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Research report

Bidirectional actions of docosahexaenoic acid on hippocampal neurotransmissions in vivo

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Abstract

Docosahexaenoic acid (DHA), a 22-carbon fatty acid with six double bonds, is one of the major polyunsaturated fatty acids in fish oils or in the mammalian central nervous system and is believed to be essential for neuronal plasticity and development. In the present study, we evaluated the effect of DHA on hippocampal neurotransmissions using anesthetized rats. Field excitatory postsynaptic potential (fEPSP) evoked by stimulation of the Schaffer collaterals was recorded from the CA1 stratum radiatum. Following intracerebroventricular injection of DHA 25 nmol, the fEPSP slope decreased gradually in 30 min and was eventually suppressed by about 30%. On the other hand, when fEPSP was evoked by stimulation of the perforant path was recorded in the molecular layer of the dentate gyrus, an increase in fEPSP slope occurred over a similar time course after DHA injection. These phenomena were independent of *N*-methyl-D-aspartate receptor activity. Linoleic acid, one of polyunsaturated fatty acids, was virtually ineffective. Furthermore, we investigated the effect of DHA on hippocampal synaptic plasticity. Although DHA did not alter the profile of paired-pulse facilitation, it inhibited the induction of long-term potentiation in the CA1 area but not in the dentate gyrus. Thus, DHA exerts regionally different effects on hippocampal neurotransmission and may be a good tool for clarifying physiological functions of the hippocampus.

Themes: Excitable membranes and synaptic transmission

Topics: Long-term potentiation: pharmacology

Keywords: Docosahexaenoic acid; Fatty acid; Hippocampus; Synaptic plasticity; Learning and memory; Neurotransmission

1. Introduction

Docosahexaenoic acid (DHA), a polyunsaturated fatty acid which consists of 22 carbon atoms and six cis-double bonds (ω3,22:6), is one of the major compositions of membrane phospholipids in the mammalian central nervous system [4,13,25,26]. DHA is endogenously released from the lipid bilayer by phospholipase A_2 [2,22,23], and thus has been supposed to play an active role in neuronal development and plasticity [5,12]. Particularly, because several reports indicate that dietary control of DHA alters visual discrimination task performance [7,18] and ischemia-induced cognitive deficit [20], much attention focused on the behavioral and physiological actions of DHA.

At the cellular and molecular levels, several lines of evidence show a broad spectrum and acute influences of DHA on ligand-gated or voltage-sensitive ionic channels. Nishikawa et al. [19] indicated that DHA promotes the channel open probability of *N*-methyl-D-aspartate (NMDA) receptor without a change in the channel conductance, predicting the existence of an unidentified DHA-binding site on NMDA receptor. DHA also inhibits voltage-sensitive K^+ channels. This effect is specific for the Kv1.2 and Kv3.1a channel, but DHA has little effect on the Kv1.5 channel [8,21]. Interestingly, extracellular Zn^{2+} abolishes the DHA-induced inhibition of the Kv1.2 channel in a concentration-dependent manner, but it does not affect the blockage of the Kv3.1a channel [21]. Furthermore, DHA

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modulates γ-aminobutyric acid (GABA) response [6,17], and produces the shift of the inactivation curve to more hyperpolarized potentials in both sodium and calcium currents in primary neuron cultures [27]. Taken together, we suspected that DHA possesses unidentified acute effects of neuronal activity and synaptic transmission.

Long-term potentiation (LTP) or long-term depression (LTD) of hippocampal synaptic transmissions is a form of activity-dependent synaptic plasticity that may underlie learning and memory [3,10,15]. Because numerous reports demonstrate that NMDA receptor activation is crucially required for the induction of LTP and LTD, it is possible that DHA has any influence on hippocampal synaptic plasticity. However, little attention has been paid to the effect of DHA on hippocampal neurotransmission and synaptic plasticity, except for a recent study reported by Young et al. [28], which indicated that exogenous DHA inhibits the induction of LTD in the CA1 area in vitro. Yet, they did not address the effect on other types of synaptic plasticity or a regional specificity among the hippocampal subregions. In the present studies, therefore, we investigated the effect of DHA on neurotransmission and various forms of synaptic plasticity in the CA1 area and the dentate gyrus (DG).

