LIVER X RECEPTORS, NERVOUS SYSTEM AND LIPID METABOLISM

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ABSTRACT

Lipids in the nervous system are represented by cholesterol and phospholipids as constituents of cell membranes and, in particular, of myelin. Therefore, lipids are finely regulated to guarantee physiological functions. In the central nervous system, cholesterol is locally synthesized due to the presence of the blood brain barrier. In the peripheral nervous system cholesterol is either up-taken by lipoproteins and/or produced by *de novo* biosynthesis. Defects in lipid homeostasis in these tissues lead to structural and functional changes that often result in different pathological conditions depending on the affected pathways (i.e. cholesterol biosynthesis, cholesterol efflux, fatty acid biosynthesis etc.). Alterations in cholesterol metabolism in the central nervous system are linked to several disorders such as Alzheimer's disease, Huntington disease, Parkinson disease, Multiple Sclerosis, Smith-Lemli-Opitz syndrome, Niemann-Pick type C disease, and glioblastoma. In the peripheral nervous system changes in lipid metabolism are associated with the development of peripheral neuropathy that may be caused by metabolic disorders, injuries, therapeutics and autoimmune diseases. Transcription factors, such as the Liver X receptors (LXRs), regulate both cholesterol and fatty acid metabolism in several tissues including the nervous system. In the last few years several studies elucidated the biology of LXRs in nervous system due to the availability of knock-out mice and the development of synthetic ligands.

Here, we review a survey of the literature focused on central and peripheral nervous system and in physiological and pathological settings with particular attention on the roles played by LXRs in both districts.

INTRODUCTION

Liver X Receptors (LXRs) are members of the nuclear receptors superfamily. LXRs have the classical structure of a nuclear receptor: a DNA binding domain, a ligand binding domain and a ligand independent activation function 2 (AF2) that, through the recruitment of coactivators and corepressors, regulates the activity of the receptor.

More than ten years ago these proteins were discovered, cloned and termed “orphan nuclear receptors” (1). Today, since the physiological ligands are known, they are classified as “adopted orphans”.

Two different isoforms, LXRα (NR1H3) and LXRβ (NR1H2) are known. LXRα is predominantly expressed in the liver and at lower levels also in the intestine, macrophages, adipose tissue, lungs, kidneys and the adrenal gland, while LXRβ is broadly expressed (2) including neurons, microglia, astrocytes (3) oligodendrocytes (4), and Schwann cells (5).

LXRs are ligand activated transcription factors that form an obligate heterodimers with the retinoic X receptor (RXR). The LXR/RXR complex, activated by ligands, binds a specific sequence, called LXR responsive element (LXRE) in the promoter of the target genes modulating their expressions. Usually the DNA sequence recognized by LXRs is a direct repeat of the core G/AGGTCA separated by four nucleotides DR-4 (1). However, it has been reported that LXR can also bind an inverted repeat (IR) sequence without spacing nucleotides (IR0) (6).

LXRs natural ligands are represented by oxysterols, an oxidized form of cholesterol produced from the cells as intermediates in steroid hormones or bile acids biosynthesis (7). Accordingly with the nature of the physiological ligands, LXRs play an important role in cholesterol, and lipid metabolism.

The role of LXRs as an intracellular cholesterol sensor is primarily due to the activation of key genes in cholesterol efflux such as the ATP binding cassette (ABC) family and in particular ABCA1, ABCG1, ABCG5 and ABCG8, apolipoprotein E (ApoE) and cholesterol ester transfer protein

(CETP) (8). This activation mediated by high affinity ligands increase HDL level and induces cholesterol efflux (9). LXRs also play a role in lipogenesis and triglyceride synthesis primarily due to the upregulation of sterol regulatory element binding protein-1c (SREBP-1c) and fatty acid synthase (FAS) (10). All these genes are regulated by LXRs in a direct fashion due to the presence of one or multiple LXRE in the promoter region of the target genes.

The literature on LXRs during the last two decades is mainly focused on the role of these nuclear receptors in the liver, adipose tissue, pancreas and skeletal muscle. During the last ten years several evidences have addressed the fundamental role of LXRs also in the nervous system~~s~~. Thus, the aim of this review is to summarize the current knowledge on LXRs in the brain, spinal cord and peripheral nerves.

