated from the unripe fruit of *Evodia rutaecarpa* Bentham, has been shown to be a non-competitive cholinesterase inhibitor (Park et al., 1996). Furthermore, single administration of dehydroevodiamine to rats significantly reverses the scopolamine-induced memory impairment in the passive avoidance and eight-arm radial maze tests (Park et al., 1996, 2000). Further study has also demonstrated that dehydroevodiamine can prevent impairment of learning and memory and neuronal loss in rat models of cognitive disturbance such as unilateral electrolytic lesion of the entorhinal cortex and the middle cerebral artery occlusion (Park et al., 2000). However, whether dehydroevodiamine has a beneficial effect in an animal model of Alzheimer's disease-type amnesia is still unknown.

It is well known that β-amyloid peptide is the major constituent of senile plaque, which is one of the pathological hallmarks of Alzheimer's disease and represents the underlying cause of the cognitive deficits observed in Alzheimer's disease (Cummings et al., 1996). It has been

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substantiated that the 11-amino acid sequence (25–35) of β-amyloid peptide is neurotoxic for primary neurons (Pike et al., 1993; Yankner et al., 1990). Intracerebroventricular (i.c.v.) administration of the aggregated form of β-amyloid peptide-(25–35) significantly induces neuronal loss and amyloid deposits in brain and impaired cognitive performance in Y-maze, passive avoidance, and water maze tasks in mice (Maurice et al., 1996a). Therefore, the present study used the passive avoidance test to examine whether dehydroevodiamine has an effect on the memory impairment induced by i.c.v. administration of aggregated β-amyloid peptide-(25–35) in mice as the first step to evaluate its potential value for the treatment of Alzheimer's disease. For comparison, the anti-amnesic effect of dehydroevodiamine on scopolamine-induced amnesia was also studied.

2. Materials and methods

2.1. Animals

Male ICR (Institute of Cancer Research) mice (25–30 g) were obtained from the Animal Center of National Yang–Ming University and maintained on a 12-h light and 12-h dark cycle (light on between 7:00 and 19:00) with food and tap water ad libitum.

2.2. Drugs

Dehydroevodiamine was prepared as before (Lin et al., 1991) and dissolved in twice-filtered water with the aid of ultrasonic vibration at 60°C for 30 min. (−)-Scopolamine hydrochloride, physostigmine hemisulfate salt, and amyloid β-protein fragment 25–35 (β-amyloid peptide-(25–35)) were purchased from Sigma. β-amyloid peptide-(25–35) was dissolved in sterile twice-filtered water and aggregated by incubation at 37°C for 4 days before use.

2.3. Passive avoidance test

2.3.1. Apparatus

The apparatus for the step-through passive avoidance test is an automated shuttle-box (Cat. 7551 Passive Avoidance Controller and Cat. 7553 Passive Avoidance Mouse Cage, UGO Basile, Italy), which is divided into an illuminated compartment and a dark compartment of the same size by a wall with a guillotine door.

2.3.2. Adaptation, training trial, and retention test

Each mouse was put through the adaptation trial by placing it in the illuminated compartment, facing away from the dark compartment. After 10 s, the door between these two boxes was opened and the mouse moved into the dark compartment freely. The latency to leave the illuminated compartment was recorded. Two hours after the adaptation trial, the mouse received the training trial. The training trial is similar to the adaptation trial except that the door is closed automatically as soon as the mouse steps into the dark compartment and an inescapable foot shock (0.6 mA, 2 s) is delivered through the grid floor (Venault et al., 1986). The responses to the electric shock were observed and scored as follows: 0, no response; 1, flinch (movement of any part of the body); and 2, run (running or jumping) or vocalization (Riekkinen, 1994). The retention test was performed 24 h after the training trial in the same manner without the electric shock and the latency of step-through to the dark compartment was recorded. The maximum cut-off time for step-through latency was 300 s (Venault et al., 1986).

2.4. Amnesia models

In scopolamine-induced amnesia, scopolamine (1 mg/kg, i.p.) was administered 20 min prior to the training trial.

In β-amyloid peptide-(25–35)-induced amnesia, the aggregated form of β-amyloid peptide-(25–35) (3 nmol) was administered i.c.v. using a microsyringe with a 28-gauge stainless-steel needle 3.0-mm-long (Hamilton), according to Maurice et al. (1996a,b). In brief, the needle was inserted unilaterally 1 mm to the right of the midline point equidistant from each eye, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. Peptides or sterile twice-filtered water (3 μl) were delivered gradually within 3 s. Mice exhibited normal behavior within 1 min after injection. It has been shown by Maurice et al. (1996b, 1998) that the scrambled β-amyloid peptide-(25–35) is without effect in mice, but decreases locomotor activity. Therefore, sterile twice-filtered water, but not scrambled β-amyloid peptide-(25–35), was used in the present study as the control. Neither insertion of the needle nor injection of the twice-filtered water had a significant influence on survival, behavioral responses, or cognitive function. After 7 days, the mice were put through the passive avoidance test (Maurice et al., 1996a).