2. Materials and methods

Male Wistar rats (7–9 weeks old) were fixed in a stereotaxic frame under anesthesia with a combination of urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.). The Schaffer collaterals (AP: −3.5 mm, LM: 3.0 mm, DV: −2.0 mm) was stimulated, and the evoked potentials were extracellularly recorded from the CA1 stratum radiatum (AP: −3.5 mm, LM: 2.0 mm, DV: −2.5 mm). In another series of experiments, the medial perforant path (AP: −8.1 mm, LM: 4.4 mm, DV: −3.0 mm) was stimulated, and the field potentials were recorded from the molecular layer of the DG (AP: −3.5 mm, LM: 2.0 mm, DV: −2.3 mm). The recordings from the CA1 region and the DG were conducted using different rats. Test stimulation (80 μs duration) was applied at intervals of 30 s and the intensity of the stimulation was adjusted to produce a field excitatory postsynaptic potential (fEPSP) with a slope that was about 50% of the maximum. DHA was dissolved in saline containing 0.2% dimethylsulfoxide and injected at 2 μl/min for 150 s through a stainless steel cylindrical cannula inserted into the lateral cerebroventricle (AP: −0.8 mm, LM: 1.5 mm, DV: −4.5 mm) contralateral to the recording site, if not especially described. In order to induce LTP, 90 min after the drug injection, theta burst stimulation consisting of five brief bursts (Ten pulses at 100 Hz) at 5 Hz was applied four times every 30 s. The effect of DHA was evaluated by measuring changes in the fEPSP slope that is defined as the maximal slope in a rise phase of the negative field potential via a computational analysis of the analog-to-digital converted signals (Wave-kun, Tokyo, Japan).

Brain temperature was measured with an intrahippocampal sensor equipped with the lesion generator system (Model RFG-4A, Radionics Inc., Burlington, MA, USA).

The following drugs were used in the present study: DHA (a gift from Maruha Co., Tokyo, Japan), linoleic acid (Sigma Chemical Co., St. Louis, MO, USA) and MK-801 (Wako Pure Chemical Industries, Ltd., Saitama, Japan).

Data were statistically analyzed with one-way or two-way analysis of variance (ANOVA) followed by Tukey's multiple range test, or *t*-test.

3. Results

First, the effect of DHA on the Schaffer collateral-CA1 neurotransmission was investigated. When DHA 25 nmol was intracerebroventricularly injected, the fEPSP slope gradually decreased in 30 min and was eventually suppressed by about 30% [$F(3,16)=19.35$, $Q(4,16)=9.08$, $P<0.01$ versus the vehicle-treated group] (Fig. 1A and B). The effect showed a dose-dependent manner (Fig. 1C). However, an unsaturated fatty acid, linoleic acid (ω6,18:2) 25 nmol, failed to affect the neurotransmission [$Q(4,16)=0.04$, $P>0.1$] (Fig. 1C). Next, fEPSP was recorded from the perforant path-DG synapse. By contrast, DHA rather potentiated fEPSP in a similar time course and in a similar dose-dependent manner [$F(3,16)=19.35$, $Q(4,16)=5.44$, $P<0.01$] (Fig. 2). Linoleic acid 25 nmol had no effect [$Q(4,16)=0.07$, $P>0.1$] (Fig. 2C). These two phenomena induced by DHA were found in all animals tested. In some cases, we observed the effects of DHA up to 3 h after the administration and found that the effects maintained for at least 3 h (CA1: $N=4$; DG: $N=3$). When DHA was injected into the same side of the lateral cerebroventricles as the recording electrode, a similar time course and a similar potency of the effect of DHA or linoleic acid were observed (DHA: $N=3$; linoleic acid: $N=2$). Therefore, possible problems in drug diffusion were ruled out. Because several reports show that brain temperature is one of the factors to inflect synaptic efficiency [11,16], we tested whether DHA altered the temperature of the hippocampus. However, DHA 25 nmol did not affect it ($N=3$, data not shown). Therefore, we ruled out the possibility that the effect of DHA was mediated by changes in brain temperature.

