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Hormone-sensitive lipase deficiency disturbs lipid composition of plasma membrane microdomains from mouse testis

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ABSTRACT

Hormone-sensitive lipase (HSL) is an intracellular neutral lipase capable of hydrolyzing acylglycerols, as well as cholesteryl and retinyl esters. In mice, HSL deficiency results in male sterility. Lipid rafts, a plasma membrane microdomain enriched in cholesterol, sphingolipids and saturated glycerophospholipids, comprise a highly dynamic clustering of proteins and lipids that play a central role in signal transduction and intercellular communication. In the present work, we examined the effect of HSL deficiency in the lipid composition of plasma membrane microdomains in mouse testis. The lack of HSL affected the density of lipid rafts, as indicated by the shifting of caveolin 1 to denser fractions in the sucrose-gradient fractionation, and altered the sterol and phospholipid composition of both lipid raft and non-raft fractions, the biochemical differences among them being less obvious in $HSL^{-/-}$ than in $HSL^{+/+}$ mice. Compared to $HSL^{+/+}$, the lipid rafts from $HSL^{-/-}$ mice had significantly less desmosterol and T-MAS, while the non-raft domain had increased cholesterol content. Lipid rafts from $HSL^{-/-}$ mice had reduced PUFA-containing phospholipid species but increased phosphatidylcholine and phosphatidylethanolamine species with monounsaturated fatty acid moieties, while the non-raft domain was enriched in phosphatidylethanolamine and phosphatidylserine species having four double bonds. The changes in both the sterols and phospholipid composition of lipid raft and non-raft microdomains may have consequences in signal transduction and contribute to the sterility observed in HSL-deficient male mice.

1. Introduction

Hormone-sensitive lipase (HSL) is an intracellular neutral lipase that hydrolyzes triacylglycerols, diacylglycerols and monoacylglycerols, as well as cholesteryl and retinyl esters [1]. This multifunctional enzyme has emerged as a key regulator of lipid metabolism in multiple tissues. Its role in testis was revealed by the phenotype of HSL-deficient mice [2,3]. Male mice homozygous for the mutant allele ($HSL^{-/-}$) are sterile as a result of oligospermia and not hypogonadism, indicating that HSL inactivation mainly affects spermatogenesis [2]. In testis, HSL is the only esterase that can hydrolyze cholesteryl ester, and the loss of this activity results in cholesteryl ester and diacylglycerol accumulation [2], and altered lipid homeostasis [4–6]. We have shown that the lack of HSL in mouse testis induces upregulation of scavenger receptors class B (SR-Bs), alters the protein composition of lipid raft microdomains, and induces activation of key proteins in cell signaling pathways [7]. In HSL deficient mouse testis, the fatty acid composition was also greatly altered, with decreases in essential polyunsaturated fatty acid (PUFA) n-6 and n-3, increases in PUFA n-6 and n-3 intermediates, and Mead acid; moreover the expression of several genes involved in fatty acid metabolism was also affected [6].

Testicular lipids strongly influence the histology and physiology of this tissue [8–10]. Lipid content and intracellular lipids distribution are fairly constant in physiological conditions. Thus their homeostasis is crucial for optimal cellular function and should be tightly regulated [11]. In addition to HSL deficiency, other lipid homeostasis alterations

Abbreviations: Abcg1, ATP-binding cassette transporter G1; BCA, bicinchoninic acid; C, carbon atoms; Cyp51, lanosterol 14α-demethylase or 14α-sterol demethylase; CypB, cyclophilin B; db, double bonds; Dhcr24, desmosterol reductase or sterol Δ^{24}-reductase; Elovl 2, elongase of very-long chain fatty acid 2; Fads, fatty acid desaturase; FF-MAS, follicular fluid meiosis activating sterol; Hmgcr, 3-hydroxy-3-methyl-glutaryl-CoA reductase; HSL, hormone-sensitive lipase; KO, knock-out; PC, phosphatidylcholine; PCA, principal component analysis; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SM, sphingomyelin; SR-B, scavenger receptors class B; T-MAS, testicular meiosis activating sterol; TfR, transferrin receptor.

