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Research Report

Involvement of the long-chain fatty acid receptor GPR40 as a novel pain regulatory system

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ABSTRACT

G-protein receptor (GPR) 40 is known to be activated by docosahexaenoic acid (DHA). However, reports studying the role and functions (including pain regulation) of GPR40 in the brain are lacking. We investigated the involvement of GPR40 in the brain on DHA-induced antinociceptive effects. Expression of GPR40 protein was observed in the olfactory bulb, striatum, hippocampus, midbrain, hypothalamus, medulla oblongata, cerebellum and cerebral cortex in the brain as well as the spinal cord, whereas GPR120 protein expression in these areas was not observed. Intracerebroventricular (i.c.v.), but not intrathecal (i.t.) injection of DHA (25 and 50 μg/mouse) and GW9508 (a GPR40- and GPR120-selective agonist; 0.1 and 1.0 μg/mouse) significantly reduced formalin-induced pain behavior. These effects were inhibited by pretreatment with the μ opioid receptor antagonist β-funaltrexamine (β-FNA), naltrindole (δ opioid receptor antagonist) and anti-β-endorphin antiserum. The κ opioid receptor antagonist norbinaltorphimine (nor-BNI) did not affect the antinociception of DHA or GW9508. Furthermore, the immunoreactivity of β-endorphin in the hypothalamus increased at 10 and 20 min after i.c.v. injection of DHA and GW9508. These findings suggest that DHA-induced antinociception via β-endorphin release may be mediated (at least in part) through GPR40 signaling in the supraspinal area, and may provide valuable information on a novel therapeutic approach for pain control.

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Abbreviations: CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPCR, G-protein-coupled receptor; GPR, G-protein receptor; β-FNA, β-funaltrexamine; i.c.v., intracerebroventricular; i.t., intrathecal; nor-BNI, nor-binaltorphimine; PC, prohormone convertases; PLC, phospholipase C; POMC, proopiomelanocortin; PUFAs, polyunsaturated fatty acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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1. Introduction

Docosahexaenoic acid (DHA) is enriched in the mammalian brain, and is important for multiple aspects of neuronal development and function (Contreras et al., 2000). Interestingly, DHA has been reported to induce antinociception without binding to opioid receptors (Nakamoto et al., 2010, 2011). We hypothesize that the release of endogenous opioid peptides plays an important part in the induction of DHA antinociception. Indeed, we proposed that the endogenous opioid peptide β-endorphin is a candidate in this mechanism in our previous study (Tokuyama and Nakamoto, 2011). That is, antinociception of DHA may be partially mediated through the μ opioid receptor stimulated by β-endorphin. However, how DHA releases β-endorphin is incompletely understood.

It is well known that fatty acids affect intracellular and intercellular signaling as well as the membrane fluidity of neurons (Horrocks and Farooqui, 2004). In addition to intracellular actions, unbound free fatty acids (FFAs) can also carry out extracellular signaling by stimulating the G-protein-coupled receptor (GPCR). Recently, a GPCR deorphanization strategy was successfully used to identify multiple receptors for FFAs (Hirasawa et al., 2008). Among these receptors, GPR40 and GPR120 have been reported to be activated by long-chain fatty acids such as DHA, eicosapentaenoic acid (EPA) and arachidonic acid (Briscoe et al., 2003; Hirasawa et al., 2005). GPR40 is coupled to an intracellular G protein (Gq) that activates the phospholipase C and phosphatidylinositol (phosphatidylinositol-4,5-bisphosphate) signaling pathway as a physiological action (Hardy et al., 2005). GPR40 is preferentially expressed in pancreatic β-cells and is known to mediate insulin secretion (Alquier et al., 2009; Itoh et al., 2003). Therefore, this receptor may contribute to the regulation of the peptide secretion in peripheral areas. Even though this receptor is widely distributed in the central nervous system (Ma et al., 2007, 2008, 2010), reports studying the role and functions of GPR40 in the brain are lacking.

In the present study, we focused on the relationship between pain regulation and GPR40. Furthermore, we investigated if GPR40 in the supraspinal area is related to the mechanism underlying the DHA-induced antinociceptive effect.

2. Results

2.1. *Expression of GPR40 and GPR120 in tissue from the brain, spinal cord and intestine*

GPR40 (31 kDa) protein expression was observed in the olfactory bulb, striatum, hippocampus, midbrain, medulla oblongata, hypothalamus, cerebellum, cerebral cortex and spinal cord. GPR120 (42 kDa) protein expression was not observed in any brain area, although expression of this protein in the small intestine was confirmed (Fig. 1A). Immunofluorescence study revealed that GPR40 was expressed in hypothalamic arcuate nucleus of the brain (Fig. 1B). In the spinal cord, on the other hand, this receptor was observed in the spinal white matter part, but not the dorsal horn (Fig. 1 C).

2.2. *Antinociceptive effect of DHA on formalin-induced nociceptive behavior*

Intraplantar injection of 5% formalin (10 μL) induced two phases of spontaneous pain behavior. In the early phase of nociceptive response, licking, shaking and biting of the injected paw normally peaked 0–10 min after formalin injection, and in the late phase, 10–30 min after formalin injection, representing the direct effect on nociceptors and tonic inflammatory nociceptive responses, respectively. DHA (25 and 50 μg/mouse, i.c.v.) significantly suppressed pain-related behavior in the second phase (10–30 min) ($P<0.01$), but not in the first phase (0–10 min) (Fig. 2A). DHA (25 and 50 μg/mouse, i.t.) had no effect on the formalin test. However, morphine (10 nmol/mouse, i.t.) significantly attenuated pain-related behavior in the early and late phase (Fig. 2B).

2.3. *Antinociceptive effect of GW9508 on formalin-induced nociceptive behavior*

GW9508 (0.1 and 1.0 μg/mouse) significantly suppressed pain-related behavior in the second phase (10–30 min) ($P<0.01$), but not in the first phase (0–10 min) (Fig. 3A). GW9508 (0.1 and 1.0 μg/mouse, i.t.) had no effect on the formalin test. However, morphine (10 nmol/mouse, i.t.) significantly attenuated pain-related behavior in the early and late phase (Fig. 3B).

2.4. *Effect of opioid receptor antagonists on GW9508-induced antinociception in the formalin test*

GW9508-induced antinociception in the second phase of the formalin test was markedly suppressed if mice were pretreated with β-FNA (20 mg/kg, i.p.) 24 h or naltrindole (5 mg/kg, i.p.) 30 min before GW9508 treatment (0.5 μg/mouse, i.c.v.), but not by norbinaltorphimine (nor-BNI) (10 mg/kg, i.p.) (Fig. 4).

2.5. *Effect of anti-β-endorphin antiserum on GW9508-induced antinociception in the formalin test*

GW9508-induced antinociception in the second phase of formalin test was markedly attenuated if mice were pretreated with anti-β-endorphin antiserum (1:25 dilution, i.c.v.) 60 min before GW9508 treatment (0.5 μg/mouse, i.c.v.) (Fig. 5).

2.6. *β-endorphin immunoreactivity in the brain after treatment with DHA or GW9508*

β-endorphin immunoreactivity at 10 min or 20 min after DHA (25 μg/mouse, i.c.v.) or GW9508 (1.0 μg/mouse, i.c.v.) treatment was increased in the hypothalamic arcuate nucleus. However, β-endorphin like immunoreactivity was restored to the control level at 60 min after the injection of DHA or GW9508 (Fig. 6).

3. Discussion

Polyunsaturated fatty acids (PUFAs) such as DHA have crucial roles in the development and function of neurons and