Since the NMDA receptor is often involved in long-lasting alternation in synaptic efficacy of the hippocampus [14], we next evaluated the possible contribution of the NMDA receptor to the DHA-induced modulation of the neurotransmission. MK-801 5 mg/kg, an NMDA receptor inhibitor, was intraperitoneally injected 30 min prior to the treatment with DHA. However, it did not prevent the DHA-induced alternation in the neurotransmission in either the CA1 region (Fig. 3A) or the DG (Fig. 3B) [CA1:

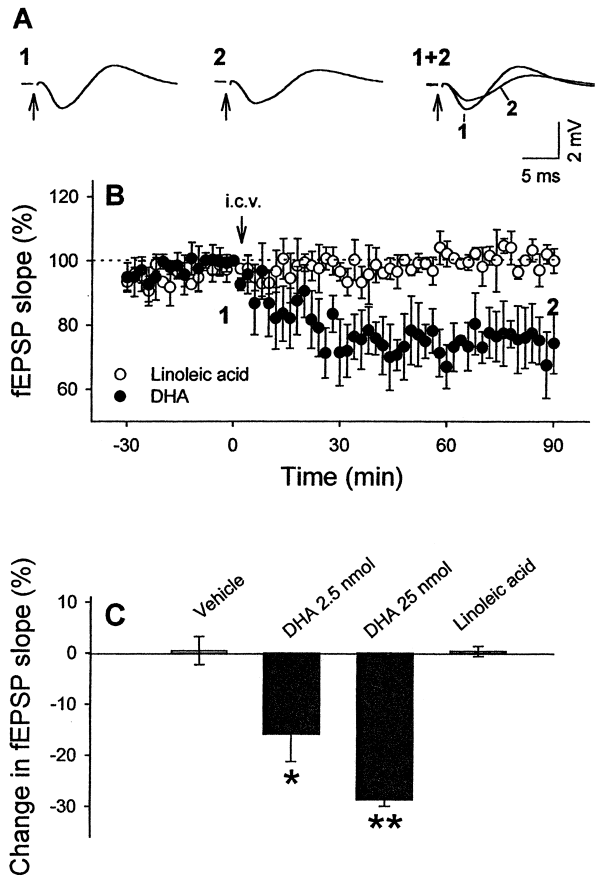

Fig. 1. Inhibitory effect of DHA on the Schaffer collateral-CA1 neurotransmission. A: Representative field potentials immediately before (1) and 90 min after intracerebroventricular injection of DHA 25 nmol (2). The Schaffer collaterals were stimulated at the time indicated by arrows. B: The time course of the fEPSP slope following the injection of DHA 25 nmol (closed circles) and linoleic acid 25 nmol (open circles). Drugs were administered at time 0 (i.c.v.). The fEPSP slope is expressed as a percentage of the baseline value immediately before the injection. C: The summary of the effects of DHA and linoleic acid. The ordinate shows an average rate of change in the fEPSP slope 85–90 min after the injection. All data are expressed as the means±S.E.M. of five cases. *$P<0.05$, **$P<0.01$ versus vehicle; Tukey's test following one-way ANOVA.

