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DHA prevents altered 5-HT1$_A$, 5-HT2$_A$, CB1 and GABA$_A$ receptor binding densities in the brain of male rats fed a high-saturated-fat diet

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Abstract

Low levels of docosahexaenoic acid (DHA) have been linked to a number of mental illnesses such as memory loss, depression and schizophrenia. While supplementation of DHA is beneficial in improving memory and cognition, the influence of dietary fats on the neurotransmitters and receptors involved in cognitive function is still not known. The aim of this study was to investigate serotonin receptor (5-HT$_{1A}$ and 5-HT$_{2A}$), cannabinoid receptor (CB1) and gamma-aminobutyric acid type A (GABA$_A$) receptor binding densities in the brain of male rats fed a high-saturated-fat (HF) diet, as well as the effect of DHA supplementation on HF diet. Alterations of these receptors in the post-mortem rat brain were detected by [^{3}H]-WAY-100635, [^{3}H]-ketanserin, [^{3}H]-CP-55,940 and [^{3}H]-muscimol binding autoradiography, respectively. In the hippocampus, the 5-HT$_{1A}$, CB1 and GABA$_A$ receptor binding densities significantly increased in response to an HF diet, while in the hypothalamus, 5-HT$_{1A}$ and CB1 binding densities significantly increased in HF-fed rats. Importantly, DHA supplementation prevented the HF-induced increase of receptors binding density in the hippocampus and hypothalamus. Furthermore, DHA supplementation attenuated 5-HT$_{2A}$ receptor binding density in the caudate putamen, anterior cingulate cortex and medial mammillary nucleus, which was also increased in HF group. This study showed that an HF diet increased 5-HT$_{1A}$, 5-HT$_{2A}$, CB1 and GABA$_A$ receptor binding densities in the brain regions involved in cognitive function and that dietary DHA can attenuate such alterations. These findings provide insight into the mechanism by which DHA supplementation ameliorates reduced cognitive function associated with an HF diet.

Keywords: DHA; High saturated fat; Serotonin receptor; CB1 receptor; GABA$_A$ receptor

1. Introduction

Different types of dietary fats affect body metabolism and cognitive function differently [1]. Studies have shown that a diet high in saturated fat promotes fat deposition and impairs memory and learning, and even contributes to the development of depression [2–4]. Conversely, a diet high in n-3 polyunsaturated fat, especially docosahexaenoic acid (DHA), can have the opposite effect [2–4]. A growing body of clinical findings implicates low DHA status with being overweight [5], impaired cognitive function and depression [6–8]. Plasma DHA was lowered in elderly subjects with depressive disorders compared to individuals without depression [8]. The tissue DHA content of the orbitofrontal cortex and cingulate cortex was also found to be lower in individuals with major depression [6,7]. Beneficial effects of DHA by improving cognition and antidepressive effects have been described in clinical trials and animal studies. There is evidence that DHA supplementation improves cognition [9], enhances memory [10] and induces an antistress response [11]; however, the underlying mechanisms remain unclear. Certain brain areas such as the hippocampus and cingulated cortex are important for cognitive function. However, there is little information on how dietary fat influences key receptors in these brain regions, which are important in the regulation of cognitive and metabolic function.

The neurotransmitter serotonin (5-HT) acts via 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors and has an important role in various central functions including control of energy intake, obesity, memory and learning [12–14]. 5-HT$_{1A}$ receptors are distributed throughout the brain and are located either pre- or postsynaptically, where they regulate various brain functions [12,15]. As presynaptic autoreceptors, the 5-HT$_{1A}$ receptors are found in dorsal and median raphe nuclei and negatively regulate 5-HT synthesis. A highly palatable diet in rats increases the density of 5-HT$_{1A}$ presynaptic receptor in these regions, suggesting a decrease in synthesis and consequently a decreased release of 5-HT [16]. 5-HT$_{1A}$ receptors as postsynaptic receptors have a wide distribution in the brain, with high density in the cortical and limbic areas, especially in the hippocampus and cortex, and low expression

