IMPACT OF BLUNTED *DE NOVO* FATTY ACID SYNTHESIS ON NEUROACTIVE STEROID LEVELS IN SCIATIC NERVE

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IMPACT OF BLUNTED *DE NOVO* FATTY ACID SYNTHESIS ON NEUROACTIVE STEROID LEVELS IN SCIATIC NERVE

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Abbreviations: 3α-hydroxysteroid oxidoreductase (3α-HSOR); 3β-hydroxysteroid oxidoreductase (3β-HSOR); 5α-androstane-3α,17β-diol (3α-diol); 5α-androstane-3β,17β-diol (3β-diol); 5α-reductase (5α-R); dehydroepiandrosterone (DHEA); dihydroprogesterone (DHP);

dihydrotestosterone (DHT); fatty acids (FAs); isopregnanolone (ISOPREG); liquid chromatography-tandem mass spectrometry (LC-MS/MS); P450 side chain cleavage (P450scc); peripheral nervous system (PNS); pregnenolone (PREG); progesterone, (PROG); sterol regulatory-binding factor-1c (SREBF-1c); testosterone (T); tetrahydroprogesterone (THP); wild type (WT); knock-out (KO).

Abstract

As we recently demonstrated, sterol regulatory-binding factor-1c (SREBF-1c) knock-out (KO) mice develop peripheral neuropathy. Neuroactive steroid levels are altered in several experimental model of peripheral neuropathy and these molecules have been proposed as protective agents. Therefore, we have assessed by liquid chromatography tandem mass spectrometry the levels of neuroactive steroids in plasma and in sciatic nerve of SREBF-1c KO male mice at two and ten months of age. These analyses were implemented analyzing in sciatic nerve the gene expression of crucial steroidogenic enzymes. Data obtained at two months of age have shown in sciatic nerve an increase of pregnenolone, associated with decrease of its first metabolite, progesterone, and further metabolites (i.e., dihydroprogesterone and isopregnanolone). The levels of testosterone were also increased. At ten months of age, the neuroactive steroid profile showed some differences. Indeed, in addition to the changes observed at two months of age, a significant decrease of pregnenolone and an increase in dihydroprogesterone, tetrahydroprogesterone and isopregnanolone were observed. Furthermore, the levels of testosterone and its metabolites (i.e., dihydrotestosterone, 3α-diol and 3β-diol) were significantly decreased. Interestingly, changes in pregnenolone, progesterone and testosterone were not observed in plasma and gene expression of steroidogenic enzymes considered was modified in the sciatic nerve suggesting a specific effect of SREBF-1c on neurosteroidogenesis. Because this peripheral neuropathy is due to the blunted fatty acid biosynthesis, data here reported support the concept that the cross-talk between this biosynthetic pathway and

neuroactive steroids, may represent a possible therapeutic strategy for peripheral neuropathy.

Introduction

Sterol regulatory-binding factor-1c (SREBF-1c) is a transcription factor of the basic helix-loop-helix-leucine zipper family that preferentially controls the synthesis of fatty acids (FA) and triglycerides (Liang *et al.* 2002). Fatty acids represent, together with cholesterol, the most abundant myelin lipids of the peripheral nervous system (PNS) (Garbay *et al.* 2000). However, while the role of cholesterol metabolism is well ascertained (Saher & Stumpf 2015), the specific contribution of FA synthesis remains to be elucidated. To this aim we have recently explored the lack of the key lipogenic transcription factor SREBF-1c on peripheral myelin compartment (Cermenati *et al.* 2015). Data we recently obtained show that SREBF-1c knock out (KO) mice, at two months of age, display a neuropathic phenotype (i.e., impairment of thermal and mechanical nociceptive threshold, subtle abnormalities of small unmyelinated C fibers) that results in hypermyelination of small-caliber fibers due to changes in myelin periodicity at ten months of age (Cermenati *et al.* 2015). In addition, these animals showed a decreased expression of biosynthesis genes as well as altered levels of FAs, such as palmitic, stearic, oleic and linoleic acids, in sciatic nerve (Cermenati *et al.* 2015). Altogether, these results indicate that impairment of FA synthesis lead to the development of peripheral neuropathy. In this context, it is important to highlight the role of neuroactive steroids. These latter are molecules acting in the nervous system including steroids produced by the nervous system (i.e., neurosteroids) and hormonal steroids coming from classical steroidogenic tissues (i.e., gonads and adrenal glands) (Melcangi *et al.* 2008). In particular, neuroactive steroids have been proposed as protective agents for different kinds of peripheral neuropathy,