LXRs AND CHOLESTEROL METABOLISM IN THE CENTRAL NERVOUS SYSTEM

Cholesterol is transported in the circulation by lipoproteins that are not able to cross the blood brain barrier. Consequently, the brain cholesterol is synthesized *in situ* by the central nervous system (CNS), mainly by neurons and glial cells (11, 12). Therefore, the brain is characterized by a large amount of cholesterol and it represents the most cholesterol rich organ. In the cerebral tissue, cholesterol is necessary for both cell functions and membrane structure of neurons and glial cells. Similar to other tissues also in the brain, the cholesterol homeostasis is regulated by LXRs, whose activation induces the expression of a plethora of genes involved in cholesterol trafficking and efflux.

The brain's cholesterol is mainly present in myelin and low cholesterol levels in CNS results in reduced myelination in oligondedrocytes (13). Thus, the maintenance of cholesterol homeostasis is crucial in the CNS. On the other hand, high cholesterol levels can lead to detrimental effects. Therefore, the brain needs to eliminate cholesterol by generating brain specific metabolites such as oxysterols which are a hydroxylated form of cholesterol and hence

more polar and able to cross the blood brain barrier. This latter mechanism represents 50% to 60% of the brain cholesterol efflux, while an unknown pathway, probably involving ApoE, eliminates the remaining 40% (12, 14).

In the brain, cholesterol is metabolized by its conversion into 24(*S*)-hydroxycholesterol and the release of this oxysterol reflects the rate of synthesis of cholesterol in the brain (15). A member of the cytochrome P450 superfamily of enzymes such as the cholesterol 24-hydroxylase (Cyp46a1) mediates the generation of 24(*S*)-hydroxycholesterol. This enzyme is exclusively expressed in the brain and in particular in hippocampal and cortical neurons that are important for learning and memory formation (16). Once produced, the 24(*S*)-hydroxycholesterol diffuses out of cells, crosses the blood brain barrier, and by systemic circulation reaches the liver for its final clearance (15). To better understand the role of Cyp46a1 in the brain, Lund and collaborators generated a mouse model lacking 24-hydroxylase that exhibited reduced cholesterol excretion (14). These animals showed severe deficiencies in spatial, associative, and motor learning, and in hippocampal long-term potentiation (LTP). Indeed, the disruption of the cholesterol 24-hydroxylase gene in the mouse leads to slower cholesterol excretion and in the suppression of the mevalonate pathway in the brain. The administration of geranylgeraniol to Cyp46a1 null hippocampal slices restored the LTP to wild type mice indicating that this molecule is essential for learning and that cholesterol turnover via the 24-hydroxylase enzyme is important to actively maintain the mevalonate pathway (16).

The ApoE is the most abundant apolipoprotein in the CNS and it is involved in lipoprotein assembly and secretion under the direct control of LXRs. Astrocytes from ApoE deficient mice secrete a low amount of free cholesterol (17) leading to neurodegeneration during aging (18) and impairment in learning and memory (19). The nascent cholesterol-containing lipoproteins are lipidated by ABC transporters such as ABCA1, ABCG1 and ABCG4, the latter highly expressed into the CNS (20). The cholesterol efflux from astrocytes is mainly mediated by the action of ABCA1

and ABCG1, while in neurons this function is predominantly carried out by ABCG4 (21). ABCA1 knock-out mice exhibit markedly decreased levels and lipidation of ApoE in the CNS. Thus, the ApoE produced in this context is resulted to be strongly amyloidogenic *in vivo* and favor the development of Alzheimer's disease (22). Moreover, the loss of both ABCG1 and ABCG4 expression in the brain results in oxysterols accumulation and reduced expression levels of LXRs and sterol regulatory element binding protein 2 (SREBP2) target genes while, ABCG4 null mice display a general deficit in associative fear memory (20). Although it is well established that both ABCA1 and ABCG1 are directly regulated by LXRs (2), the expression of ABCG4 is not affected by LXRs activation (23). Despite the generation of several knock-out models of the ABC transporters, it still seems difficult to establish which one may play a major role in mediating cholesterol efflux. Probably the expression of the ABC transporters in different CNS cells accounts for a differential efflux of cholesterol regulated in a different manner. However, this is a hypothesis that needs to be supported by further studies.

Another role of LXRs in controlling cholesterol uptake is the regulation of inducible degrader of LDL receptor (IDOL). IDOL is an E3 ubiquitin ligase that favors the degradation of the LDL receptor (LDLR) (24). IDOL is expressed in neurons and it has been suggested to inhibit neurite outgrowth (25). Moreover, Hong and collegues extended these observations by demonstrating that IDOL modulates the levels of the VLDL receptor (VLDLR) and of the apolipoprotein E receptor 2 (ApoER2 or known also as LDL receptor-related protein 8, LRP8), through a mechanism involving receptors ubiquitination, leading to reduced reelin binding. These data suggest that IDOL, through LXR activation, may have a role during neurons development (26).

