Biochemical and Biophysical Research Communications 404 (2011) 254–260

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Abrogation of neutral cholesterol ester hydrolytic activity causes adrenal enlargement

Keisuke Ohta [a], Motohiro Sekiya [a,*], Hiroshi Uozaki [b], Masaki Igarashi [a], Satoru Takase [a], Masayoshi Kumagai [a], Mikio Takanashi [a], Yoshinori Takeuchi [a], Yoshihiko Izumida [a], Midori Kubota [a], Makiko Nishi [a], Hiroaki Okazaki [a], Yoko Iizuka [a], Naoya Yahagi [a], Hiroaki Yagyu [c], Masashi Fukayama [b], Takashi Kadowaki [a], Ken Ohashi [a], Shun Ishibashi [c], Jun-ichi Osuga [c]

[a] The Departments of Metabolic Diseases, University of Tokyo, Tokyo 113-8655, Japan
[b] Pathology, Faculty of Medicine, University of Tokyo, Tokyo 113-8655, Japan
[c] The Division of Endocrinology and Metabolism, Department of Medicine, Jichi Medical University, Tochigi 329-0498, Japan

ARTICLE INFO

Article history:
Received 22 November 2010
Available online 25 November 2010

Keywords:
Adrenal glands
Nceh1
Lipe
Cholesterol ester

ABSTRACT

We have previously demonstrated that neutral cholesterol ester hydrolase 1 (Nceh1) regulates foam cell formation and atherogenesis through the catalytic activity of cholesterol ester hydrolysis, and that Nceh1 and hormone-sensitive lipase (Lipe) are responsible for the majority of neutral cholesterol ester hydrolase activity in macrophages. There are several cholesterol ester-metabolizing tissues and cells other than macrophages, among which adrenocortical cells are also known to utilize the intracellular cholesterol for steroidogenesis. It has been believed that the mobilization of intracellular cholesterol ester in adrenal glands was facilitated solely by Lipe. We herein demonstrate that Nceh1 is also involved in cholesterol ester hydrolysis in adrenal glands. While Lipe deficiency remarkably reduced the neutral cholesterol ester hydrolase activity in adrenal glands as previously reported, additional inactivation of Nceh1 gene completely abrogated the activity. Adrenal glands were enlarged in proportion to the degree of reduced neutral cholesterol ester hydrolase activity, and the enlargement of adrenal glands and the accumulation of cholesterol esters were most pronounced in the Nceh1/Lipe double-deficient mice. Thus Nceh1 is involved in the adrenal cholesterol metabolism, and the cholesterol ester hydrolytic activity in adrenal glands is associated with the organ enlargement.

1. Introduction

Adrenocortical cells require constant supply of cholesterol as a precursor for the conversion of steroid hormones. Cholesterol delivery in the adrenal glands involves three major processes: (1) uptake of lipoprotein-derived cholesterol via low density lipoprotein receptor (LDLR) mediated endocytic pathways and scavenger receptor class B member 1 (SCARB1)-mediated "selective" uptake pathways (2) endogenous cholesterol biosynthesis in endoplasmic reticulum (ER) (3) cholesterol mobilization from intracellular cholesterol esters (CEs) stored in lipid droplets [1]. Regarding the cholesterol uptake, adrenal glands of rodents are largely dependent on SCARB1-mediated selective uptake pathway while LDLR pathway plays a pivotal role in human adrenal cholesterol metabolism [2–4]. In the selective uptake process, lipoproteins bind to SCARB1 through which CEs are delivered into the cells. The delivered CEs should be hydrolyzed to be utilized for steroidogenesis by non-lysosomal neutral lipase(s). Therefore, CE hydrolysis plays a pivotal role not only in the break down of stored lipids but also in the lipoprotein uptake and utilization. The resultant unesterified cholesterol is transported to mitochondria by the steroidogenic acute regulatory protein (StAR) [5], where it is converted into the different steroid hormones by a battery of oxidative enzymes [6].

Hormone-sensitive lipase (Lipe) was reported to catalyze the liberation of free cholesterol from its esterified form in adrenal glands and to be involved in adrenal steroidogenesis [7]. Lipe is an intracellular neutral lipase which catalyzes the hydrolysis of triacylglycerol (TG), diacylglycerol, monoacylglycerol, CEs, phospholipids, retinyl ester and other lipids in a wide variety of tissues and cells [8–11]. Among the cells expressing Lipe, the hydrolysis of intracellular CEs in macrophages has been a matter of debate,

Abbreviations: LDL, low density lipoprotein; CE, cholesterol ester; Scarb1, scavenger receptor class B member 1; ER, endoplasmic reticulum; nCEH, neutral cholesterol ester hydrolase; StAR, steroidogenic acute regulatory protein; Nceh1, neutral cholesterol ester hydrolase 1; Lipe, hormone-sensitive lipase; ACTH, adrenocorticotropic hormone; Hmgcs1, 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1; Abca1, ATP-binding cassette sub-family A member 1; Soat1, sterol O-acyltransferase 1; Soat2, sterol O-acyltransferase 2.

* Corresponding author. Fax: +81 3 5802 2955.
E-mail address: mseki-tky@umin.ac.jp (M. Sekiya).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.11.103

and Lipe has been one of the candidates of macrophage CE hydrolases. However, circumstantial evidences have suggested the existence of unknown lipase(s). We recently identified a promising lipase, neutral cholesterol ester hydrolase 1 (Nceh1) [12], and demonstrated that Nceh1 and Lipe are the major CE hydrolases in macrophages taking advantage of targeted gene deletion [13].