such as those due to aging, physical injury, chemotherapy and diabetes (Giatti *et al.* 2015, Melcangi *et al.* 2011). PNS is not only a target for the action of neuroactive steroids but is also able to synthesize and metabolize them. Indeed, Schwann cells (i.e., cells forming peripheral myelin) express the machinery of the first step of steroidogenesis, such as the enzyme cytochrome P450 side chain cleavage (P450scc), responsible of the conversion of cholesterol into pregnenolone (PREG) (Zhu & Glaser 2008, Olbrich *et al.* 2013). In addition, Schwann cells as well as dorsal root ganglia express steroidogenic enzymes such as 3β-hydroxysteroid dehydrogenase, which converts PREG into PROG (Rodriguez-Waitkus *et al.* 2003, Chan *et al.* 2000, Schumacher *et al.* 2001, Coirini *et al.* 2003) and 5α-reductase (5α-R) type 1, which converts PROG and testosterone (T) into dihydroprogesterone (DHP) and dihydrotestosterone (DHT) respectively (Giatti *et al.* 2015, Melcangi *et al.* 2011). Moreover, DHP and DHT are then further metabolized by the action of 3α- or 3β-hydroxysteroid oxidoreductase (3α- or 3β-HSOR) (Giatti *et al.* 2015, Melcangi *et al.* 2011). In particular, DHP is converted in tetrahydroprogesterone (THP) or in isopregnanolone (ISOPREG), while T is converted in 5α-androstane-3α,17β-diol (3α-diol) or in 5α-androstane-3β,17β-diol (3β-diol). In agreement, with the neurosterodogenic capacity of PNS, altered levels of neuroactive steroids were reported in different experimental models of peripheral neuropathy (Melcangi *et al.* 2014, Melcangi *et al.* 2016, Giatti et al. 2015). Interestingly, a link between neuroactive steroids and FA synthesis in the PNS has been also reported. Indeed, activation of liver X receptors exerts important neuroprotective effects in diabetic peripheral neuropathy induced by streptozotocin restoring in sciatic nerve the altered SREBF-1c function, myelin FAs as well as neuroactive steroids levels (Cermenati *et al.* 2010, Cermenati *et al.*

2012). In addition, in the same experimental model treatment with DHP or 3α-diol exerts neuroprotective effects modulating myelin lipid profile in the sciatic nerve. Indeed, both neuroactive steroids were able to normalize the levels of stearic and oleic acids, as well as of SREBF-1c and expression of enzymes involved in the *de novo* lipogenic pathways (Mitro *et al.* 2014). All these effects resulted in a reduction of the myelin alterations (i.e., myelin infoldings) observed in the sciatic nerve of diabetic animals (Mitro et al. 2014).

On the basis of these results, levels of neuroactive steroids were evaluated in plasma as well as in sciatic nerve of SREBF-1c KO mice at two and ten months of age and compared with levels observed in wild type (WT) littermate animals. These analyses were implemented analyzing in sciatic nerve the gene expression of crucial steroidogenic enzymes, such as P450scc, 5α-R and 3α- HSOR.

Materials and Methods

PREG, PROG, DHP, THP, ISOPREG, T, DHT, 3α-diol, 3β-diol, DHEA were purchased from Sigma-Aldrich. 17β-Estradiol-2,3,4-$^{13}C_3$ (1ng/sample), PREG-20,21-$^{13}C_2$ (10ng/sample) and PROG-2,3,4-$^{13}C_3$ (0.4ng/sample) were used as internal standards (Sigma-Aldrich, Italy). Samples were prepared with a solid phase extraction (SPE) cartridges (Thermo Scientific™ HyperSep™ C18, San Jose, CA, USA). All solvents and reagents were LC-MS grade (CARLO ERBA Reagents, Milano, Italy).

Animals

Srebp-1c KO mice, an animal model of reduced *de novo* lipogenesis, were purchased from The Jackson Laboratory (B6;129S6-*Srebf1tm1Jdh*, stock number: 004365) and were bred to generate a colony of wild type, heterozygous and homozygous mice. Animals were maintained in standard cages (four animals per cage), and were exposed to light/dark cycles of 12:12 hours at constant temperature (23°C) with food and water available ad libitum. The genotyping was performed, according to The Jackson Laboratory instructions, with the GoTaq Green Master Mix (Promega). The experiments were conduct with either 2 or 10-month-old male mice.

Procedures including animals and their care were performed in agreement with institutional guidelines that are in compliance with national (D.L. No. 26, March 4, 2014, G.U. No. 61 March 14, 2014) and international laws and policies (EEC Council Directive 2010/63, September 22, 2010: Guide for the Care and Use of Laboratory Animals, United States National Research Council, 2011). The Italian

Ministry of Health approved the animal protocols of this study (ministerial decree n 579/2015-PR).