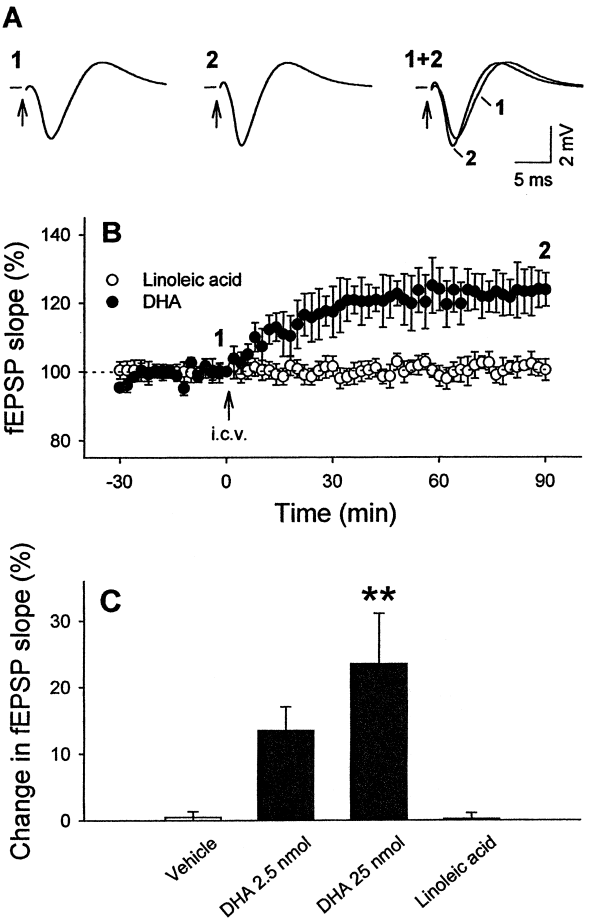

Fig. 2. Facilitatory effect of DHA on the perforant path-DG synaptic transmission. A: Typical traces immediately before (1) and 90 min after the injection of DHA 25 nmol (2). The perforant path was stimulated at the time indicated by arrows. B: The time course of the fEPSP slope following the injection of DHA 25 nmol (closed circles) and linoleic acid 25 nmol (open circles). C: The effects of DHA and linoleic acid are shown by an average rate of change in fEPSP slope 85–90 min after the injection. All data are expressed as the means±S.E.M. of five cases. **$P<0.01$ versus vehicle; Tukey's test following one-way ANOVA.

$t(8)=0.44$, $P=0.67$; DG: $t(8)=0.49$, $P=0.63$]. We confirmed the effectiveness of the intraperitoneal MK-801 by the result of another experiment, which indicated that it did completely inhibit the formation of LTP in both subregions ($N=5$, data not shown). Therefore, we concluded that the depression of the CA1 synaptic transmission and the facilitation of the DG neurotransmission after the treatment with DHA are independent of NMDA receptor activation.

We next examined whether synaptic plasticity was affected by intracerebroventricular administration of DHA. First, we explored the effect on paired-pulse facilitation (PPF), which is a type of short-term synaptic plasticity and thought to be produced predominantly by presynaptic modulation [29]. Although the PPF profile was assessed immediately before and 90 min after DHA injection, little changes in the PPF ratios were noticed in both subregions [CA1: $F(1,40)=0.59$, $P=0.45$; DG: $F(1,40)=0.387$, $P=0.54$] (Fig. 4).

Next, the effect of DHA on LTP was investigated. Theta burst stimulation was applied 90 min after DHA treatment. The CA1 fEPSP recorded from the animals that received the injection of DHA 25 nmol did not display LTP [$F(3,16)=31.27$, $Q(4,16)=6.12$, $P<0.01$] (Fig. 5A and Fig. 6A). The effect showed a dose-dependent manner (Fig. 5B). Linoleic acid 25 nmol had no effect on CA1 LTP [$Q(4,16)=0.22$, $P>0.1$] (Fig. 5B). Because DHA alone attenuated baseline neurotransmission, the preexisting alternation of basal responses may affect the induction of LTP induction. Therefore, 30 min before the theta burst stimulation, the intensity of the test stimulation was adjusted so that the fEPSP slope was approximately the same as the baseline responses before the DHA injection, but LTP was not generated (Fig. 6B). Neither DHA nor