Nceh1 is highly expressed in macrophages and the expression is also observed in brain, kidney, heart and other tissues. Nceh1 liberates fatty acids from CEs, and also from TG to a lesser extent [12]. In terms of structural aspects, Nceh1 is inserted into the ER membrane by an N-terminal single-spanning transmembrane domain [14]. Lipe possesses an N-terminal domain which interacts with fatty acid binding protein 4 [15] whereas Nceh1 lacks such a domain. Lipe is also reported to interact with StAR [16] and perilipin [17]. The C-terminal domain of Nceh1 is composed of α/β hydrolase folds that accommodate the catalytic motif which is almost identical to that of Lipe in amino acid sequence. Adenovirus-mediated overexpression of Nceh1 blocked foam cell formation in THP-1 macrophages [12].

We herein demonstrate that Nceh1 is also involved in the adrenal CE metabolism. To the best of our knowledge, this is the first published report that lack of Lipe resulted in a significant increase of adrenal weight, which was more pronounced in adrenal glands in *Nceh1/Lipe* double-deficient mice. Lipe was the major neutral CE hydrolase (nCEH) in adrenal glands while Nceh1 was also responsible for lesser but significant amounts of nCEH activity. The extent of reduction of nCEH activity was correlated with the adrenal enlargement as well as adrenal lipid contents.

2. Materials and methods

2.1. Mice

Nceh1-deficient, Lipe-deficinet, or Nceh1/Lipe double-deficient mice were generated as described previously [13,18]. All mice used in this study were crossed onto the C57BL/6J background for more than 10 generations. Mice were maintained on a 12 h dark/light cycle and were fed a normal chow diet (Lab Diet 5053, PMI Nutrition International). Mice were euthanized at 9 weeks at the beginning of the dark cycle on ad libitum, unless otherwise stated. All experimental procedures handling animals were conducted according to our institutional guidelines.

2.2. Preparation of adrenal glands

Adrenal glands were removed, dissected free of fat under stereoscopic microscope, rinsed in PBS, blotted dry and weighed individually on an electronic platform scale (AG135, Mettler Toledo, Tokyo, Japan).

2.3. Determination of adrenal CE content

Adrenal lipids were extracted by methanol/chloroform, and CE content was determined by an enzymatic fluorometric microassay [13].

2.4. Determination of adrenal DNA content

Adrenal glands were homogenized and sonicated. The DNA content was measured by fluorescent method with Hoechst 33258 [19].

2.5. nCEH activity assay

Enzyme activity was assayed basically as described previously [18]. The homogenates were incubated at 37 °C for 60 min in a final volume of 200 μl of a reaction mixture containing 6.14 μmol/l cholesterol [1-^{14}C] oleate (48.8 μCi/μmol), 23.7 μmol/l lecithin, 12.5 μmol/l sodium taurocholate, and 85 mmol/l potassium phosphate (pH 7.0).

2.6. Histology

Sections were stained with hematoxylin–eosin or immunostained as follows. After incubation with primary antibody (anti-mouse ki-67 clone TEC-3 1:50; DAKO or anti-Nceh1 antibody 1:8000, respectively) for overnight at 4 °C, the sections were incubated with secondary antibody for 30 min at room temperature and then with EnVision plus system (DAKO) or Vectastain elite ABC standard kit (Vector), respectively for 30 min at room temperature. Finally, the sections were developed with DAB (Wako Pure Chemicals Co.) and counterstained with hematoxylin.

2.7. Statistics

Statistical differences between groups were analyzed by one-way ANOVA and a Tukey–Kramer posthoc test, unless stated otherwise.

3. Results

3.1. Genetical inactivation of Nceh1 and/or Lipe results in an increase in adrenal weights

While attempting to reveal as-yet-unidentified physiological roles of Nceh1, we noted the considerable enlargement of adrenal glands in *Nceh1/Lipe* double-deficient mice (Fig. 1A). Consistent with the visual observations, deficiency of Nceh1 and/or Lipe increased adrenal weights although the effect of Nceh1 deficiency was relatively small compared to Lipe deficiency (increased by 10% and 26% in *Nceh1*-deficient mice and *Lipe*-deficient mice, respectively (Fig. 1C). The enlargement of adrenal glands was most pronounced in *Nceh1/Lipe* double-deficient mice (62% increase). Adrenal glands of male mice are normally smaller than those of female mice [20], that was also reproduced in our experimental setting, and the enlargement of adrenal glands caused by the deficiency of lipase(s) was observed in both genders (data not shown). Although the adrenal mass is under systemic hormonal control, it has been reported that several intra-adrenal molecules directly affect adrenal mass [21–23]. Indeed, we were able to detect NCEH1 protein expression in adrenal glands as well as LIPE (Fig. 1B), therefore we attempted to reveal the physiological roles of Nceh1 and Lipe in adrenal glands.

To address whether the adrenal enlargement is due to cellular hypertrophy and/or hyperplasia, adrenal DNA content was measured (Fig. 1D). DNA content was mildly increased solely in *Nceh1/Lipe* double-deficient adrenal glands, and weight/DNA ratio, which reflects the average weight per cell, was significantly increased solely in *Nceh1/Lipe* double-deficient adrenal glands (Fig. 1E). These results indicated that the adrenal enlargement in *Nceh1/Lipe* double-deficient mice was caused by both cellular hypertrophy and hyperplasia. Since plasma adrenocorticotropic hormone (ACTH) levels were similar among groups, the adrenal enlargement was not due to the activation of hypothalamic pituitary adrenal axis (Fig. 1F).

3.2. Nceh1 and/or Lipe deficiency reduced adrenal nCEH activity and caused cellular CE accumulation

To explore the underlying mechanism, the adrenal glands of each genotype were examined histologically (hematoxylin–eosin