Quantification of neuroactive steroids by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Neuroactive steroids were quantified in sciatic nerve and plasma from wild type and Srebp-1c KO mice at 2 and 10 months of age. Sciatic nerves (15 mg per animals) from the two experimental groups were prepared according to (Caruso *et al.* 2013). Plasma samples (250 µl per animals), added with internal standards, were first extracted with acetonitrile and then follow the protocol described in (Caruso *et al.* 2013). Calibration curves freshly prepared were used for the quantitative analysis. The LC/MS was carried out using a linear ion trap-mass spectrometer with an APCI source working in positive ion mode (LTQ, ThermoElectron Co., San Jose, CA, USA) equipped with a Surveyor liquid chromatography (LC) Pump Plus and a Surveyor Autosampler Plus (ThermoElectron Co., San Jose, CA, USA). The analytical conditions were performed as previously described (Caruso *et al.* 2010).

Quantitative real-time PCR

For the gene expression analysis, total mRNAs were extracted from wild type and Srebp-1c knockout sciatic nerves using the Nucleospin RNA II kit (Macherey–Nagel, Duren, Germany). The gene expression profile of the enzymes involved in steroidogenesis was done with a TaqMan qRT-PCR instrument (CFX96 real time system, Bio-Rad Laboratories, Segrate, Italy) using the iScriptTM one-step RT-PCR kit for probes (Bio-Rad Laboratories, Segrate, Italy). Samples were run in 384-well

formats in triplicate as multiplex reactions and the 36B4 was used as housekeeping gene for all the analyses.

Statistical analysis

Data obtained from the two experimental groups were analyzed by two ways unpaired Student's *t*-tests. All statistical analyses were performed in GraphPad PRISM (version 5) considering a $p<0.05$ as statistically significant (the number of animals is reported in the figure legends).

Results

The levels of PREG, DHEA, PROG and its derivatives (i.e., DHP, THP and ISOPREG), T and its derivatives (DHT, 3α-diol, 3β-diol) were assessed by LC-MS/MS in sciatic nerve of SREBF-1c KO mice at two different ages (i.e., two and ten months) and in WT animals. As reported in Figure 1, sciatic nerve of SREBF-1c KO mice at two months of age shows a significant increase of PREG levels associated with decrease of its first metabolite, PROG, and further metabolites (i.e., DHP and ISOPREG). On the contrary, the levels of another PROG metabolite, such as THP, were unaffected. Among androgens only T was significantly modified, showing an increase in its levels. On the contrary, the levels of DHEA and T metabolites (i.e., DHT, 3α-diol and 3β-diol) were unmodified. The levels of PREG [WT (n=5) under detection limit (0.05) vs SREBF-1cKO (n=5) 0.15 ± 0.05 pg/μl], PROG [WT (n=5) 0.28 ± 0.09 vs SREBF-1cKO (n=5) 0.15 ± 0.01 pg/μl] and T [WT (n=5) 0.23 ± 0.03 vs SREBF-1cKO (n=5) 8.24 ± 4.6 pg/μl] were unmodified in plasma of SREBF-1c KO mice vs WT animals.

An increase of PREG levels in sciatic nerve without corresponding changes in the plasma, was further supported by the gene expression of P450scc. Indeed, as reported in Figure 2, the expression of this enzyme, involved in the first step of steroidogenesis, was significantly increased. Concomitantly both 5α-R and 3α-HSOR mRNA levels (i.e, enzymes converting PROG and T into their further metabolites) were also significantly induced.

At ten months of age when the peripheral neuropathy is more pronounced (Cermenati et al. 2015), the neuroactive steroid profile in sciatic nerve of SREBF-1cKO mice showed a different pattern of that observed at two months of age. As

shown in Figure 3, a significant decrease of PREG and PROG levels was observed. On the contrary, the levels of PROG metabolites (i.e., DHP, THP and ISOPREG) were significantly increased. The levels of androgens in sciatic nerve were also modified, but in an opposite manner. Indeed, with the exception of the levels of DHEA, that were unmodified, the levels of T and its metabolites (i.e., DHT, and 3β-diol) were significantly decreased in SREBF-1cKO mice, while 3α-diol significantly increases (Figure 3). Similarly, to what observed at two months, also at ten months of age, the plasma levels of PREG [WT (n=6) 0.10 ± 0.02 vs SREBF-1cKO (n=6) 0.18 ± 0.06 pg/μl], PROG [WT (n=6) 0.09 ± 0.04 vs SREBF-1cKO (n=6) 0.14 ± 0.05 pg/μl] and T [WT (n=6) 0.87 ± 0.69 vs SREBF-1cKO (n=6) 0.24 ± 0.09 pg/μl] were unmodified in SREBF-1c KO mice vs. WT animals. Assessment of steroidogenic enzymes, indicate a significant decrease of P450scc associated with an increase of 5α-R and 3α-HSOR mRNA levels (Figure 4).