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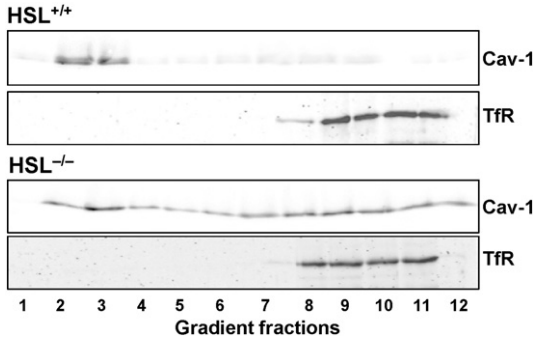

Fig. 1. Effects of HSL deficiency on testis plasma membrane microdomains. Western blotting of gradient fractions from $HSL^{+/+}$ and $HSL^{-/-}$ mouse testis with anti-caveolin-1 (Cav-1) and anti-transferrin (anti-TfR) antibodies, shows the expression of Cav-1 in lipid raft fractions (fractions 2 and 3) and TfR in non-raft fractions (fractions 8–11). Representative immunoblots are shown.

may cause infertility, as shown in ATP-binding cassette transporter $1^{-/-}$ [12], $Fads2^{-/-}$ (fatty acid desaturase 2) [13], $Elovl2^{-/-}$ (elongase of very-long chain fatty acid 2) [14] and $Nr1h2^{-/-}$ (Lxrβ, liver X receptor β) [15] mice.

There is also considerable evidence indicating that cholesterol is required for the development of gametes and fertility in both sexes. Disruption of *Dhcr24*, which encodes the cholesterol biosynthetic enzyme desmosterol reductase or sterol Δ^{24}-reductase, causes infertility in male mice [16]. In addition to cholesterol sterol intermediates, such as follicular fluid meiosis activating sterol (FF-MAS) and the testicular meiosis activating sterol (T-MAS), were described as stimulators of meiosis in the gonads in vitro [17]. More recently, however, it has been reported that a male mouse model with germ cell-specific knockout (KO) of Cyp51 (lanosterol 14α-demethylase), which catalyzes the conversion of lanosterol to FF-MAS, showed no effects on gametogenesis and fertility [18]. Other cholesterol biosynthesis intermediates have been also detected in the testis [18] and the possibility exists that cholesterol intermediates accumulating in the testis may have additional physiological roles in this organ.

Lipid rafts, a plasma membrane microdomain, have been established as an important feature in cell membranes with regard to signal transduction, trafficking, endocytosis and exocytosis [19]. These microdomains are enriched in cholesterol, sphingolipids and saturated glycerophospholipids. Lipid rafts are heterogeneous in both protein and lipid content. The presence of cholesterol is critical for membrane raft formation; some rafts require less cholesterol than others in order to maintain their integrity [20]. Changes in lipid composition of lipid raft have been shown to alter the specific localization of raft associated proteins and their function [21–24]. Many key signaling molecules have been shown to function via lipid rafts; examples of such molecules are SR-Bs [7]. Thus, it is not surprising that lipid homeostasis dysfunction in the testis may lead to changes in plasma membrane microdomains that could cause infertility in mice.

The hypothesis of present study was that the metabolic changes induced by HSL deficiency may result in the alteration of the composition of the plasma membrane microdomains and of the lipid homeostasis in mice testis. The main objective of the present work was to study the effect of the lack of HSL in the lipid composition of plasma membrane microdomains, both lipid raft and non-raft, in mice testis, to gain insight into the mechanisms mediating the infertility in these male mice.

2. Materials and methods

2.1. Mice and tissue collection

We used wild-type (or $HSL^{+/+}$) and HSL KO (or $HSL^{-/-}$) mice from our colony weighing 25–35 g and approximately 4 months old. Mice were kept in a temperature-controlled environment with a 12 h light/dark cycle. The mice were given a standard rodent chow (SAFE-A04) ad libitum and had continuous access to tap water. The experimental protocol was approved by the Animal Research Ethics Committee (CEEA) at the Hospital Universitario Ramón y Cajal (Madrid, Spain).