In conclusion, cholesterol is synthesized directly in the brain and an abnormal accumulation of cholesterol may result in neurodegeneration and favor the onset of cholesterol-associated nervous system disorders. Thus, the main role of LXRs in the brain is to promote cholesterol efflux to maintain cholesterol homeostasis in this tissue.

LXRs AND NEURODEGENERATIVE DISEASES

In the CNS both LXRα and LXRβ isoforms are expressed, however, LXRβ is the most abundant isotype in the central nervous system (27).

The generation of LXRs knock-out mice and the development of different synthetic compounds shed the light on the physiology and the functions regulated by these transcription factors. Indeed, double LXR knock-out (LXRDKO) mice exhibit numerous severe abnormalities such as loss of neurons, proliferation of astrocytes, disorganization in myelin sheaths, accumulation of lipid deposits and closure of ventricules (28) in the CNS.

On this ground, we will examine the roles of LXRs in neurodegenerative diseases as also summarized in figure 1.

Alzheimer's disease. Cholesterol accumulation in the CNS increases the risk of developing Alzheimer's disease (AD) (29). AD is characterized by progressive neuronal degeneration, gliosis, extraneuronal deposition of amyloid-β peptides (Aβ) forming senile plaques and intraneuronal accumulation of hyperphosphorylated tau protein (neurofibrillary tangles) (30, 31). The lack of either LXRα or LXRβ in APP23 transgenic mice (a mouse model of AD) causes an increased amyloid deposition (32). AD is also characterized by microglial activation and neuroinflammatory processes. In this regard, LXRs activation by synthetic agonists decreased the inflammatory response of primary mixed glial cultures to fibrillar amyloid β peptide (fAβ). On the other hand, cells lacking LXRs displayed a higher expression of inflammatory genes suggesting that LXRs may act as endogenous inhibitors of the innate CNS response induced by fAβ (32). The anti-inflammatory properties of LXRs agonists on a mouse model of AD may be due, at least in part, to inhibition of NF-κB signaling pathway (33). Fitz and collegues reported that a high fat diet exacerbated the AD phenotype. In this context, the authors demonstrated that LXRs activation by reducing amyloid load and facilitating its clearance improves AD phenotype (34). Another protein that is considered associated with the development of AD is selective Alzheimer's disease

indicator-1 (Seladin 1) (35). This protein catalyzes the conversion of desmosterol into cholesterol and therefore is also known as 3-β-hydroxysterol delta-24-reductase (Dhcr24) (35). During the onset of AD, seladin is downregulted, thus favoring the production of β-amyloid. Moreover, it has been demonstrated that the overexpression of Seladin 1 had protective effects by increasing the cholesterol content of the membrane and conferring resistance against β-amyloid aggregates in neuroblastoma cells. On the other hand, the specific inhibition of this enzyme increased cell susceptibility (36). In addition, a whole genome screen for the identification of novel direct LXR target genes, an LXRE, was detected in the second intron of Seladin 1 gene (37). This is another potential mechanism by which LXR activation protects against AD. However, to test the hypothesis that LXR activation requires Seladin 1 to exert protective effects on AD, further studies need to be performed in Seladin 1 knock-out mice that have an altered membrane composition due to brain cholesterol deficiency. Finally, all these studies suggested that LXRs activation might be a useful tool for the treatment of AD.

Parkinson's disease. Microgliosis, astrogliosis, progressive degeneration of dopaminergic neurons, presence of Lewy bodies in dopaminergic neurons, and α-synuclein accumulation in substantia nigra pars compacta defines Parkinsons's disease (PD) (38). Male LXRβ knock-out mice show an adult-onset motor neuron degeneration after 7 months of age (39) and the LXRβ deficiency in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD, exacerbates the already affected dopaminergic neuron condition (40). LXRs activation in MPTP treated wild type mice protected against the MPTP-induced neurodegeneration of dopaminergic neurons and reduced glial cells activation (40). Since LXRβ was not found to be expressed in dopaminergic neurons, the authors concluded that LXR activation exerts the protective effects in PD by acting on the cytotoxic functions of microglia (40).