Discussion

As we have recently demonstrated, lack of the key lipogenic transcription factor SREBF-1c favors the development of peripheral neuropathy (Cermenati et al. 2015). Data here reported indicate for the first time that, accordingly to what observed in other experimental models of acquired (i.e., aging process, physical injury, diabetes) and hereditary (i.e., CMT1A) peripheral neuropathy (Melcangi et al. 2014, Melcangi et al. 2016, Giatti et al. 2015), also SREBF-1cKO mice show an alteration of neuroactive steroid levels in sciatic nerve. In particular, as here reported, changes in the levels of PREG (i.e., the first neuroactive steroid formed from cholesterol) occurred. Interestingly, the effect is different depending on the age of animals considered that parallels the severity of peripheral neuropathy. Indeed, at two months of age we observed a significant increase of this neuroactive steroid, while at ten months of age a decrease in the levels of PREG was detected. In agreement, the gene expression of the enzyme converting cholesterol into PREG (i.e., P450scc) followed this pattern. This finding, together with the observation that PREG levels were not modified in plasma, strongly suggests a specific effect of SREBF-1c on neurosteroidogenesis of the peripheral nerve. Indeed, in several experimental models we observed that changes in plasma did not reflect corresponding changes in nervous tissues (Melcangi et al. 2014, Melcangi et al. 2016, Giatti et al. 2015, Caruso et al. 2013). As here reported, changes in the levels of PREG induce consequences also in the levels of its further metabolites. Indeed, either at two and ten months of age the levels of the first metabolite of PREG (i.e., PROG) are decreased, while the further metabolites of PROG are differently modulated depending on the aging of the animals. Indeed, at two months of age

DHP and ISOPREG are decreased while at ten months of age an increase of these two neuroactive steroids together an increase in THP levels was observed. A similar pattern was also evident for T and derivatives. Indeed, at two months an increase of T levels was observed, while at ten months of age a decrease of T and some of its metabolites (i.e., DHT and 3β-diol) together with an increase of 3α-diol occurred. We previously demonstrated that, at two months of age, SREBF-1c KO mice begin to show the first signs of peripheral neuropathy (i.e, impairment of thermal and mechanical nociceptive threshold, subtle abnormalities of small unmyelinated C fibers), but only at ten months of age this became evident with abnormal nerve conduction velocity, intraepidermal nerve fiber density, decreased *g*-ratio of small-caliber fibers indicating a selective hypermyelination (Cermenati *et al.* 2015). Therefore, since neuroactive steroids exert a variety of protective effects on peripheral neuropathy (Giatti *et al*. 2015, Melcangi *et al.* 2003, Melcangi *et al.* 2005) the increase in their sciatic nerve levels might be interpreted like a possible endogenous response to neurodegenerative events. Interestingly, these effects seem to involve different steps of neurosteroidogenesis depending on the age of animals reflecting peripheral neuropathy progression. Indeed, the first step of neurosteroidogenesis (i.e., formation of PREG) reacts at the beginning of peripheral neuropathy (i.e., at two months of age), while conversion of PROG into its metabolites (i.e., DHP, THP and ISOPREG) as well as T into 3α-diol was associated with ascertained peripheral neuropathy (i.e. at ten months of age). Therefore, the observed increase in PROG or T metabolite levels may further support a protective role for these molecules in peripheral neuropathy. Indeed, PROG derivatives are effective in protecting peripheral nerve by aging damage (Azcoitia *et al.* 2003), physical injury (Melcangi *et al.* 2000,

Chavez-Delgado *et al.* 2005, Roglio *et al.* 2008), chemotherapy-induced peripheral neurotoxicity (Roglio *et al.* 2009) and diabetic peripheral neuropathy (Leonelli *et al.* 2007, Veiga *et al.* 2006, Cermenati *et al.* 2012, Mitro *et al.* 2014). In addition, T metabolites are considered protective agents in the same experimental models of peripheral neuropathy (Huppenbauer *et al.* 2005, Ayhan *et al.* 2003, Calabrese *et al.* 2014, Mitro *et al.* 2014, Roglio *et al.* 2007) with the exception of aging process (Azcoitia *et al.* 2003). In this context, it is important to highlight that while DHP, like its precursor PROG, still interacts with the progesterone receptor (PR), the further metabolites THP and ISOPREG modulate the activity of GABA-A receptor (Melcangi *et al.* 2008). In particular, THP binds and activates GABA-A receptor (Belelli & Lambert 2005, Lambert *et al.* 2003, Lambert *et al.* 2009), while ISOPREG, albeit unable to bind GABA-A receptor, antagonizes the effect of THP (Melcangi *et al.* 2008). Similarly, 3α-diol (i.e., a T metabolite) is a GABA-A receptor agonist (Melcangi et al. 2008). Thus, modulation of PR or GABA-A receptor might be considered as a possible therapeutic approach for peripheral neuropathy.