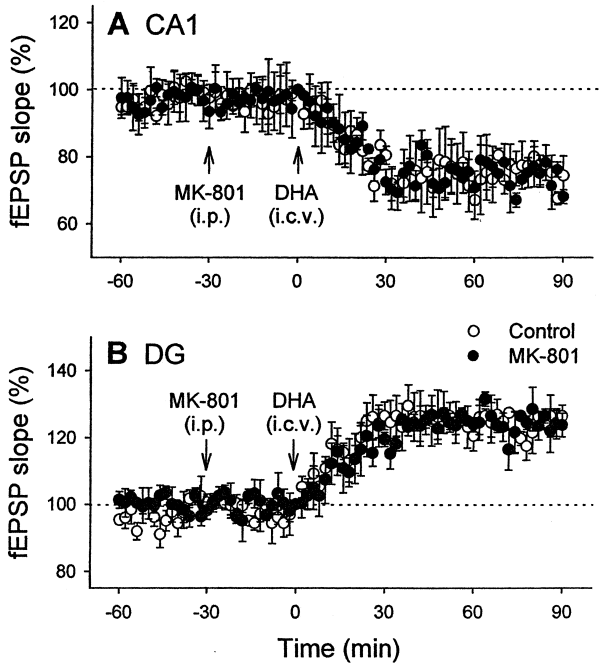

Fig. 3. Effect of the NMDA receptor inhibitor MK-801 on the DHA-induced alternation of the neurotransmission in the CA1 area (A) and the DG (B). MK-801 5 mg/kg (closed circles) or its vehicle (open circles) was intraperitoneally injected 30 min before the injection of DHA 25 nmol. Data represent the means±S.E.M. of five animals.

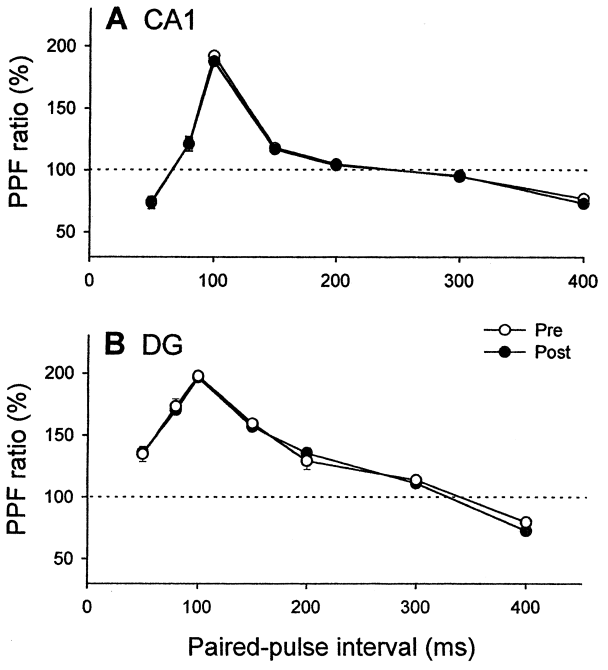

Fig. 4. Lack of the effect of DHA on PPF in the CA1 area (A) or the DG (B). The PPF ratio is expressed as a percentage of the second fEPSP slope to the first fEPSP. Open circles indicate the PPF profile immediately before the injection of DHA 25 nmol (Pre), and closed circles represent the PPF profile 90 min after the administration (Post). Vertical bars on data points are S.E.M.; when not indicated, S.E.M. fell within the data symbols.

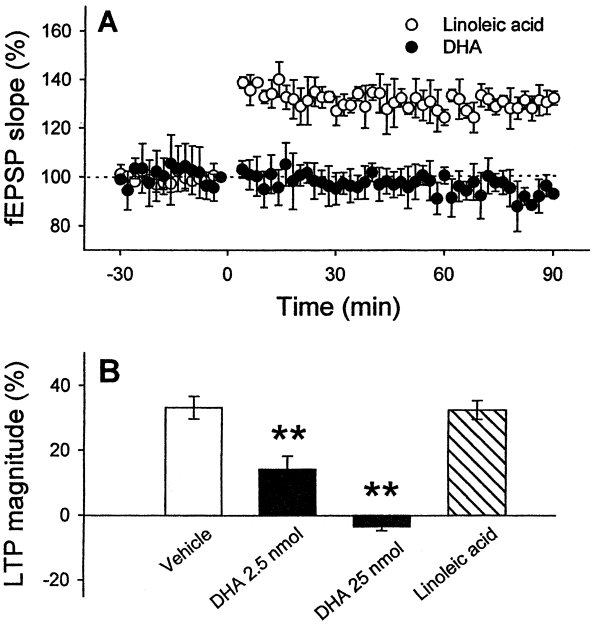

Fig. 5. Inhibitory effect of DHA on CA1 LTP. A: The time course of the fEPSP slope following theta burst stimulation applied at time 0. DHA 25 nmol (closed circles) or linoleic acid 25 nmol (open circles) was injected 90 min before the theta burst stimulation. The fEPSP slope is expressed as a percentage of the response immediately before the theta burst stimulation. B: The effects of DHA and linoleic acid are summarized through LTP magnitude which was defined as an average rate of change in the fEPSP slope at time 85–90. All data are expressed as the means±S.E.M. of five cases. $^{**}P<0.01$ versus vehicle; Tukey's test following one-way ANOVA.

