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Short communication

Oral administration of fisetin promotes the induction of hippocampal long-term potentiation *in vivo*

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ABSTRACT

To explore memory enhancing effect of the flavonoid fisetin, we investigated the effect of oral administration of flavonoids on the induction of long-term potentiation (LTP) at hippocampal CA1 synapses of anesthetized rats. Among four flavonoids (fisetin, quercetin, luteolin and myricetin) tested, only fisetin significantly facilitated the induction of hippocampal LTP. The effect of oral fisetin was abolished by intracerebroventricular injection of U0126, an agent that was previously found to inhibit its effect in hippocampal slices *in vitro*. These results suggest that orally administered fisetin crosses the blood–brain barrier and promotes synaptic functions in the hippocampus.

Impaired cognition and memory are associated with a large number of clinical disorders including neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease as well as head trauma, stroke and a variety of drug-associated toxicities. A decrease in cognition also occurs with age. Therefore, there is a critical need for drugs that improve cognitive performance.

The excitatory synapses of the hippocampus display a long-lasting increase in synaptic potentials following high-frequency stimulation (HFS) of presynaptic fibers. This phenomenon is termed long-term potentiation (LTP) and widely believed to a cellular basis of learning and memory.[1] Therefore, compounds that promote the induction of hippocampal LTP could be useful for improving the memory impairment.

Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a flavonoid presented in a number of commonly eaten foods, such as strawberries,[2] and has a variety of biological effects that may be beneficial for the treatment of neurodegenerative disorders including Alzheimer's disease. For example, fisetin protects nerve cells from oxidative stress-induced death,[3] promotes the differentiation of nerve cells[4] and inhibits the aggregation of amyloid β protein that may cause the progressive neuronal loss in Alzheimer's disease.[5] In addition, we have previously found that fisetin facilitated the induction of hippocampal LTP in an electrophysiological experiment with rat hippocampal slices and enhanced object recognition memory in a behavioral experiment with mice,[6] demonstrating that fisetin may be a promising compound for treating the impairment of cognition and memory. However, since the LTP facilitating and memory enhancing effects of fisetin were shown by bath application *in vitro* and oral administration *in vivo*, respectively,[6] the linkage between these effects remained unclear. To fill this gap, we investigated the effect of oral administration of fisetin on the induction of LTP at Schaffer collateral-CA1 pyramidal cell synapses *in vivo* by using anesthetized rats in the present study.

Recording of evoked potentials in the CA1 region of the hippocampus was made as described in our previous paper[7] with some modifications.[8] Briefly, male Wistar rats, 7–9 weeks old, were anesthetized by intraperitoneal injection of urethane (1 g/kg) and α-chloralose (25 mg/kg), and their heads were firmly fixed with ear bars in a stereotaxic frame (Narishige, Tokyo, Japan). A heat lamp was used to maintain adequate body temperatures under anesthesia. For oral administration, a polyethylene tube connected to a syringe was inserted from the mouth so that its tip reached the stomach. For intracerebroventricular injection, a stainless steel cylindrical cannula (0.5 mm outer diameter) connected to a Hamilton syringe was stereotaxically inserted into the lateral ventricle (0.8 mm posterior to bregma, 1.5 mm lateral to midline, 3.7 mm ventral to dura). Drug solutions were slowly injected by hand over 150 s. The Schaffer collaterals were stimulated by a bipolar tungsten electrode (catalog #UEWMGCSEKNNM; FHC, Bowdoin, ME USA) positioned in the stratum radiatum of the CA1 region near the CA2/CA1 border (3.6 mm posterior to bregma, 3.0 mm lateral to midline,

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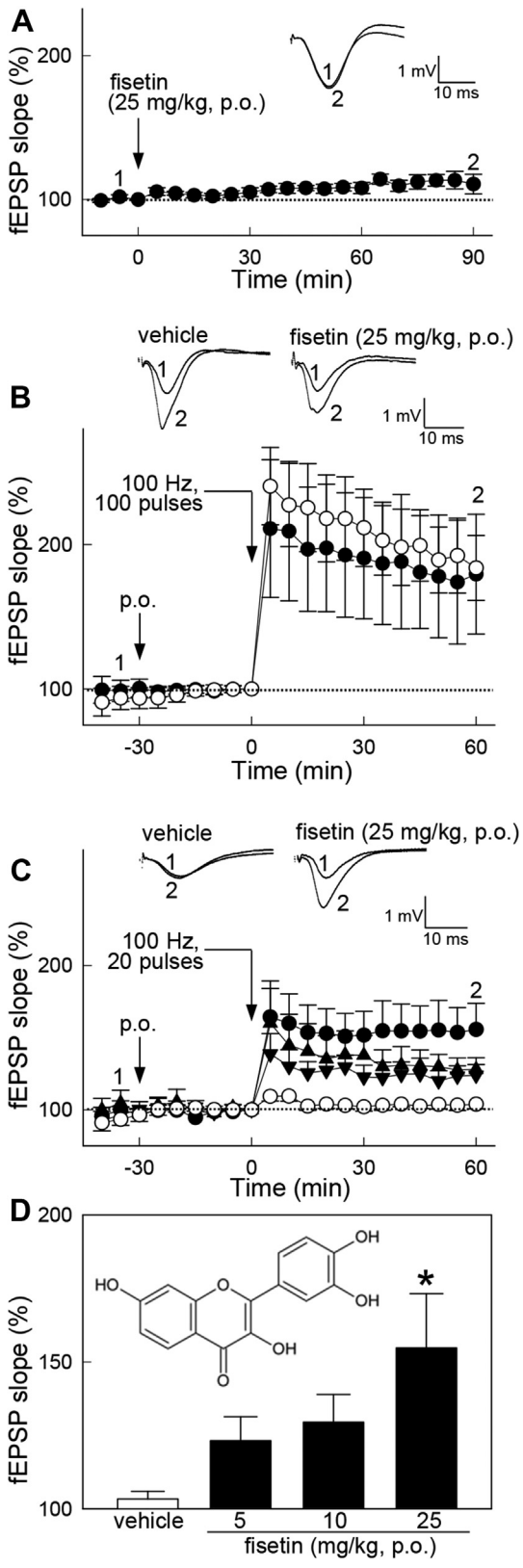