The generation of mice with a targeted disruption of the Lipe gene encoding HSL ($HSL^{-/-}$) has been reported; these mice have no detectable HSL activity or protein in any tissue [2,3]. HSLþ/F1 mice were bred for >5 generations into a C57BL/6 background. Given that homozygote males are infertile, heterozygote females were mated with heterozygote males to maintain the transgenic mice colony. The pups were maintained with the mothers until the end of the lactating phase. Two weeks after weaning, males and females were separated into individual cages. Mice were genotyped by PCR analysis of tail DNA, as described previously [2].

At least 5 mice from each experimental group were analyzed when they were 4 months old. Mice were anesthetized by i.p. injection of 100 μl/25 g of body weight of ketamin (50 mg/ml) and 2% xilacine (3:1 vol/vol) and the blood samples were drawn by intracardiac puncture. Mice were sacrificed after anesthesia by cervical dislocation. Whole animal body and testicular weights were recorded. Testes were removed, frozen in liquid nitrogen and stored at −80 °C until processing.

2.2. Sucrose gradient fractionation of membranes

Membrane fractions were separated according to a modified version of a previously described detergent-free method [7,24]. Briefly, frozen testes were homogenized in 2 ml of 500 mM sodium carbonate (pH 11.0) supplemented with protease inhibitor cocktail (Thermo Scientific, Pierce, Rockford, IL, USA) using a Dounce homogenizer. Then the homogenates were centrifuged for 30 min at 15,000 g to obtain a Golgi-free supernatant. This was further disrupted by sonication with 3 bursts of 30 s each. Equal amounts of membrane protein were then adjusted to 42.5% sucrose by the addition of 2 ml 85% sucrose prepared in MBS (25 mM 2-(*N*-morpholino)ethanesulfonic acid [MES]) pH 6.5 and 0.5 M NaCl) and placed at the bottom of an ultracentrifuge tube. A 5–35% discontinuous sucrose gradient was formed in MES

Table 1
Sterol composition of total membrane and lipid raft and non-raft membrane domains in $HSL^{+/+}$ and $HSL^{-/-}$ mouse testis.
Lipids were extracted from each fraction and analyzed by GC/MS. Total membrane data are shown as ng of each membrane sterol per mg of tissue protein. Lipid raft and non-raft data are shown as μg of each sterol species per mg of fraction protein (raft, fractions 2 + 3; non-raft, fractions 8 + 9 + 10). Data are shown as means ± SEM of 4–5 mice per experimental group. Statistical comparisons are shown versus $HSL^{+/+}$ (* $P < 0.05$, ** $P < 0.01$). T-MAS ($\Delta^{8,24}$-dimethylcholestadienol).

	$HSL^{+/+}$			$HSL^{-/-}$		
Sterol	Total membrane (ng/mg)	Lipid raft (μg/mg)	Non-raft (μg/mg)	Total membrane (ng/mg)	Lipid raft (μg/mg)	Non-raft (μg/mg)
Cholesterol	15,289 ± 1455	382.6 ± 56.3	52.10 ± 4.74	15,815 ± 1105	285.5 ± 28.2	64.0 ± 3.5*
Desmosterol	109.3 ± 13.9	1.91 ± 0.40	0.39 ± 0.09	72.0 ± 9.2*	0.96 ± 0.18*	0.39 ± 0.04
Lathosterol	27.2 ± 4.2	0.42 ± 0.06	0.11 ± 0.02	22.4 ± 5.4	0.38 ± 0.10	0.10 ± 0.01
T-MAS	46.3 ± 8.1	0.82 ± 0.07	0.15 ± 0.03	23.9 ± 4.6*	0.37 ± 0.06**	0.12 ± 0.01
Lanosterol	13.1 ± 4.0	0.20 ± 0.04	0.04 ± 0.003	11.4 ± 2.7	0.11 ± 0.03	0.04 ± 0.006