linoleic acid affected the indication of LTP in the DG [$F(3,16)=0.004$, $P>0.90$] (Fig. 7).

4. Discussion

Here we have shown for the first time that intracerebroventricular administration of DHA influenced hippocampal neurotransmission and synaptic plasticity in the anesthetized rats. The effect appeared to reflect the pharmacological action of DHA because of its dose-dependent fashion and the ineffectiveness of linoleic acid. Although the mechanism remains to be determined, DHA is more likely to act on the postsynaptic sites because it did not change the PPF profile [29].

It is remarkably interesting that the effect of DHA showed a regional difference. Particularly, its effect on basal synaptic transmission was the opposite; DHA decreased the fEPSP slopes in the Schaffer collateral-CA1 neurotransmission but increased the fEPSP slopes in the perforant path-DG synaptic transmission. Incidentally, the suppressive effect on the CA1 baseline response is in accord with the in vitro study described by Young et al., which indicates that exogenous DHA attenuated the baseline response of the Schaffer collateral-CA1 synapses in hippocampal slices [28]. The initial possible explanation

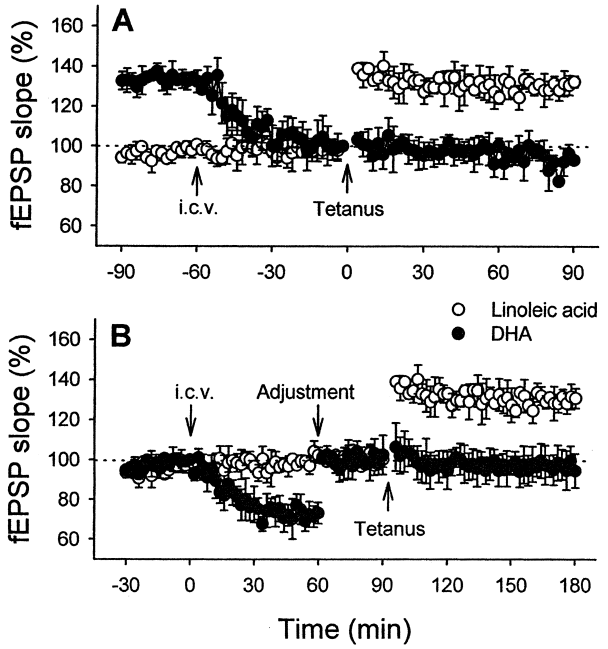

Fig. 6. Effect of DHA 25 nmol (closed circles) or linoleic acid 25 nmol (open circles) on CA1 LTP. A: The time course of the fEPSP slope following theta burst stimulation at time 0 (Tetanus). The ordinate is expressed as a percentage of the response theta burst stimulation. The same data as in Fig. 5A appear again. B: The fEPSP slope was expressed as a percentage of the response immediately before the drug injection at time 0 (i.c.v.). Test stimulus intensity was adjusted at time 60 (Adjustment) so that the fEPSP slope was approximately the same as the baseline response before the DHA injection. All data represent the means±S.E.M. of five cases.