Hungtington's Disease. This pathology is characterized by abnormal expansion of the polyglutamine tract located in the N-terminus of an ubiquitous expressed protein known as

huntingtin. It was demonstrated that the cholesterol biosynthetic pathway is altered in HD mice brains and postmortem human striatal and cortical tissues (41). Recently, it was reported that wild type huntingtin activates LXRs mediated transcription and thus unravels a new LXRs coactivator. Furthermore, the overexpression of mutant huntingtin has negative effects on LXRs target genes such as ABCA1, an effect partially rescued by LXR activation by a synthetic ligand (42). These data propose a role of LXR in the pathophysiology of HD probably involving cholesterol homeostasis. However, more work needs to be done to ascertain the role of LXRs in HD.

Multiple Sclerosis. This is an inflammatory disease characterized by demyelination in CNS due to extensive damages of the myelin around the axons. Since LXRs have potent anti-inflammatory activities (43), recent studies have demonstrated the efficacy of LXRs agonists in the treatment of multiple sclerosis (MS). It is known that Th1 and Th17 cells contribute to the development of autoimmune diseases among with MS (44, 45). An established animal model of MS is the experimental autoimmune encephalomyelitis (EAE), an inflammatory disease of the central nervous system characterized by demyelination, with a histopathology similar to the human disease (46, 47). It has been reported that the LXRs agonist T0901317 blocked the production of nitric oxide and inhibited the induction of proinflammatory cytokines and chemokines by LPS-stimulated primary mouse astrocytes and microglia (48). A more recent study also showed that T0901317 suppressed IL-23 and IL-17 expression in primary glial cells and in splenocytes from EAE mice (49). Moreover, administration of this LXRs agonist prior to the onset of the disease blocked the development of EAE through suppression of T-cell proliferation and cytokines release (50). Only recently Cui and colleagues (51) performed experiments in wild type mice and in LXRDKO with two different LXRs synthetic ligands, GW3965 and T0901317, since T0901317 cross-reacts also with other nuclear receptors such as the bile acids receptor FXR and the xenobiotic receptor PXR (52). They demonstrated that both ligands ameliorated EAE in wild type mice but not in LXRDKO mice, indicating that the beneficial effects showed are due to LXRs

activation. The positive effects exerted by LXRs activation are mediated by the induction of the LXRs target genes SREBP-1, which mediated the suppression of Th17 by binding to the IL-17 promoter (51). Collectively, these studies suggest that the LXRs ligands may be useful therapeutics for treatment of MS.

Niemann-Pick type C1. The Niemann-Pick type C1 (NPC1) protein is a fundamental molecule for the intracellular trafficking of cholesterol, favoring the shuttling of LDL to lysosome to hydrolized and released free cholesterol. The disease is characterized by a mutation in the NPC1 protein that results in the accumulation of unesterified cholesterol, sphingomyelin and glycolipids into the lysosomes of neurons and glia cells. Accumulation of these lipids in the CNS leads to ataxia, dysarthria, dysphagia, and in severe cases can also cause dementia and epileptic seizures (53). Given the large accumulation of cholesterol into the cells, treatments favoring cholesterol efflux may improve the Niemann-Pick type C1 disease. In this regard, Repa and collegues demonstrated that LXR activation in a mouse model of NPC1 disease increased cholesterol excretion from the brain, blunted inflammation, slackened neurodegeneration, and ultimately extended lifespan and improved the NPC1 phenotype (54). Moreover, cholesterol removal mediated by 2-hydroxypropyl-β-cyclodextrin in NPC1 knock-out mice blocked cholesterol biosynthesis by inhibiting the action of SREBP2 and activated LXR target genes. In addition, constant administration of cyclodextrin avoided the neurodegeneration observed in NPC1 knock-out mice. These results demonstrated that cyclodextrin treatment restored cholesterol trafficking from lysosomes to cytosol, thus such cholesterol disposal compound may be useful for the treatment of the NPC1 disease (55).

Stroke. Anti-inflammatory strategies can be a useful tool also for the treatment of ischemic injury. In this field, two independent studies demonstrated the protective role of LXRs activation on brain ischemia. The first study reported that a single dose of GW3965, delivered 2 hours post-injury, ameliorates the extent of cytotoxic edema that result from ischemic insult. The authors

demonstrated that LXR activation leads to reduced neuroinflammation and to the promotion of vascular endothelial grow factor (VEGF) expression, another protective agent in brain ischemia (56). The second study confirmed the neuroprotective properties of LXRs activation in the experimental model of stroke and provided evidences that the lack of LXRs had detrimental effects on the infarct volume in an animal model of stroke (57). Another study reported similar results described above and provided a potential mechanism whereby LXRs activation reduced NF-κB activation, which led to a decrease in cyclo-oxygenase-2 (COX-2) expression and finally ameliorated brain inflammation (58). All these data highlight that activation of the LXRs exerts a neuroprotective effect in the experimental model of stroke.

