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Pretreatment with 5-hydroxymethyl-2-furaldehyde blocks scopolamine-induced learning deficit in contextual and spatial memory in male mice

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ABSTRACT

5-Hydroxymethyl-2-furaldehyde (5-HMF) is a compound derived from the dehydration of certain sugars. The aim of the present study was to evaluate the effect of 5-HMF on the cognitive impairment induced by scopolamine, a muscarinic receptor antagonist. To measure various cognitive functions, we conducted the step-through passive avoidance task, the Y-maze task and the Morris water maze task. A single administration of 5-HMF (5 or 10 mg/kg, p.o.) significantly attenuates scopolamine-induced cognitive impairment in these behavioral tasks without changes in locomotor activity, and the effect of 5-HMF on scopolamine-induced cognitive impairment was significantly reversed by a sub-effective dose of MK-801, an NMDA receptor antagonist. In addition, a single administration of 5-HMF (10 mg/kg, p.o.) enhanced the cognitive performance of normal naïve mice in the passive avoidance task. Furthermore, Western blot analysis revealed that the levels of phosphorylated Ca^{2+}/calmodulin-dependent protein kinase II-α (CaMKII) and extracellular signal-regulated kinases (ERK) were significantly enhanced by the single administration of 5-HMF in the hippocampal tissues. Taken together, the present study suggests that 5-HMF may block scopolamine-induced learning deficit and enhance cognitive function via the activation of NMDA receptor signaling, including CaMKII and ERK, and would be an effective candidate against cognitive disorders, such as Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is an age-related disorder characterized by the accumulation of senile plaques and neurofibrillary tangles and the loss of synaptic functions (Hyman et al., 2012; Spires-Jones and Hyman, 2014). It is well known that the brain of AD patients exhibits a significant loss of the cholinergic nervous system (Mufson et al., 2008), and the degeneration of cholinergic neurons in the basal forebrain, cerebral cortex and other areas contributes significantly to the cognitive decline in AD (Francis et al., 1999; Hasselmo, 2006). Current therapies for AD include acetylcholinesterase (AChE) inhibitors, such as donepezil, rivastigmine and galantamine, which promote the activity of the cholinergic neurotransmitter system by accumulating neurotransmitters in the synaptic cleft of cholinergic neurons (McGleenon et al., 1999). In addition, N-methyl-D-aspartate (NMDA) receptor antagonists (e.g., memantine) are also prescribed for severe AD patients (Kilpatrick and Tilbrook, 2002). Although such agents are mainly used in clinical settings, their therapeutic potential is limited, and several adverse effects have been identified (Farlow et al., 2011).

5-Hydroxymethyl-2-furaldehyde (5-HMF, Fig. 1) is a compound derived from the dehydration of certain sugars (Rosatella et al., 2011). 5-HMF is barely found in fresh food, but it is naturally generated in sugar-containing food during heat-treatments, such as drying or cooking. It has been reported that 5-HMF has various beneficial effects, such as anti-oxidant activity and a protective effect against hypoxic injury (Li et al., 2011; Zhao et al., 2013). Although several in vitro tests and studies on rats show a possible carcinogenic potential, there has been no report about correlation between intakes of 5-HMF and any disease in humans (Husoy et al., 2008). In addition, it has been found to bind specifically to intracellular sickle hemoglobin. Preliminary studies showed that the administration of 5-HMF inhibits the formation of sickled cells in the blood (Abdulmalik et al., 2005; Hannemann et al., 2014). Currently, 5-HMF is undergoing clinical trials for the treatment of sickle cell disease under the developmental code Aes-103 (National Institutes of Health, 2013). However, despite the diverse pharmacological

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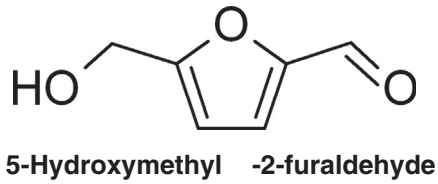

Fig. 1. The structure of 5-hydroxymethyl-2-furaldehyde.

activities of 5-HMF that have been reported, its effects on cognitive function remain to be established.

In the present study, to determine the effects of 5-HMF on cognitive dysfunction, we employed the scopolamine-treated animal model which is a widely used effective mean to search for potential drug candidates for cognitive dysfunction, including AD (Polster, 1993). Scopolamine, a muscarinic cholinergic receptor's antagonist, impairs learning and memory in both rodents and humans (Beatty et al., 1986; Kopelman and Corn, 1988). We conducted behavioral tasks, including the step-through passive avoidance, the Y-maze and the Morris water maze tasks, in order to evaluate the effects of 5-HMF on various types of cognitive functions in mice. Moreover, Western blot analysis was conducted to confirm the alteration of molecular signaling cascades related to learning and memory, including the phosphorylation levels of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and extracellular signal-regulated kinases (ERK) in mouse hippocampal tissues.

