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## Palmitic acid and docosahexaenoic acid opposingly regulate the expression of insulin-degrading enzyme in neurons

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Previous results show that treatment with saturated fatty acids, such as palmitic acid (PA), induces the pathology of Alzheimer's disease (AD), while treatment with polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), protects against AD pathology. However, the pharmacological mechanism underlying these opposite effects of fatty acids on AD is not well understood. Here, we show that PA treatment significantly reduced the expression of insulin-degrading enzyme (IDE), an important protease responsible for the degradation of amyloid- $\beta$  (A $\beta$ ) in neural cells, while incubation with DHA up-regulated IDE levels in primary hippocampal neurons. Moreover, pre-incubation with PA attenuated the DHA-induced IDE expression. Taken together, these results suggest the opposite effects of saturated fatty acids and polyunsaturated fatty acids on the expression of IDE, indicating a novel mechanism underlying the pharmacological function of fatty acids in AD intervention.

Many epidemiological and animal studies suggest that diet with a high content of saturated of fatty acids promotes Alzheimer's disease (AD) pathology and the degree of saturation of fatty acids is critical in determining the risk for AD (Skoog et al. 1996; Grant 1999; Solfrizzi et al. 2005; Scarmeas et al. 2006). On the other hand, some natural unsaturated fatty acids, such as docosahexaenoic acid (DHA) etc., have been suggested to be favorable in treatment of AD (Simopoulos 1999; Calon et al. 2004). However, the pharmacological mechanism underlying these opposite effects of fatty acids on AD is not well understood. The initial pathological cause of AD has been ascribed to the accumulation of amyloid- $\beta$  (A $\beta$ ). The level of A $\beta$  is determined by the balance between its generation and turnover (Selkoe 1993). A $\beta$  is degraded by endopeptidases, among which insulin-degrading enzyme (IDE) is a major one. IDE mediates much of the degradation of soluble monomeric A $\beta$  (Selkoe 2001). Emerging studies have revealed a significant linkage of IDE and AD pathology (Bertram et al. 2000; Ertekin-Taner et al. 2000; Farris et al.

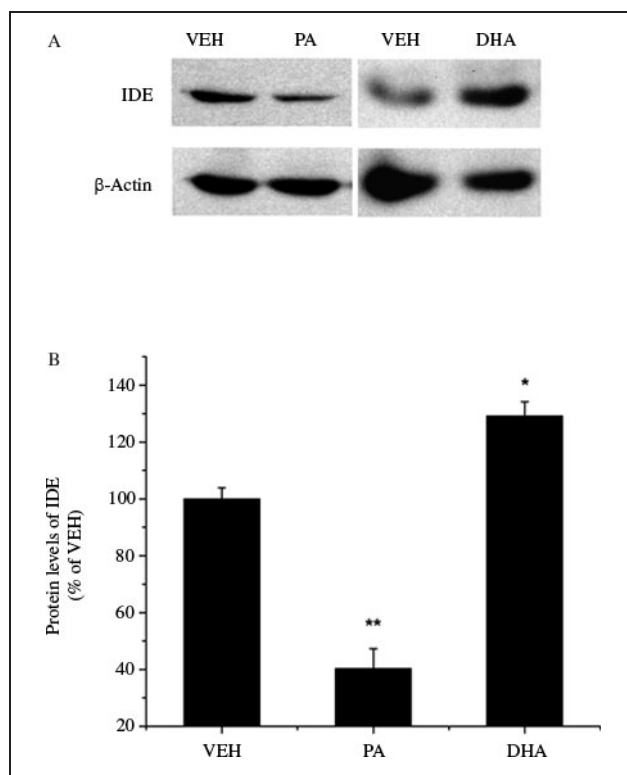


Fig. 1: Palmitic acid and docosahexaenoic acid opposingly regulated the expression of IDE in neurons. A, Primary hippocampal neurons were treated with palmitic acid (PA, 100 nM), docosahexaenoic acid (DHA, 100 nM) or vehicle (VEH), followed with determination of IDE expression by Western blot. B, The statistical analysis of results in A.  $\beta$ -actin served as standards for analyses of the protein samples. Data are presented as the mean  $\pm$  S.E. Asterisks indicate statistical difference from control. \* $p$  < 0.05, \*\* $p$  < 0.01, t-test,  $n$  = 4

2003; Leissring et al. 2003). However, the cellular and molecular regulation of IDE is not well understood.

In the present study, one of the most abundant saturated fatty acids in human tissues, palmitic acid (PA) and a major polyunsaturated fatty acid, DHA were used to treat primary hippocampal neurons, in order to investigate their effects on the expression of IDE in neurons. The results show that treatment with PA (100 nM) significantly reduced the level of IDE expression in neurons (0.4-fold,  $p$  < 0.01, Fig. 1). Conversely, incubation with DHA (100 nM) induced IDE expression in neurons (1.3-fold,  $p$  < 0.05, Fig. 1).