Amyotrophic lateral sclerosis. A study performed in male LXRβ null mice showed that these animals presented motor neuron degeneration related with lipid accumulation in the spinal cord (39). Chronic motor neuron degeneration is associated with amyotrophic lateral sclerosis (ALS). The main feature that spinal cords of ALS patients have in common is a pathological accumulation of sphingomyelin, ceramides, and cholesterol esters (59). These lipid classes seem to be responsible for motor neuron sensitization for programmed cell death. Moreover, it has been demonstrated that in LXRβ knock-out mice, motor dysfunction progresses with age and finishes with paralysis (39). The authors reported that the onset of disability occurs in mice lacking LXRβ between 3 and 7 months of age. At 7 months of age those knock-out animals showed a reduced control of muscle action and problems with motor coordination associated with lipid accumulation (39). Because LXRβ null mice displayed motor neuron phenotype the same team investigated the toxicity of a known motor neuron toxin such as β-sitosterol. In this study, the administration to 8-month-old LXRβ null mice of β-sitosterol induced motor neuron death in the lumbar region of the spinal cord. Moreover, in 16-month-old LXRβ knock-out mice, β-sitosterol caused severe paralysis and symptoms correlated with dopaminergic dysfunctions leading to an ALS phenotype. A possible explanation of this phenotype is that the lack of LXRβ leads to high cholesterol efflux from the

brain through 24-hydroxycholesterol and neuronal toxicity probably due to the LXRα activation by β-sitosterol (60). In conclusion, the correct balance of cholesterol in the brain is fundamental for the functionality of this organ and because LXRs play a part in the regulation of cholesterol homeostasis, it can be a useful target for the treatment of the neurodegenerative disease associated with defects in this pathway. However, a long-lasting activation of LXRs may result in detrimental effects on the brain such as neurotoxicity.

Glioblastoma. Glioblastoma (GBM) is the most common and malignant primary brain tumor and among different kind of cancer it is one with a poor prognosis. Guo and collegues have recently elucidated the role of cholesterol homeostasis in this lethal tumor (61). Indeed, they provide evidences of increased cholesterol uptake in the GBM due to the mutated epidermal growth factor receptor (EGFR) activated pathway involving phosphoinositide 3-kinase (PI3K) and SREBP-1 leading to up-regulation of the LDL receptor (LDLR) (61). Moreover, LXRs activation promoted LDLR degradation by inducing its target gene IDOL and increased expression of the cholesterol efflux transporter ABCA1. These effects promoted cell death and inhibited tumor growth in vivo (58).

Smith-Lemli-Opitz syndrome. Smith-Lemli-Opitz syndrome (SLOS) is characterized by the deficiency of 7-dehydrocholesterol reductase (Dhcr7). Therefore, affected subjects display the inability to correctly produce or synthesize cholesterol. Cholesterol supplementation after birth is the current approach, however, the lack of cholesterol in utero drives to developmental malformations. On this ground, it has been proposed that in utero treatment with a LXRs agonist of a model of SLOS pregnant female mice (Dhcr7 knock-out mice), incapable of *de novo* synthesis of cholesterol, increase the placental expression of ABCA1 allowing the maternal to fetus cholesterol transfer. These data suggest that LXRs activation may have potential for in utero therapy of SLOS (62).

ENDOCRINOLOGICAL IMPLICATIONS OF LXRs ACTIVATION IN THE CENTRAL NERVOUS SYSTEM

Despite the synthesis of steroids in peripheral tissues (i.e. adrenal gland and gonads), CNS produces these hormones to regulate physiological functions in the brain and in the spinal cord. Thus, they are named neuroactive steroids (63, 64). Several neurodegenerative conditions (such as Alzheimer's disease, Parkinson's disease, Multiple Sclerosis and Charcot-Marie-Tooth type 1A) are associated with modified levels of neuroactive steroids (65, 66, 67, 68). In this regard our laboratory provided evidences that age related neuropathological changes in AD brains were associated with modified levels of specific neuroactive steroids such as changes in the levels of progesterone and testosterone metabolites (65). Moreover, another example is represented by the improvement of the MS phenotype in the experimental autoimmune encephalomyelitis (EAE) rat model by the treatment with progesterone, which blunted the neuroinflammation (67).