Fig. 1. Oral fisetin facilitated the induction of LTP at Schaffer collateral-CA1 pyramidal cell synapses in anesthetized rats. (A) Effect of oral administration of fisetin (25 mg/kg) on basal synaptic transmission (n = 5). Fisetin was orally administered at time 0 min. The fEPSP slope is expressed as the percentage of the value immediately before administration of fisetin. Insets are representative records immediately before (1) and 90 min after (2) administration of fisetin. (B) Effect of oral administration of fisetin on LTP induced by strong HFS (100 pulses at 100 Hz). Vehicle (○, n = 5) or 25 mg/kg fisetin (●, n = 6) was orally administered at time −30 min, and strong HFS was applied at time 0 min. The fEPSP slope is expressed as the percentage of the value immediately before application of HFS. Insets are representative records immediately before (1) and 60 min after (2) application of strong HFS in vehicle- or fisetin-treated rats. (C) Effect of oral administration of fisetin on the induction of LTP following application of weak HFS (20 pulses at 100 Hz). Vehicle (○, n = 7) or fisetin (▼, 5 mg/kg, n = 5; ▲, 10 mg/kg, n = 5; ●, 25 mg/kg, n = 6) was orally administered at time −30 min, and strong HFS was applied at time 0 min. Insets are representative records immediately before (1) and 60 min after (2) application of weak HFS in vehicle- or 25 mg/kg fisetin-treated rats. (D) Dose dependency of the LTP facilitating effect of oral fisetin. The data in (C) was summarized by calculating the average of fEPSP slopes 30–60 min after HFS as an index of LTP magnitude. The chemical structure of fisetin is shown as an inset of D. All data are means ± S.E.M. *P < 0.05 vs vehicle, Dunnett's test.

2.5 mm ventral to dura), and the evoked field excitatory postsynaptic potentials (fEPSPs) were recorded with a monopolar tungsten electrode from the stratum radiatum of the ipsilateral CA1 region (3.6 mm posterior to bregma, 2.0 mm lateral to midline, 2.0 mm ventral to dura). Test stimulation (0.08 ms duration) was applied at intervals of 30 s, and the stimulus intensity was adjusted to evoke fEPSPs of 50% of the maximum amplitude. Signals were stored using a data acquisition system for off-line analysis (PowerLab 8/30; ADInstruments, Nagoya, Japan). After stable responses were obtained for at least 20 min, test drug was administered at a volume of 10 ml/kg for oral administration or 5 μl/brain for intracerebroventricular injection. Strong HFS (100 pulses at 100 Hz) or weak HFS (20 pulses at 100 Hz) was applied to the Schaffer collaterals at the same stimulus intensity through the same electrode as that used for test stimulation. The rising slope of fEPSP was measured as an index of synaptic efficacy. At the end of each experiment, brains were dissected to verify the positions of electrode tips. All animal experiments were approved by the Institutional Animal Care and Use Committee of Musashino University (No. 16006, No. 17008), and performed in accordance with the guidelines of Musashino University on animal experiments.

Fisetin, quercetin, luteolin and myricetin were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All flavonoids were dissolved in 10% Tween 80 in saline immediately before use. U0126 (Promega Co., Madison, WI, USA) was dissolved at 2.34 mM in 50% dimethyl sulfoxide in saline and stored at −30 °C until use. U0126 was used at the dose that has been reported to specifically inhibit responses mediated by the extracellular signal-regulated kinase in the rat brain in previous papers.[9–11] Data are presented as the means ± S.E.M. of n independent observations. Significance of the differences was determined with Dunnett's test or Tukey–Kramer's test.

Since oral administration of fisetin was effective at the doses of 10 and 25 mg/kg in enhancing object recognition memory in mice in our previous study,[6] the effect of fisetin was tested at same doses in the present study. Oral administration of fisetin (25 mg/kg) did not affect the fEPSP slopes evoked by test stimulation (Fig. 1A). As shown in Fig. 1B, application of strong HFS produced robust LTP in control rats with oral administration of vehicle (10% Tween 80 in saline), and the magnitude of LTP induced by strong HFS was not significantly affected by oral administration of fisetin (25 mg/kg). As shown in Fig. 1C, application of weak HFS failed to induce LTP in control rats with oral administration of vehicle, but clearly produced LTP in rats with oral administration of fisetin. The facilitation of LTP induction by oral fisetin was dose dependent and statistically significant at a dose of 25 mg/kg (Fig. 1D). To ask if the LTP facilitating effect is specific for fisetin, we also investigated the effects of three structurally related flavonoids, quercetin, luteolin and myricetin. Oral administration of quercetin (5–25 mg/kg) had no effect on the induction of LTP (Fig. 2A). Oral administration of luteolin or myricetin (5–25 mg/kg) tended to promote the induction of LTP, but the effect was not statistically significant (Fig. 2B and C).

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