SREBF-1c KO sciatic nerve at ten months of age experience energy depletion and mitochondrial dysfunction from a metabolic point of view (Cermenati *et al.* 2015). In this regards, it is important to underline that Schwann cells and neurons rely on glycolysis as fuel source. The lack of SREBF-1c imposes both cells to use fatty acid instead of glucose and lactate; a driver towards mitochondrial dysfunction in sciatic nerve of SREBF-1c null mice. On this basis, it might be possible that at two months of age, when subtle signs of peripheral neuropathy are present and the metabolic switch it is still not dramatic, mitochondria try to counteract the initial neurodegeneration by inducing P450scc

expression, the first enzyme regulating the whole neurosteroidogenesis, and consequently the levels of its product PREG. On the contrary, at ten month of age mitochondria are boosted to their maximum capacity to cope the energy depletion due to the metabolic switch from glucose towards fatty acid utilization. At this age, P450scc levels were reduced probably due to altered mitochondrial function. Indeed, Schwann cells mitochondrial dysfunction is intimately associated with peripheral neuropathy development (Ino & Iino 2016). To compensate this altered mitochondrial function, SREBF-1c KO sciatic nerve increased both 5α-R and 3α-HSOR expression levels and relative metabolites.

The SREBF-1c null mice shows also alteration in peripheral nerve FA composition (Cermenati *et al.* 2015). Here we also shed the light on an altered neuroactive steroid pattern in both two and ten months old animals developing peripheral neuropathy. These data along with our previous observations, in which we showed that diabetes altered FA composition and DHP treatment was able to bring it back to control (Mitro *et al.* 2014), strongly hint to a tight cross talk between fatty acid and neuroactive steroid pathways.

In conclusion, our data suggest that during the development of peripheral neuropathy due to the blunted FA biosynthesis, neuroactive steroids at the beginning of the pathology (two months of age) try to counteract the early stages of neurodegeneration. During the progression to peripheral neuropathy (ten months of age), when the peripheral nerve boosts mitochondria to cope energy depletion due to reduced glycolysis, the levels of mitochondria-produced hormone such as PREG are decreased and downstream neuroactive steroids are instead increased. These data further support the concept of that neuroactive

steroids, by a cross-talk with FA biosynthetic pathway, may represent a possible therapeutic strategy for peripheral neuropathy.

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Conflict of interest statement

The authors declare no conflict of interest

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Legends to Figures

Figure 1- Levels of neuroactive steroids in sciatic nerve of wild type (WT) and of SREBF-1c KO mice at two months of age. Data (n= 5 for each experimental group) are expressed as pg/mg ± SEM. Student's t-test analysis: *p<0.05 and ***p<0.001 vs. WT.

Figure 2- Gene expression of P450 side chain cleavage (P450scc), 5α-reductase (5α-R) and 3α-hydroxysteroid oxidoreductase (3α-HSOR) in sciatic nerve of wild type (WT) and of SREBF-1c KO mice at two months of age. The columns represent the mean ± SEM (n= 5 for each experimental group). Student's t-test analysis: *p<0.05 and **p<0.01 vs. WT.

Figure 3- Levels of neuroactive steroids in sciatic nerve of wild type (WT) and of SREBF-1c KO mice at ten months of age. UDL: under detection limit. Detection limits for 3β-diol were 0.05 pg/mg. Data (n= 6 for each experimental group) are expressed as pg/mg ± SEM. Student's t-test analysis: *p<0.05 and **p<0.01 vs. WT.

Figure 4- Gene expression of P450 side chain cleavage (P450scc), 5α-reductase (5α-R) and 3α-hydroxysteroid oxidoreductase (3α-HSOR) in sciatic nerve of wild type (WT) and of SREBF-1c KO mice at ten months of age. The columns represent

the mean ± SEM (n= 5 for each experimental group). Student's t-test analysis: *p<0.05 and **p<0.01 vs. WT.