Since PA and DHA coexist in brain tissue, we further examined their combined effect on IDE expression in neurons. As shown in Fig. 2, co-incubation with PA almost blocked the DHA-induced IDE expression.

Taken together, the present study suggests, for the first time, the opposite effects of saturated fatty acids and polyunsaturated fatty acids on the expression of IDE in neurons, indicating a novel mechanism underlying the pharmacological function of fatty acids in AD intervention.

## Experimental

### 1. Primary neuronal culture

Hippocampi were dissected from the brains of embryonic day 18 (E18) Sprague Dawley rat fetuses, treated with 0.05% trypsin for 5 min at 37 °C, and dissociated by repeated passage. Nerve cells were grown in Neurobasal medium (NBM; Invitrogen, USA) supplemented with B27, 5 U/ml penicillin, 5  $\mu$ g/ml streptomycin, 0.5 mM glutamine, and 25  $\mu$ M glutamate at 37 °C in 10% CO<sub>2</sub>. The culture media were exchanged with glutamate-free NBM 3 days after the start of cell culture. Cultures grown in serum-free

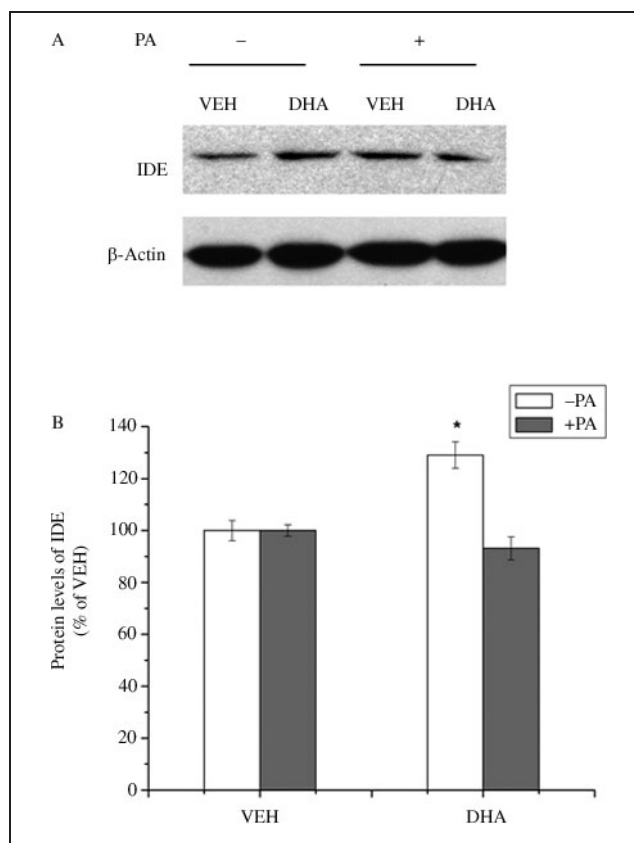


Fig. 2: Palmitic acid attenuated the up-regulation of IDE expression induced by docosahexaenoic acid. A, Primary hippocampal neurons were treated with docosahexaenoic acid (DHA, 100 nM) or vehicle (VEH), in the presence of palmitic acid (PA, 0 or 100 nM), followed by determination of IDE expression by Western blot. B, The statistical analysis of results in A.  $\beta$ -actin served as standards for analyses of the protein samples. Data are presented as the mean  $\pm$  S.E. Asterisks indicate statistical difference from control. \* $p < 0.05$ , t-test,  $n = 3$

NBM yielded 99.5% neurons and 0.5% glia after 7 to 10 days of culture and were then treated with fatty acids or vehicle (0.5% BSA) in B27-free NBM.

## 2. Western immunoblotting

Protein concentrations were determined by use of the Amersham Pharmacia Biotech electrophoresis machine. First, samples (50  $\mu$ g of total protein/lane) were separated by electrophoresis through SDS-PAGE gels and transferred to PVDF membranes (Millipore, USA). After transfer, the membranes were blocked for 1.5 h in TTBS containing 5% nonfat milk. The membranes were then incubated with primary antibodies specific for IDE (diluted 1:4,000, Abcam, USA) or  $\beta$ -actin (diluted 1:500, Santa Cruz Biotechnology, USA). After they were rinsed in TTBS, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse IgG (1:10,000) or anti-rabbit IgG (1:10,000) in TTBS. The membranes were developed by Kodak medical X-ray processors (Kodak, Japan).

## 3. Chemicals

Palmitic acid and docosahexaenoic acid were purchased from Sigma-Aldrich (USA).

## 4. Statistical analyses

Data were presented as group means  $\pm$  S.E. Differences between means were determined using the Student's t-test for paired or unpaired observations. Differences were considered statistically significant when  $p$  value  $< 0.05$ .

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