The precursor of neuroactive steroids is cholesterol, thus CNS expresses all key enzymes for the neuroactive steroids synthesis (69). Because cholesterol is central for the neuroactive steroid synthesis and LXRs are cholesterol sensing transcription factors, it is possible that LXRs may regulate the amount of cholesterol available for the neuroactive steroids to be synthesized. Indeed, our laboratory evaluated whether the LXRs activation may regulate neuroactive steroid levels in the CNS of diabetic animals compared with non-diabetic controls. We found that diabetes reduced neuroactive steroid levels in different areas of the CNS. The LXRs activation selectively increased the levels of some neuroactive steroids and rescued the CNS symptoms due to diabetes (70). Our results suggest that LXRs activation also influences neuroactive steroid levels in the CNS. Thus, it might be possible that the neurodegenerative diseases associated with decreased levels of neuroactive steroids where LXRs showed protective effects may have involved also the regulation of the neuroactive steroid biosynthetic pathway.

However, more studies are necessary to prove the protective role of the LXR-neuroactive steroid axis in the CNS.

CENTRAL LXRs IN THE CONTROL OF ENERGY METABOLISM

Nowadays several studies demonstrated the fundamental role of the brain in the control of whole body energy homeostasis (71). This control is the result of hormonal signals generated by peripheral organs and nutrients such as glucose, free fatty acids and amino acids and of the consequent CNS responses to adapt the body to the nutritional status (72). The main area involved in the control of central energy balance is the hypothalamus. Dysregulation of hypothalamic functions lead to development of metabolic disorders. In fact, rodent models challenged with high fat diet and obese subjects developed hypothalamic inflammation and gliosis leading to neuronal injury and decrease number of pro-opiomelanocortin (POMC) neurons that are crucial components of the network controlling energy balance. These detrimental effects may be associated with increased body weight (73). Moreover, several studies draw the attention to the association between metabolic syndrome, a complex disease characterized by insulin resistance, dyslipidemia and elevated blood pressure, and the development of cognitive disorders including also Alzheimer's disease (74).

Recently, Kruse and collegues reported that the paraventricular and ventromedial nuclei express mainly LXRα whereas the arcuate nucleus expresses LXRβ. Moreover, fructose fed rats selectively decreased hypothalamic LXRβ levels while LXRα increased. This work highlighted a possible relationship between glucose and the expression of LXRs in the hypothalamus, indicating a role for LXRs in the control of food intake and energy expenditure (75). In addition to the possibility of energy intake control it has been also demonstrated that hypothalamic LXRβ regulates arginine vasopressin and consequently body water balance. When this pathway is not properly regulated as in the LXRβ knockout mice showing reduced number of hypothalamic vasopressin positive neurons, these animals developed diabetes insipidus (76). Despite the role of LXRs in hypothalamus that still needs to be completely addressed to prove a role for these

receptors in the control of energy metabolism, in peripheral organs LXRs activation directly impact hormone levels that may affect brain such as insulin and leptin.

The effects on insulin relies on the ability of LXRs to reduce the expression of gluconeogenic genes in the liver such as phosphoenolpyruvate carboxykinase (PEPCK), glucose 6-phosphatase (G6Pase) and Peroxisome Proliferator Activated receptor γ coactivator-1α (PGC-1α), while on the other hand induces glucokinase (GK) the first enzyme in the glycolysis pathway (77). Furthermore, in white adipose tissue the activation of LXRs induce the expression of the insulin-sensitive glucose transporter-4 (GLUT-4) (77). Therefore LXRs activation reduced blood glucose and improved insulin sensitivity in animal models of type 2 diabetes (78). LXRs play also an important role in pancreatic beta cells. These cells exclusively express LXRβ (79). LXRβ knock-out mice do not secrete insulin in response to glucose showing an impaired glucose tolerance phenotype (80). To sustain a role of LXRs in beta cells it also has been demonstrated that T0901317, a synthetic LXR agonist, caused, in pancreatic islets and in MIN6 cells, an increase in glucose-dependent insulin secretion and in islet insulin content (81). Remain controversial the role of LXRs agonists in the stimulation of insulin secretion, some studies showed an increased in plasma insulin concentration (82, 83) while others report no significant effects (84, 85, 86). In pancreatic beta cells the glucose and lipid metabolism appears responsible for the insulin secretion induced by LXRs agonists. In this view seems important the upregulation of the lipogenic LXRs target genes such as SREBP-1c, FAS and ACC which regulate the fatty acids and malonyl Co-A synthesis that ultimately stimulate insulin production and secretion (79). On the other hand a prolonged exposure to the synthetic LXRs ligand T0901317 has been associated with lipotoxicity due to triglycerides and free fatty acids accumulation in pancreatic islets (86). In addition, the activation of LXRs with synthetic ligands induces hypertriglyceridemia. These effects worsened when the db/db diabetic mouse models were treated for 12 days with T0901317. These animals