Figure 1

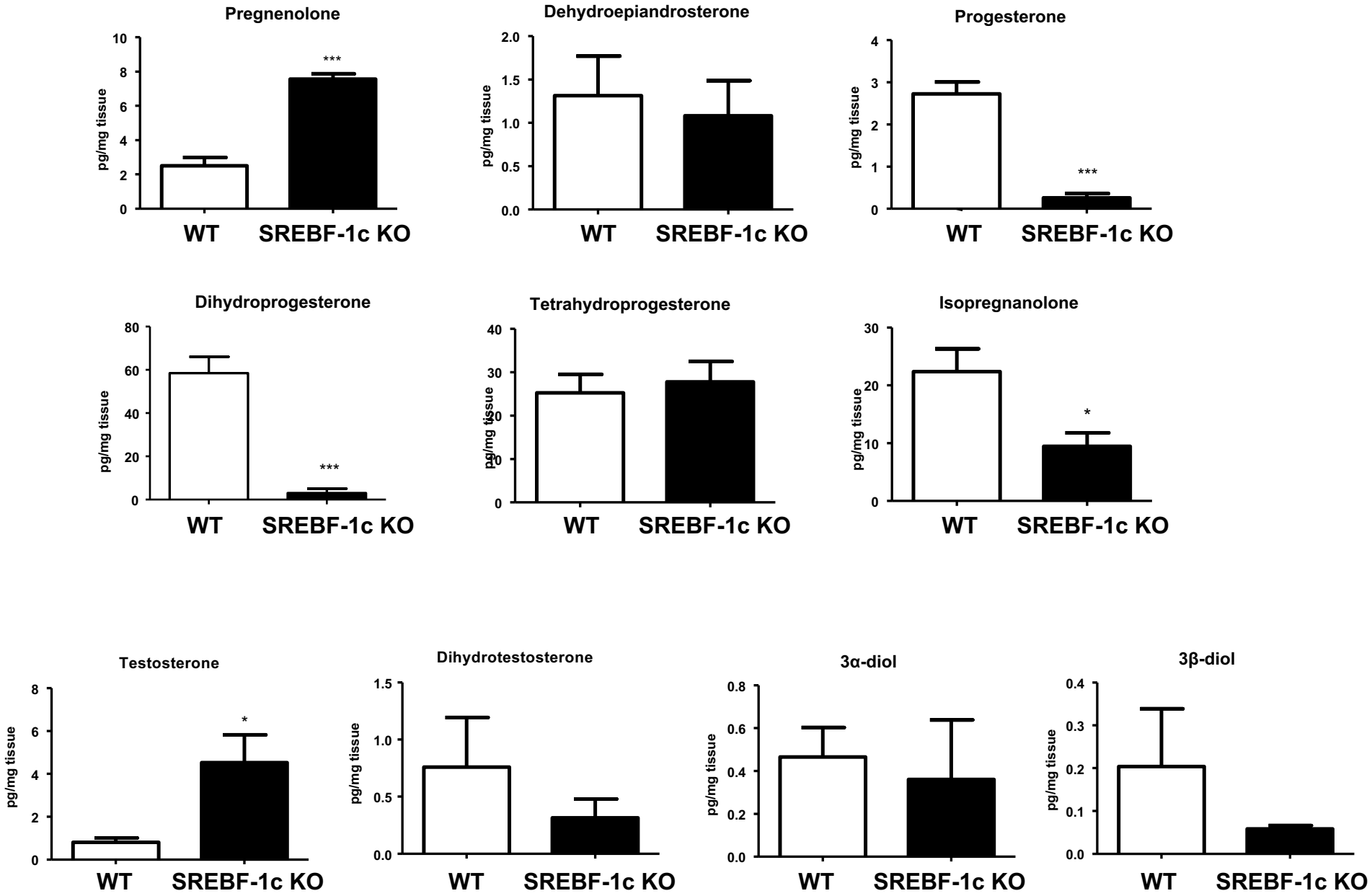

Pregnenolone
pg/mg tissue
0
2
4
6
8
10
***
WT
SREBF-1c KO
Dehydroepiandrosterone
pg/mg tissue
0.0
0.5
1.0
1.5
2.0
WT
SREBF-1c KO
Progesterone
pg/mg tissue
0
1
2
3
4
***
WT
SREBF-1c KO
Dihydroprogesterone
pg/mg tissue
0
20
40
60
80
***
WT
SREBF-1c KO
Tetrahydroprogesterone
pg/mg tissue
0
10
20
30
40
WT
SREBF-1c KO
Isopregnanolone
pg/mg tissue
0
10
20
30
*
WT
SREBF-1c KO
Testosterone
pg/mg tissue
0
2
4
6
8
*
WT
SREBF-1c KO
Dihydrotestosterone
pg/mg tissue
0.0
0.5
1.0
1.5
WT
SREBF-1c KO
3α-diol
pg/mg tissue
0.0
0.2
0.4
0.6
0.8
WT
SREBF-1c KO
3β-diol
pg/mg tissue
0.0
0.1
0.2
0.3
0.4
WT
SREBF-1c KO