2. Materials and methods

2.1. Animals

Male ICR mice (26–30 g, 6 weeks old) were purchased from the Orient Co., Ltd., a branch of the Charles River Laboratories (Gyeonggi-do, Korea), and kept in the University Animal Care Unit for 1 week prior to the experiments. All experimental protocols were approved by the Institutional Animal Care and Use Committee (Approved No. KHP-2014-02-01). The mice were housed 5 per cage, provided food and water ad libitum and kept on a 12-h light/dark cycle (the light was on from 07:30 to 19:30 h) at constant temperature (23 ± 1 °C) and relative humidity (60 ± 10%). Animal treatment and maintenance were carried out in accordance with the Animal Care and Use Guidelines issued by Kyung Hee University, Korea.

2.2. Materials

5-HMF, scopolamine hydrobromide, donepezil hydrochloride monohydrate and dizocilpine (MK-801) were purchased from the Sigma Chemical Co. (St. Louis, MO). 5-HMF, scopolamine hydrobromide, donepezil and MK-801 were dissolved in 0.9% saline. Rabbit polyclonal anti-phosphorylated ERK (pERK) antibody was purchased from Cell Signaling Technology (Cell Signaling, MA). Rabbit polyclonal anti-ERK, rabbit polyclonal anti-phosphorylated CaMKIIα (pCaMKIIα), rabbit polyclonal anti-CaMKII and horseradish peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). All other materials were of the highest grade available and were obtained from normal commercial sources.

2.3. Step-through passive avoidance task

The acquisition and retention trials of the passive avoidance task were carried out over 2 days. Testing was performed in a box consisting of two identically sized chambers (20 × 20 × 20 cm) separated by a guillotine door (5 × 5 cm), as previously described (Jung et al., 2014); one chamber was illuminated with a 50 W bulb, and the other was not illuminated. The floor of the non-illuminated compartment was composed of 2 mm stainless steel rods spaced 1 cm apart. The mice were administered either 5-HMF (2.5, 5, 10 or 20 mg/kg, p.o.) or donepezil (5 mg/kg, p.o.) 1 h before an acquisition trial. Donepezil, widely used for treating against dementia in clinical fields, was employed as a positive control to compare with anti-dementia effect of 5-HMF, as previously described (Hasanein and Mahtaj, 2015; Jung et al., 2014). The control group received 0.9% saline solution rather than 5-HMF. The mice were treated with scopolamine (1 mg/kg, i.p.) or 0.9% saline solution 30 min before the acquisition trial. During the acquisition trial, the mice were first placed in the illuminated compartment. The door between the two compartments was opened 10 s later. The door automatically closed when the mice entered the non-illuminated compartment, and a 3-s electrical shock (0.5 mA) was delivered through the stainless steel rods. Mice that did not enter the non-illuminated compartment within 60 s after the door opened were excluded from the retention trial. The retention trial was conducted 24 h after the acquisition trial by returning the individual mice to the illuminated compartment. The time it took for the mouse to enter the dark compartment after the door opened was defined as the latency in both trials. Latencies were recorded for up to 300 s.

In a memory enhancing study, 5-HMF (2.5, 5, 10 or 20 mg/kg, p.o., without scopolamine) was administered 1 h before the acquisition trial. When the mice entered the non-illuminated compartment, a 3-s electrical shock (0.25 mA) was delivered through the stainless steel rods to avoid a ceiling effect. In the retention trial, the latencies were recorded for up to 600 s. Other procedures were the same as described above.

In a separate antagonism study, 5-HMF (10 mg/kg) was administered 1 h before the acquisition trial, and a sub-effective dose of MK-801 (0.0125 mg/kg) was given 30 min after the administration of 5-HMF. Scopolamine (1 mg/kg) was administered 5 min after the MK-801 treatment. The acquisition trial was conducted at 25 min after the administration of scopolamine. When the mice entered the non-illuminated compartment, a 3-s electrical shock (0.5 mA) was delivered through the stainless steel rods. The dose of MK-801 in the present study was used so that the passive avoidance task performance would not be impaired when administered alone (Lee et al., 2013; Park et al., 2010). Other procedures were the same as described above.

2.4. Y-maze task

The Y-maze is a three-arm maze with equal angles between all arms, which were 40 cm long and 3 cm wide, with walls 12 cm high. The maze floor and walls were constructed from dark opaque polyvinyl plastic as described previously (Jung et al., 2014). Mice were initially placed within one arm, and the sequence and number of arm entries were recorded manually for each mouse over an 8-min period. The percentage of triads in which all three arms were represented, i.e., ABC, CAB, or BCA but not BAB, was recorded as an alternation to estimate short-term memory. One hour before the test, the mice were administered 5-HMF (5 or 10 mg/kg, p.o) or donepezil (5 mg/kg, p.o.) as a positive control. After 30 min, memory impairment was induced by administering scopolamine (1 mg/kg, i.p.). The control group animals received 0.9% saline solution rather than 5-HMF or donepezil. The arms were cleaned between tests to remove odors and residues. The alternation score (%) for each mouse was defined as the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100 as shown by the following equation: % Alternation = [(Number of alternations) / (Total arm entries − 2)] × 100. The number of arm entries was used as an indicator of locomotor activity.

2.5. Morris water maze task

The Morris water maze is a circular pool (90 cm in diameter and 45 cm in height) with a featureless inner surface. The pool was filled to a depth of 30 cm with water containing black dye (24 ± 1 °C). The

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