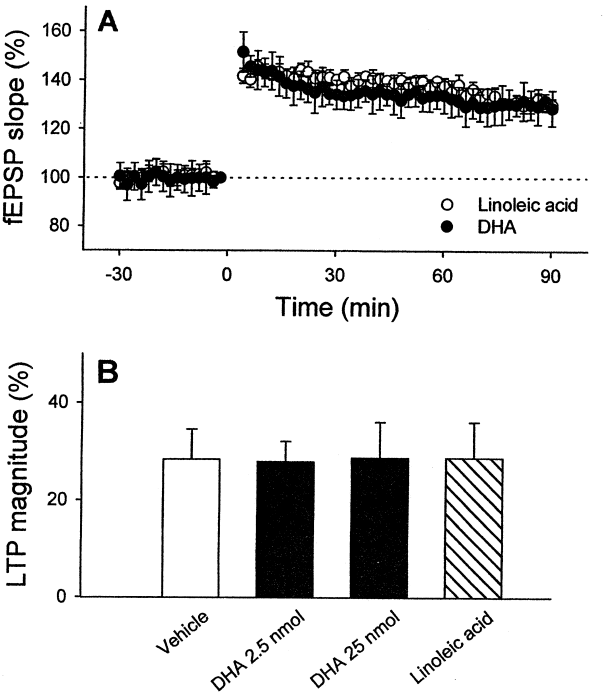

Fig. 7. Lack of the effect of DHA on DG LTP. A: The time course of the fEPSP slope following the theta burst stimulation applied at time 0. DHA 25 nmol (closed circles) or linoleic acid 25 nmol (open circles) was injected 90 min before the theta burst stimulation. The fEPSP slope is expressed as a percentage of the response immediately before the theta burst stimulation. B: The summary of the effects of DHA and linoleic acid. All data are expressed as the means±S.E.M. of five cases.

for the regional difference was that the degree of the NMDA receptor activation by DHA is different between the CA1 area and the DG because the $[Ca^{2+}]$ level tightly regulates the direction of the long-term synaptic modulation. Based on this idea, we tested the effect of the NMDA receptor inhibitor on the DHA-induced modulation of the neurotransmission, but we unexpectedly found that the NMDA receptor activation was not involved in the effect of DHA in either hippocampal subregion. However, there remains another possibility. A previous study using the patch-clamp technique showed that DHA inhibits the K^+ channel function [8,21]. Since the K^+ channel blocker, tetraethylammonium, induces NMDA-independent long-lasting modulation in the Schaffer collateral-CA1 synapse of hippocampal slices [1,9], it is possible that the effect of DHA in the present study is mediated by the modulation of the K^+ channel. Importantly, DHA-induced inhibition of the Kv1.2 channel was abolished by Zn^{2+} [21]. Although the hippocampal synapses contains a significant amount of Zn^{2+}, but its distribution is not uniform; Zn^{2+} is characteristically abundant in the molecular layer of the DG [24]. Therefore, the effect of DHA in the DG would be more susceptive to the cancellation by Zn^{2+} than that in the CA1 area. Furthermore, Nabekura et al. [17] indicated that the modulation of GABA response by DHA is highly dependent on the receptor subunit combinations; DHA 10 μM potentiates the GABA response on $\alpha_1\beta_2\gamma_{2S}$ receptor complexes but inhibits the response on $\alpha_1\beta_2$ receptor complexes. It is possible that the distribution of the GABA receptor subunits is different between the CA1 region and the DG. Further investigations would verify this possibility.

The effect of DHA on LTP also showed a regionally difference. DHA blocked the induction of CA1 LTP whereas it did not affect DG LTP. In the DG, DHA alone enhanced baseline response, but LTP was not occluded, which suggests that the mechanism of the effect of DHA on basal neurotransmission is different from that on LTP. This idea is further supported by the result that the NMDA receptor inhibitor prevented the induction of LTP but not DHA-induced alternation of the basal neurotransmission. Therefore, the effect of DHA on neurotransmission and LTP should be investigated separately. Anyway, it should be noted that DHA produced long-term changes in synaptic efficacy through a mechanism independent of LTP. This might be a novel type of synaptic plasticity. Because DHA is assumed to be endogenously released by phospholipase A_2 [2,22,23], DHA possibly serves as an intrinsic modulator of hippocampal neurotransmission and synaptic plasticity

To our knowledge, except for DHA as we have shown

here, there has been no report about the substance or reagent that displays such a remarkably regionally different effect on hippocampal neurotransmission and synaptic plasticity. Thus, DHA will be a good tool for clarifying physiological functions of the hippocampus.

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