Figure 2

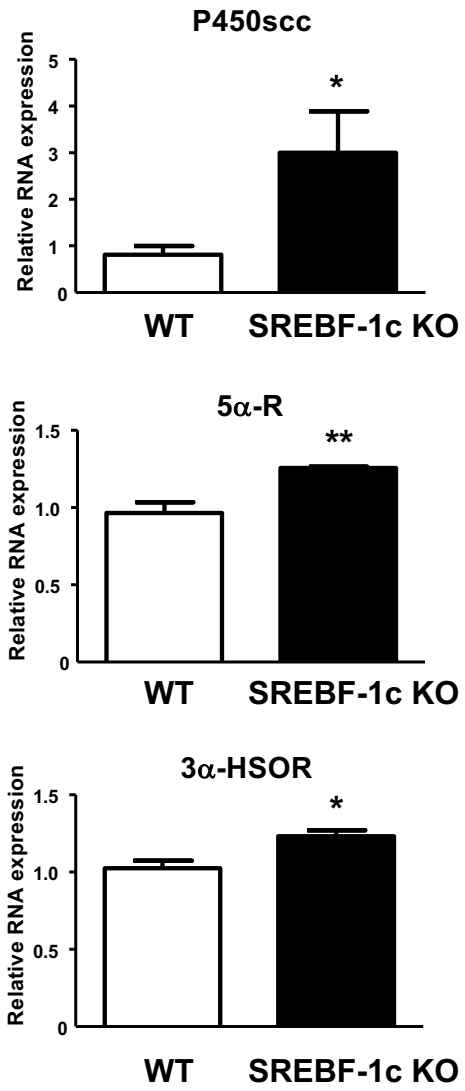

P450scc
Relative RNA expression
5
4
3
2
1
0
*
WT
SREBF-1c KO
5α-R
Relative RNA expression
1.5
1.0
0.5
0
**
WT
SREBF-1c KO
3α-HSOR
Relative RNA expression
1.5
1.0
0.5
0
*
WT
SREBF-1c KO