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in other brain regions such as the hypothalamus, striatum and amygdala [17]. Clinical studies have shown that 5-HT_{1A} receptor expression is negatively associated with memory function [18]. Postsynaptic 5-HT_{2A} receptors can be found in high levels in cerebral cortical areas and at intermediate levels in the hypothalamus, striatum and hippocampus [19,20]. Using [^{125}I] DOI binding autoradiography, a high-saturated-fat diet increased 5-HT_{2A} binding density in the ventromedial hypothalamic nucleus and anterior olfactory nucleus in diet-induced obese mice, but not in mice resistant to obesity development [21]. Furthermore, using [^{3}H]-ketanserin autoradiography, 5-HT_{2A} receptor binding densities were significantly increased in postmortem tissue from the temporal cortex of patients with dementia [22]. Based on the accumulated evidence of clinical trials, blockade of 5-HT_{2A} receptor ameliorates both the positive and negative symptoms and, to some extent, the cognitive deficits in schizophrenia [23,24]. The highly selective 5-HT_{2A} antagonists MDL 100907 and EMD 281014, both developed as antipsychotics, have also been shown to enhance cognitive function in animal models [25,26].

The cannabinoid CB1 receptor plays an important role in various aspects of neural functions including learning and memory, anxiety, depression, addiction, appetite and feeding behaviour. Both CB1 knockout mice and CB1 antagonist (SR141716)-treated wild-type mice exhibited deficits in extinction of spatial memory [27,28]. The systemic administration of the CB1 agonist WIN55,212-2 in rats impaired the acquisition of contextual fear conditioning [29], which is known to depend on the hippocampus [30]. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. There are two receptors that mediate GABA neurotransmission in the brain: $GABA_A$ and $GABA_B$. The inhibitory function of $GABA_A$ is increasingly being recognised as important in the regulation of cognition, emotion, memory and obesity. It has been reported that the density of $GABA_A$ receptors was increased in the cortex of schizophrenia patients in order to compensate for the lowered levels of GABA [31,32]. Allelic variants in the $GABA_A\alpha 6$ receptor subunit gene were also associated with abdominal obesity [33]. Furthermore, the majority of leptin's antiobesity effects were mediated by GABAergic neurons reducing inhibitory tone to postsynaptic anorexigenic POMC neurons in the hypothalamus [34].

The effect of a DHA-supplemented, high-saturated-fat diet on these receptor binding densities in brain regions associated with cognition has not been thoroughly investigated. To address this issue, we have used multiple ligands including [^{3}H]-WAY-100635, [^{3}H]-ketanserin, [^{3}H]-CP-55,940 and [^{3}H]-muscimol to examine the regional changes of 5-HT_{1A}, 5-HT_{2A}, CB1 and $GABA_A$ receptor in the rat brain. Rats were fed either high-saturated-fat diet, DHA supplement in high-statured-fat diet or low-fat diet for 4 weeks. We examined alterations in receptor expression in response to a high-saturated-fat diet and if these alterations could be prevented by a supplementation of dietary DHA.

2. Experimental procedure

2.1. Animals and dietary treatments

Thirty male Wistar rats (300–320 g) were obtained from the Animal Resources Centre (Perth, Western Australia, Australia) and housed in environmentally controlled conditions (22°C, 12-h light–dark cycle with light cycle from 06:00 to 18:00 h and dark cycle from 18:00 to 06:00 h) with *ad libitum* access to standard laboratory chow and water. Rats were allowed 1 week to adapt to their new environment before experiments began. They were randomized into three groups with different diets: (a) standard laboratory chow as the low-fat control (LF, fat content 10% in kcal, saturated fat 1%), (b) high-fat diet (HF, 25% in kcal, saturated fat 10%) and (c) high-fat diet+0.5% DHA. The dose of DHA supplementation used in this study was based on the dose recommended for humans at 250 mg/70 kg/day (European Food Safety Authority) [35]. After 4 weeks of dietary treatment, rats were sacrificed by rapid CO_2 asphyxiation between 07:00 and 09:00 h in order to minimize the impact of circadian variation, and the brains were immediately removed and frozen in liquid nitrogen. Five rats per group were used to examine [^{3}H]-WAY-100635, [^{3}H]-ketanserin, [^{3}H]-CP-55,940 and [^{3}H]-muscimol binding in the brain. The study was approved by the University of Wollongong Animal Ethics Committee, and all animal experiments were conducted in compliance with the National Health and Medical Research Council Australian, Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