showed increased liver mass and severe hepatic steatosis along with reduced blood glucose and PEPCK expression (87). On this ground the antidiabetic potential of LXRs ligands has been limited.

Leptin is a hormone controlling nutritional status of the body and defects in its function leads to obesity (88). It has been reported that leptin is directly regulated by LXRs indicating a connection with the nutritional status. Indeed, mice fed with the LXRs agonist T0901317 resulted in a two-fold downregulation of leptin expression in white adipose tissue (89). These data suggest that LXRs agonist may increase food intake by blunting leptin expression an undesired effects in terms of obesity development. Taken together these data indicate a potential role of LXRs in the brain in the regulation of energy metabolism, however more careful investigations are necessary to better elucidate the role of LXRs in hypothalamus. Moreover, new ligands discerning between lowering glucose levels and inducing lipogenesis may be useful for the treatment of metabolic disorders associated to altered energy homeostasis.

LXRs AND CHOLESTEROL METABOLISM IN PERIPHERAL NERVOUS SYSTEM

The peripheral nervous system (PNS) consists of nerves and ganglia and different from the CNS is much more exposed to injuries due to the lack of blood brain barrier and/or bones.

Peripheral neuropathies are common disorders affecting PNS. Peripheral neuropathies may arise during the aging process, after mechanical injury, by metabolic disorders (e.g., diabetes mellitus), by infections and autoimmune diseases, or after exposure to such type of therapeutics or toxic compounds. Damages to the PNS can also be inherited as in the case of the Charcot–Marie–Tooth (CMT) disease (63).

As previously described in the CNS, in the PNS also cholesterol plays a crucial role. Similarly to the CNS, in the PNS the majority of cholesterol is found in myelin and in particular in peripheral myelin producing cells such as the Schwann cells. To investigate the role of cholesterol in Schwann cells, two different mouse models have been generated and characterized. The first is a mouse

model where the squalene synthase, a key enzyme to produce cholesterol, has been inactivated (SQS mice). The second model is a Schwann cells specific knock-out of the SREBP cleavage activation protein (SCAP). The SREBPs are a family of transcription factors regulating genes in cholesterol and fatty acid biosynthesis, the major components of the myelin sheath. Both models developed peripheral neuropathy due to a reduced myelination of the sciatic nerve. Moreover, in the SQS mutant Schwann cells it has been demonstrated that the export from the endoplasmic reticulum of the major myelin protein P0 to growing myelin is dependent on cholesterol. These data undoubtedly prove the important role of cholesterol and fatty acids in myelin generation (13, 90). Given the key role of lipids in the PNS and that LXRs are central in the regulation of cholesterol homeostasis and fatty acid metabolism, it is important to address their biology in this tissue either in physiological or in pathological conditions. In this regard, our laboratory demonstrated that both LXR isoforms are expressed and functional in the sciatic nerve (91). Makoukji and colleagues highlighted the importance of LXRs in regulating myelin peripheral nerves. Indeed, this team demonstrated that LXRDKO mice displayed thinner myelin compared with the age matched in wild type control animals (5). Thus, the lack of LXRs in the sciatic nerve alters the myelin structure (5).

From an endocrinological point of view, extensive literature reported the vital role of neuroactive steroids in PNS. As described in the CNS, the PNS also expresses the genetic makeup for the biosynthesis of neuroactive steroids. On this ground, several studies showed that neuroactive steroids are protective agents on peripheral neuropathy (63, 64, 69). Given that neuroactive steroids are cholesterol-derived molecules, we hypothesized that LXRs may have protective effects on peripheral neuropathy by modulating the levels of these hormones. Thus, we investigated the effects of LXRs activation in the contest of peripheral neuropathy induced by a metabolic disorder such as diabetes. In this contest, as previously reported by other studies, we showed that diabetes decreased the neuroactive steroid levels in the sciatic nerve. However, upon

