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Short Communication

27-Hydroxycholesterol mediates negative effects of dietary cholesterol on cognition in mice

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HIGHLIGHTS

- Dietary cholesterol has a negative effect on memory in wildtype mice.
- This negative effect was not seen in mice lacking 27-hydroxycholesterol.
- 27-Hydroxycholesterol appears to mediate negative effects of cholesterol on memory.

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ABSTRACT

In spite of the fact that cholesterol does not pass the blood–brain barrier, treatment of mice with dietary cholesterol causes significant effects on a number of genes in the brain and in addition a memory impairment. We have suggested that these effects are mediated by 27-hydroxycholesterol, which is able to pass the blood–brain barrier. To test this hypothesis we utilized *Cyp*27−/− mice lacking 27-hydroxycholesterol. The negative effect on memory observed after treatment of wildtype mice with dietary cholesterol was not observed in these mice. The cholesterol diet reduced the levels of the "memory protein" Arc (Activity Regulated Cytoskeleton associated protein) in the hippocampus of the wildtype mice but not in the hippocampus of the *Cyp*27−/− mice.

The results are consistent with 27-hydroxycholesterol as the mediator of the negative effects of cholesterol on cognition.

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1. Introduction

Despite lack of conclusive evidence that cholesterol intervention prevents Alzheimer's disease (AD), a substantial body of data supports the contention that high mid-life cholesterol levels are associated with development of AD [1–3]. In view of the fact that cholesterol itself does not pass the blood–brain barrier, the possibility has been discussed that the link between circulating cholesterol and neurodegeneration may be a consequence of vascular and inflammatory effects of high cholesterol on the brain vascular system [4–6]. At the present state of knowledge, however, alternative explanations cannot be excluded.

In marked contrast to cholesterol itself, side-chain oxidized metabolites of cholesterol are able to pass the blood–brain barrier. One of these metabolites is 27-hydroxcholesterol (27OH), and we have shown that there is a substantial continuous flux of this oxysterol from the circulation into the brain [7]. High levels of circulating cholesterol are associated with high levels of 27OH and thus it is likely that hypercholesterolemia is associated with increased flux of 27OH into the brain. The brain of patients who had died with AD contained markedly increased levels of 27OH [8]. Under in vitro [9–11] as well as under some in vivo conditions [11], 27OH seems to accelerate neurodegenerative processes.

Treatment of mice with dietary cholesterol have significant effects on the expression of a number of genes in the brain and in addition a memory impairment [10,12]. In a previous work we showed that a high fat diet containing high cholesterol reduces

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the level of the "memory protein" activity-regulated cytoskeleton-associated protein, (Arc) in mouse brain [9]. Addition of 27OH to cultures of primary hippocampal neurons was found to reduce expression of Arc.

We [9,10] and others [13] have suggested that the negative effects of hypercholesterolemia on the brain may be mediated by 27OH. However, this hypothesis has not been demonstrated firmly in vivo.

In the present work we have tested the hypothesis that the impaired memory function caused by dietary cholesterol is mediated by 27OH and that part of this effect is mediated by Arc. In this study we have utilized *Cyp*27−/− mice lacking 27OH in the circulation and brain [14]. Since such mice have a reduced production of bile acids and reduced capacity to absorb cholesterol and other lipids, these mice were substituted with 0.05% cholic acid in the diet [15].

2. Materials and methods

2.1. Mice

Male *Cyp*27−/− knockout (KO) and wild type (WT) of the inbred C57BL/6J background were used for the study. The KO and WT mice were generated from heterozygotes as described previously [15]. Mice were kept on a 12-h light/12-h dark cycle and with ad libitum access to food and water. They were housed in Makrolon type II cages with wire top (Tecniplast, Sweden). The study was carried out in 4 cohorts of 43 animals. At about 18–20 g of body weight and at 30–42 days old, the mice were randomly assigned to 4 different types of diet as follows: Lab chow feed: R34 (CHOW); cholesterol feed: R34 with 0.5% cholesterol (CHOL); cholic acid feed: R34 with 0.05% cholic acid (CA); cholesterol with cholic acid feed: R34 with 0.5% cholesterol and 0.05% cholic acid (CHOL + CA). The different diets were obtained from Lantmännen. The four groups of mice were WT-CHOW ($n = 12$); WT-CHOL ($n = 12$); *Cyp*27KO-CA ($n = 9$); *Cyp*27KO-CHOL + CA ($n = 9$). Two additional groups of *Cyp*27KO mice were used: *Cyp*27KO-CA ($n = 8$); *Cyp*27KO-CHOW ($n = 8$).