2.2. Histological procedures

Coronal brain sections (14 μm) were cut in a cryostat at −18°C from the level of bregma −0.24 mm to −5.16 mm [36], thaw-mounted onto poly-L-lysine-coated microscope slides (Polysine, Menzel GmbH & Co, KG) [37] and stored at −20°C.

2.3. [^{3}H]-WAY-100635, [^{3}H]-ketanserin, [^{3}H]-CP-55,940 and [^{3}H]-muscimol binding autoradiography

[^{3}H]-WAY-100635 autoradiography was performed to examine 5-HT_{1A} receptor binding density following procedures as described in previous work from our laboratories [38]. Brain sections were warmed to room temperature and preincubated in 50 nM Tris–HCl buffer (pH 7.4) for 30 min. The sections were then incubated with 5 nM [^{3}H]-WAY-100635 (specific activity 83.0 Ci/mmol, Amersham Biosciences, UK Limited) at room temperature for 2.5 h in 50 mM Tris–HCl (pH 7.4) containing 10 μM pargyline (Sigma). Nonspecific binding was determined by incubating consecutive sections exposed to 10 μM 5-HT. All sections were washed for 2 min and then 3 min in ice-cold 50 mM Tris–HCl buffer.

[^{3}H]-ketanserin autoradiography was performed as described previously [19]. Binding of [^{3}H]-ketanserin (67.0 Ci/mmol; PerkinElmer Life Sciences, Boston, MA, USA) to 5-HT_{2A} receptors was measured by preincubating sections in 170 mM Tris–HCl buffer (pH 7.4) for 15 min at room temperature. Sections were then incubated for 120 min at room temperature in buffer containing 2 nM [^{3}H]-ketanserin. Nonspecific binding was determined by the addition of 2 μM spiperone to consecutive sections. Sections were washed in ice-cold buffer (2×10 min), dipped in distilled water and dried.

Binding of [^{3}H]-CP-55,940 was used to assess binding density of CB1 receptor [39]. Sections were allowed to defrost and then preincubated for 30 min in Tris–HCl buffer [5% bovine serum albumin (BSA), 50 mM Tris–HCl, pH 7.4] at room temperature. The binding sites of CB1 receptor were defined by incubation with 10 nM [^{3}H]-CP-55,940. Nonspecific binding was determined in the presence of 10 μM CP-55,940. Following incubation for 2 h at room temperature, slides were washed firstly for 1 h and then 3 h in ice-cold buffer (1% BSA, 50 mM Tris–HCl, pH 7.4), and then finally washed for a further 5 min in buffer containing no BSA. Slides were then dipped briefly in ice-cold distilled water and dried under a gentle stream of cool air.

[^{3}H]-muscimol binding was performed to examine $GABA_A$ receptor binding density based on the method described in previous work from our laboratories [31]. Briefly, all sections underwent three 5-min preincubations at 4°C in 50 mM Tris–citrate (pH 7.0). Sections were then incubated for 45 min at 4°C in the same buffer containing 3 nM [^{3}H]-muscimol (specific activity 29.5 Ci/mmol, PerkinElmer, USA). Nonspecific binding was determined by incubating adjacent sections in [^{3}H]-muscimol plus 100 μM GABA. Following incubation, sections were rinsed four times for 2 s each in 4°C buffer.