LXRs activation by a synthetic ligand, the levels of some neuroactive steroids were restored to the levels detected in non-diabetic animals (91). The molecular mechanism involved the induction of a classical LXRs target gene such as the sterol acute regulatory protein (StAR) (8, 91). In fact, this protein is a cholesterol transporter that favored the cholesterol shuttling into mitochondria. Once in this organelle, cholesterol is the substrate of the cytochrome P450 side chain cleavage (P450scc or Cyp11a1) that generates pregnenolone, the first steroid in the biosynthetic pathway (91). From this study we concluded that LXRs activation promoted cholesterol utilization within the sciatic nerve. Similar results were also obtained in another study by using a synthetic activator of the translocator protein 18 kDa (TSPO). Similar to StAR, the main function of this protein is to transport cholesterol into the mitochondria which is a required step for the steroidogenesis (92).

Moreover, Verheijen and colleagues, by using the SCAP Schwann cells-specific knock-out demonstrated that not only cholesterol is important for myelin formation but also fatty acids (90). One of the lipogenic regulators is represented by SREBP-1c, a transcription factor under the direct control of LXRs. Our laboratory provided evidences that diabetic peripheral neuropathy is characterized by myelin abnormalities due to an altered myelin cholesterol and fatty acid profile (93). LXRs activation in the contest of diabetes restored myelin lipids to those found in the myelin of control animal peripheral nerves. These effects were mediated by a restored nuclear localization of SREBP-1c, after the LXRs activation, which was blunted by diabetes. Along with a restored lipid profile, LXRs also restored the levels of the myelin protein P0 (93). The positive effects of LXRs activation on diabetic peripheral neuropathy were associated with improved functional tests as an outcome to score the pathology (93). The roles of LXRs in the PNS are summarized in figure 2.

CONCLUSIONS

Lipid homeostasis is crucial to maintain the physiological function of the central nervous system and the peripheral nervous system. Diseases affecting both cholesterol and/or fatty acid metabolism have negative effects either in the CNS and/or in the PNS. Several evidences based on mice lacking LXRs in the nervous system indicated the critical role of this transcription factor in preserving a healthy phenotype, thus highlighting its importance in both the CNS and the PNS. Interestingly, it is of outmost importance that the activation of LXRs in neurodegenerative diseases, generally, had protective effects. Most of the beneficial effects of LXRs activation in neurodegenerative diseases have been ascribed to its ability to blunt the inflammatory response. However, LXRs also modulate the levels of neuroactive steroids. These molecules have been extensively studied for their beneficial effects on neurodegenerative diseases. However, their therapeutic potential has been limited by their systemic side effects. The LXRs ligand may by-pass these side effects of the hormones because they activate locally, in the nervous system, the steroidogenesis that ultimately confers neuroprotection. On the other hand, LXRs activation may leads to hypertriglyceridemia and liver steatosis.

In conclusion, LXRs may represent an attractive target for the treatment of neurodegenerative diseases in the CNS and/or the PNS. However, the discovery of new ligands and/or trials with different protocols of drug administration may result in maintaining the LXRs beneficial effects and avoiding those adverse.

LEGEND TO FIGURES

Figure 1. Role of LXRs in the central nervous system. Green arrows and red symbols represent the induced and the repressed genes and pathways after LXRs activation, respectively.

Liver X Receptors (LXR), ATP binding cassette A1 (ABCA1), ATP binding cassette G1 (ABCG1), Apolipoprotein E (Apo E), Low Density Lipoprotein Receptor (LDLR), Inducible Degrader of the LDLR (IDOL), Very Low Density Lipoprotein Receptor (VLDLR), Apolipoprotein E Receptor 2 (ApoER2), Sterol Regulatory Element Binding Protein-1c (SREBP-1c), Interleukin-17 (IL-17), selective Alzheimer's disease indicator-1 (Seladin 1)/3-β-hydroxysterol delta-24-reductase (Dhcr24), Smith-Lemli-Opitz syndrome (SLOS).

Figure 2. Role of LXRs in the peripheral nervous system. Green arrows represent the induced and genes and pathways after LXRs activation.

Liver X Receptors (LXR), Cholesterol (Chol), Sterol Acute Regulatory Protein (StAR), Translocator Protein 18 kDa (TSPO), Cytochrome P450 side chain cleavage (P450scc), Pregnenolone (PREG), Sterol Regulatory Element Binding Protein-1c (SREBP-1c).

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