To examine the anti-amnesic effect of dehydroevodiamine or physostigmine, a test drug was i.p. administered 30 min before or immediately after the training trial, or 30 min before the retention test (Matsuno et al., 1994).

2.5. Data analysis

The results were expressed as medians and interquartile ranges. The data were analyzed by U-test or Kruskal–Wallis test and followed by a Dunn test with a significance level of $P < 0.05$.

3. Results

Throughout the passive avoidance test, it was found that none of the treatments affected either the step-through

latency in the adaptation and training trials or the sensitivity to electric shocks, as compared with that of the control animals (data not shown).

3.1. Effects on the scopolamine-induced amnesia

As shown in Fig. 1, the step-through latency of the scopolamine (1 mg/kg, i.p.)-treated group was significantly reduced as compared with that of the control group ($P < 0.01$). Physostigmine and dehydroevodiamine markedly improved scopolamine-induced amnesia when administered 30 min before the training trial (physostigmine: $H(3) = 15.2$, $P < 0.01$; dehydroevodiamine: $H(3) = 11.8$, $P < 0.01$), immediately after the training trial (physostigmine: $H(3) = 16.0$, $P < 0.01$; dehydroevodiamine: $H(4) = 18.9$, $P < 0.01$), or 30 min before the retention test (physostigmine: $H(3) = 22.0$, $P < 0.01$; dehydroevodiamine: $H(3) = 14.8$, $P < 0.01$) (Fig. 1). The rank order of anti-amnesic potency was post-training > pre-training = pre-test for both physostigmine and dehydroevodiamine. When administered immediately after the training trial, the lowest effective doses for physostigmine and dehydroevodiamine to significantly improve amnesia were 0.03 and 3 mg/kg, respectively.

Table 1
Effect of centrally administered β-amyloid peptide-(25–35) in the step-through passive avoidance test in mice

Treatment (i.c.v.)	*n*	Step-through latency (s)	
		Median	Interquartile range
None	13	300.0	181.4–300.0
Vehicle (H_2O)	18	253.0	136.9–300.0
β-Amyloid peptide-(25–35) (3 nmol)	23	26.7[a]	18.0–56.1

[a] $P < 0.01$, as compared with the vehicle control group.