The mice were weighed before they started the diet. They were on these diets for approximately 4 months and were all behaviorally tested in the water maze at 4 months of age. After the completion of behavioural testing, the mice were killed by cervical dislocation and trunk blood was collected. The brain was dissected out; the left- and right-hippocampus and cortex, and the cerebellum were collected. The brain pieces were put in liquid nitrogen and stored in a −70 freezer (Hotlab freezer) until analyzed.

All experimental procedures in this study were in compliance with National Institutes of Health Guide for Care and Use of Laboratory Animals, the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by The Southern Stockholm Research Animal Ethics Committee. The experiments were performed when possible by examiners blinded to the genotypes or treatments of the mice.

2.2. Morris water maze test

The Morris water–maze (MWM) is a circular pool constructed of grey PVC, 160 cm in diameter and 45 cm in height [16]. MWM protocol was adapted from Gao et al. [17]. Briefly, the pool is filled with water and renders opaque using non-toxic tempera paint to form a contrast with the mouse's body for the automated tracking software to detect the mouse. The water is kept at a temperature of $21 \pm 1\,^{\circ}$C. A transparent plastic square platform (9 cm × 9 cm) is placed approximately 1 cm below the water surface and 10 cm from the edge of the pool. Distal visual cues consist of several wall posters approximately 0.50 × 0.75 m in size that surround the pool. The whole experiment consists of three phases. (1) Water adaptation trial: to test for stamina and to habituate the mice to the water, on Day 1, all the mice are given 3 habituation swimming trials of 60 s/trial with no platform present in the pool. This was done to see if individual mice retained the stamina necessary to complete three tests. (2) Acquisition learning test and probe test: the entire acquisition training procedure occurs over the next 3 days. The mice are trained in hidden platform position, to locate and escape onto the submerged platform over 6 sessions (2 sessions per day, 4 h apart) each with 3 trials. The maximum time allowed to locate the hidden platform was 60 s/trial with inter-trial intervals of 15 s. The entry points are changed randomly between trials. During the acquisition phase, mouse that did not find the platform during the 60 s trial period was placed on the platform for 15 s at the end of the trial, to assist its learning. At the end of the learning phase the platform was removed from the position and a 60 s probe test was done. The entry point for probe trials was in the quadrant opposite the target quadrant. (3) Visual cue tests: three visual cue tests were performed where the hidden platform with black/white stripes panel on one side is raised above the water surface and becomes visible. Performance in the MWM test was monitored with EthoVision video-tracking system (Noldus Information Technology, Wageningen, The Netherlands).

2.3. Measurements of Arc

Total RNA from hippocampus of mouse brain was extracted following the manufacturer's instructions using the RNAeasy lipid tissue mini kit acquired from Qiagen (CA, USA). Total RNA was reverse-transcribed using High-capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). Real-time RT-PCR amplification assays for ARC gene were performed with a total volume of 20 μl in each well containing 10 μl of PCR Master Mix (Applied Biosystems), 2 μl of cDNA corresponding to 10 ng of RNA, and 1 μl of TaqMan® Gene Expression Assays (Applied Biosystems). GAPDH was used as an endogenous control. The relative quantification of ARC expression was carried out using the comparative cycle threshold method, $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{ARC} - Ct_{GAPDH})_{treated} - (Ct_{ARC} - Ct_{GAPDH})_{untreated}$ [18]. Each sample was measured in triplicate. Relative transcription levels ($2^{-\Delta\Delta Ct}$) were expressed as a mean ± SEM.

2.4. Statistical analysis

Morris water–maze tests were analyzed with repeated measurements analysis of variance (ANOVA) using SPSS software. When appropriate, ANOVA was followed by Bonferroni post hoc test. RT PCR data were analyzed with Mann–Whitney test. The level of statistical significance was set at $p < 0.05$ for all parameters. All data are presented as group mean values ±SEM.

3. Results and discussion

3.1. Body weight

All mice gradually increased their body weight from the baseline starting point of 4 months of feeding on their assigned diets. The data for body weight was analyzed by repeated measures ANOVA with the four different groups as the independent variable. As can be expected in young adult mice, a significant effect was apparent ($p < 0.001^{**}$) due to the increase in body weight from baseline to the starting of behavioural testing of the experiment (data not shown). There was no significant difference in body weight between the groups ($p = 0.09$).

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