Figure 3

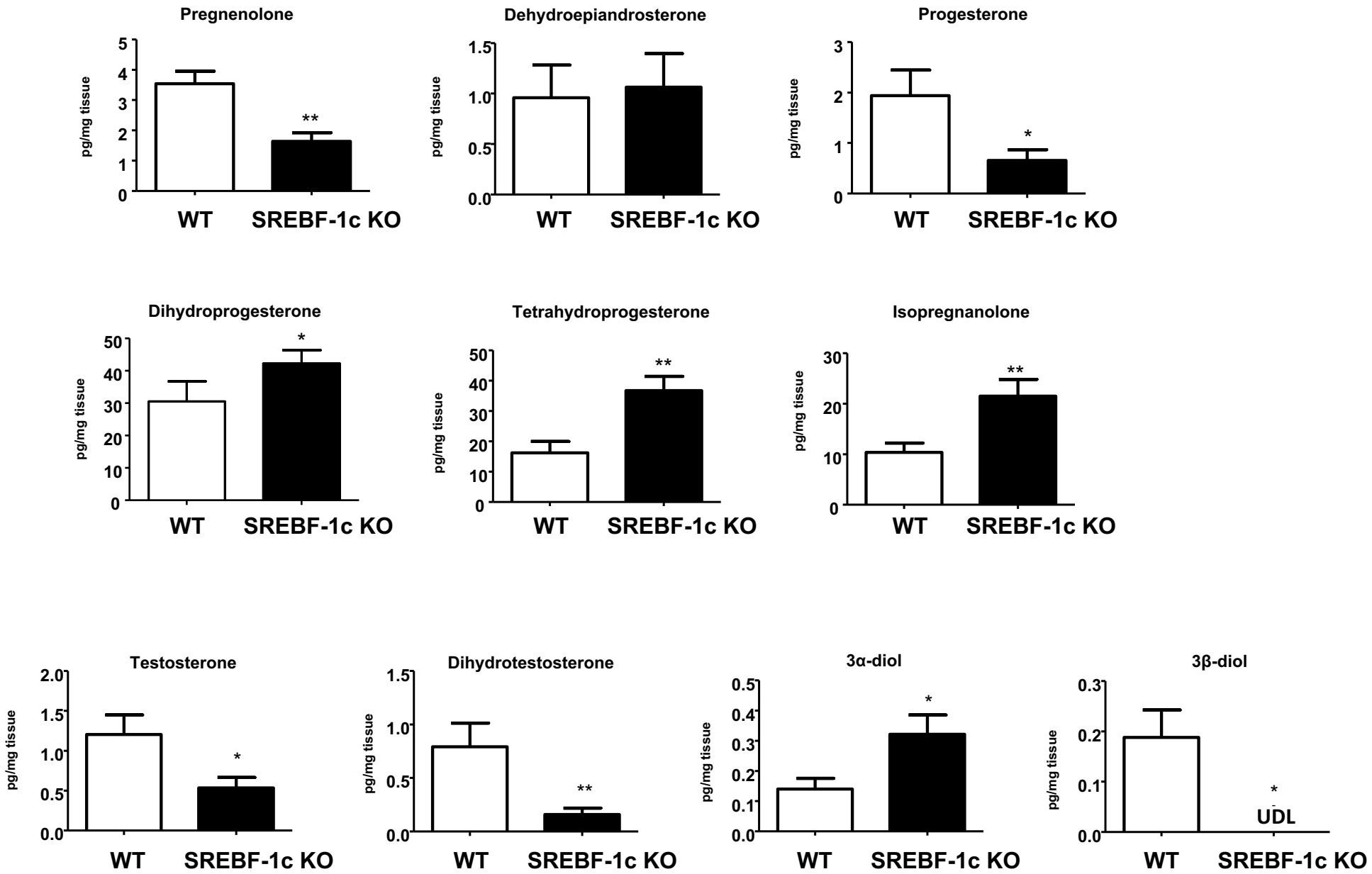
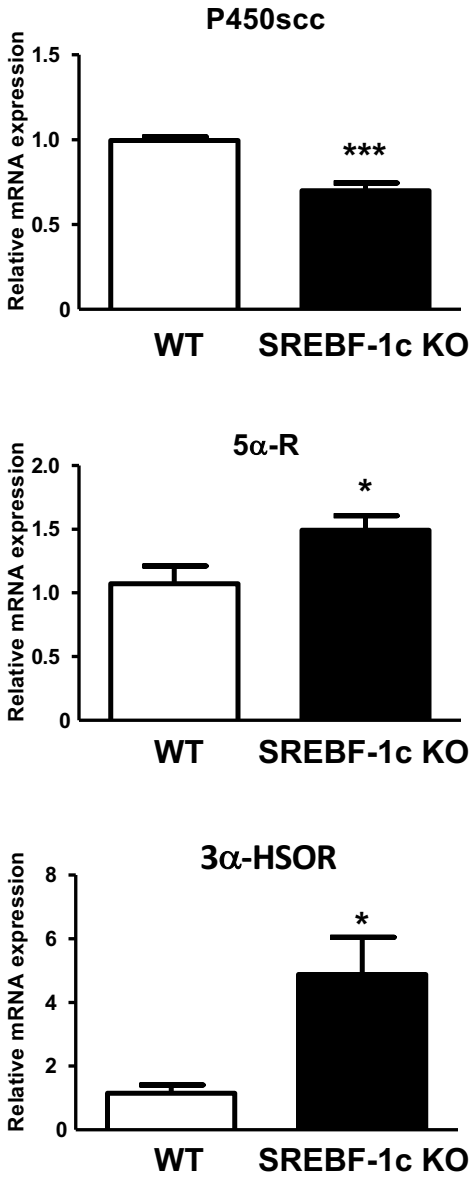

Pregnenolone
pg/mg tissue
5
4
3
2
1
0
**
WT
SREBF-1c KO
Dehydroepiandrosterone
pg/mg tissue
1.5
1.0
0.5
0.0
WT
SREBF-1c KO
Progesterone
pg/mg tissue
3
2
1
0
*
WT
SREBF-1c KO
Dihydroprogesterone
pg/mg tissue
50
40
30
20
10
0
*
WT
SREBF-1c KO
Tetrahydroprogesterone
pg/mg tissue
50
40
30
20
10
0
**
WT
SREBF-1c KO
Isopregnanolone
pg/mg tissue
30
20
10
0
**
WT
SREBF-1c KO
Testosterone
pg/mg tissue
2.0
1.5
1.0
0.5
0.0
*
WT
SREBF-1c KO
Dihydrotestosterone
pg/mg tissue
1.5
1.0
0.5
0.0
**
WT
SREBF-1c KO
3α-diol
pg/mg tissue
0.5
0.4
0.3
0.2
0.1
0.0
*
WT
SREBF-1c KO
3β-diol
pg/mg tissue
0.3
0.2
0.1
0.0
*
UDL
WT
SREBF-1c KO

Figure 4


P450scc
Relative mRNA expression
1.5
1.0
0.5
0
***
WT
SREBF-1c KO
5α-R
Relative mRNA expression
2.0
1.5
1.0
0.5
0
*
WT
SREBF-1c KO
3α-HSOR
Relative mRNA expression
8
6
4
2
0
*
WT
SREBF-1c KO