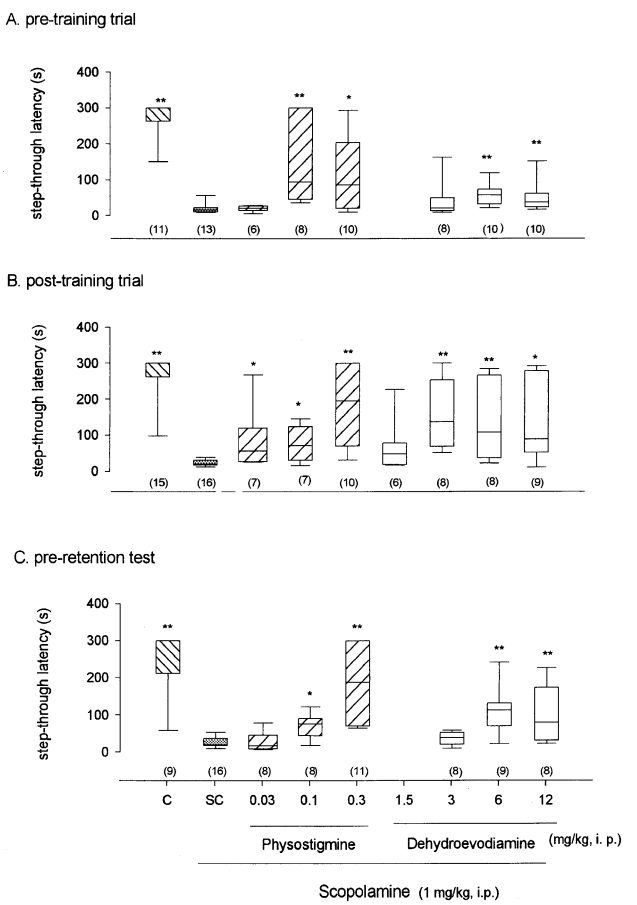

Fig. 1. Effects of physostigmine and dehydroevodiamine on scopolamine-induced amnesia in the step-through passive avoidance test in mice. Mice were treated with scopolamine (1 mg/kg, i. p.) 20 min prior to the training trial to induce amnesia. Various doses of physostigmine (0.03–0.3 mg/kg, i.p.) or dehydroevodiamine (1.5–12 mg/kg, i.p.) were administered 30 min before the training trial (A), immediately after the training trial (B), or 30 min before the retention test (C). Data are expressed as medians (horizontal bar) and interquartile ranges (column). The number of mice in each group is indicated in parentheses. $^{*}P < 0.05$ and $^{**}P < 0.01$, as compared with the scopolamine-treated control group (SC).

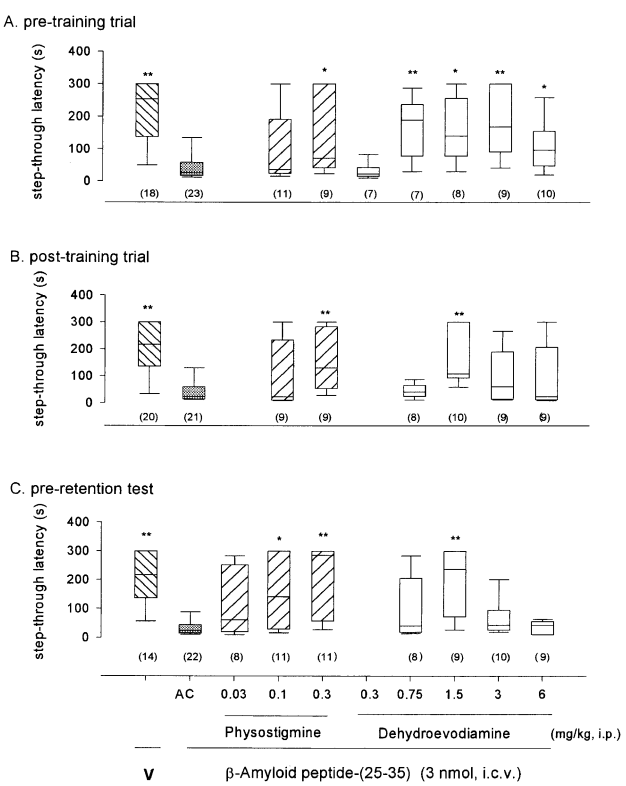

Fig. 2. Effects of physostigmine and dehydroevodiamine on β-amyloid peptide-(25–35)-induced amnesia in the step-through passive avoidance test in mice. Seven days after i.c.v. administration of the vehicle, twice-filtered water (V), or the aggregated form of β-amyloid peptide-(25–35) (3 nmol), mice were subjected to the passive avoidance test. Various doses of physostigmine (0.03–0.3 mg/kg, i.p.) or dehydroevodiamine (0.3–6 mg/kg, i.p.) were administered 30 min before the training trial (A), immediately after the training trial (B), or 30 min before the retention test (C). Data are expressed as medians (horizontal bar) and interquartile ranges (column). The number of mice in each group is indicated in parentheses. $^{*}P < 0.05$ and $^{**}P < 0.01$, as compared with the β-amyloid peptide-(25–35)-treated control group (AC).

3.2. Effects on the β-amyloid peptide-(25–35)-induced amnesia

Seven days after i.c.v. administration of twice-filtered water (vehicle control) or the aggregated form of β-amyloid peptide-(25–35) (3 nmol), mice were trained for the step-through passive avoidance task. As shown in Table 1, the vehicle control group showed a step-through performance comparable to that of the non-treated group, whereas the β-amyloid peptide-(25–35)-treated group showed a significant decrease in latency ($H(2) = 23.1$, $P < 0.01$). As shown in Fig. 2, physostigmine and dehydroevodiamine markedly improved β-amyloid peptide-(25–35)-induced amnesia when administered 30 min before the training trial (physostigmine: $H(2) = 6.5$, $P < 0.05$; dehydroevodiamine: $H(5) = 20.8$, $P < 0.01$), immediately after the training trial (physostigmine: $H(2) = 6.5$, $P < 0.05$; dehydroevodiamine: $H(4) = 10.5$, $P < 0.05$), or 30 min before the retention test (physostigmine: $H(3) = 14.7$, $P < 0.01$; dehydroevodiamine: $H(4) = 10.3$, $P < 0.01$). The rank order of anti-amnesic potency for physostigmine was pre-test > post-training = pre-training, and that for dehydroevodiamine was pre-training > post-training = pre-test. The lowest effective doses for test drugs to significantly improve $A\beta_{25-35}$-induced amnesia were, 0.1 mg/kg physostigmine administered 30 min before the retention test, and 0.75 mg/kg dehydroevodiamine administered 30 min before the training trial.

4. Discussion

The processes of learning and memory involve information acquisition, consolidation, and retrieval. To evaluate the effect of a test drug on information acquisition, consolidation, or retrieval in the passive avoidance test, the drug is administered before the training trial, immediately after the training trial, or before the retention test (Matsuno et al., 1994). Previous studies have shown that dehydroevodiamine administered prior to the training trial has an anti-amnesic effect on scopolamine-induced amnesia in rats (Park et al., 1996, 2000). Consistent with results of these studies, the present study in mice further demonstrated that dehydroevodiamine administered immediately after the training trial and 30 min before the retention test also had an anti-amnesic effect on scopolamine-induced amnesia. The most important aspect of this study was to demonstrate that dehydroevodiamine improved β-amyloid peptide-(25–35)-induced amnesia.

Some studies have shown that cholinesterase inhibitors such as physostigmine and tetrahydroaminoacridine administered according to the above-mentioned three schedules all attenuate scopolamine-, cycloheximide-, and *p*-chloroamphetamine-induced amnesia in mice (Matsuno et al., 1994; Nabeshima et al., 1988; Senda et al., 1997). This suggests that cholinesterase inhibitor can facilitate cognitive functions in the acquisition, consolidation, and retrieval stages of learning and memory processes. Since dehydroevodiamine is a cholinesterase inhibitor, it is expected that the same results should be obtained. Indeed, the present study showed that dehydroevodiamine, like physostigmine administered according to three schedules, attenuated both scopolamine- and β-amyloid peptide-(25–35)-induced amnesia in mice. However, there are some differences between dehydroevodiamine and physostigmine when the rank order of anti-amnesic potency in three administration schedules and the lowest effective dose in both amnesic models are compared. In scopolamine-induced amnesia, the rank order of anti-amnesic potency for both drugs was the same, i.e. post-training > pre-training = pre-test. In β-amyloid peptide-(25–35)-induced amnesia, the order became pre-test > post-training = pre-training for physostigmine, and pre-training > post-training = pre-test for dehydroevodiamine. Physostigmine appeared to have a more potent anti-amnesic effect on scopolamine- (effective doses, 0.03–0.1 mg/kg) than on β-amyloid peptide-(25–35)-induced amnesia (effective doses, 0.1–0.3 mg/kg) when administered at the post-training session. In contrast, dehydroevodiamine appeared to be more potent on β-amyloid peptide-(25–35)- (effective doses, 0.75–6 mg/kg) than scopolamine-induced amnesia (effective dose, 6–12 mg/kg) when administered at the pre-training session. The potency of dehydroevodiamine (IC_{50} value = 37.8 μM) to inhibit acetylcholinesterase activity is 300-fold less than that of physostigmine (IC_{50} value = 0.12 μM) (Park et al., 1996). However, dehydroevodiamine (minimum effective doses, 0.75 and 1.5 mg/kg) was about 2.5–15-fold less potent than physostigmine (0.1 and 0.3 mg/kg) to improve β-amyloid peptide-(25–35)-induced amnesia. This suggests that the anti-amnesic effect of dehydroevodiamine on β-amyloid peptide-(25–35)-induced amnesia seems attributable not only to the acetylcholinesterase inhibition but also involves some other action, for example, enhancing cerebral blood flow (Haji et al., 1994). Because both cholinergic and glutamatergic dysfunctions are involved in the β-amyloid peptide-(25–35)-induced amnesia (Maurice et al., 1996a,b), it is worth studying whether dehydroevodiamine also has an action on the glutamatergic system. Recently, σ_1 receptor agonists have been reported to attenuate both scopolamine- and β-amyloid peptide-(25–35)-induced amnesia (Senda et al., 1997; Maurice et al., 1998). The results obtained with our established method for σ_1 receptor binding assay (Chou et al., 1999) indicated that dehydroevodiamine at 10^{-4} M had no significant interaction with σ_1 receptors on mouse brain membranes (Liao et al., unpublished data).

In conclusion, this study showed for the first time that dehydroevodiamine, given according to three administration schedules of passive-avoidance testing, has anti-amnesic effects on both scopolamine- and β-amyloid peptide-(25–35)-induced amnesia in mice. The beneficial

effect of dehydroevodiamine on Alzheimer's disease-type dementia is worth further study.

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