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Pleiotropic effects of statins on brain cells

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ABSTRACT

Starting with cholesterol homeostasis, the first part of the review addresses various aspects of cholesterol metabolism in neuronal and glial cells and the mutual crosstalk between the two cell types, particularly the transport of cholesterol from its site of synthesis to its target loci in neuronal cells, discussing the multiple mechanistic aspects and transporter systems involved.

Statins are next analyzed from the point of view of their chemical structure and its impingement on their pharmacological properties and permeability through cell membranes and the blood-brain barrier in particular. The following section then discusses the transcriptional effects of statins and the changes they induce in brain cell genes associated with a variety of processes, including cell growth, signaling and trafficking, uptake and synthesis of cholesterol.

We review the effects of statins at the cellular level, analyzing their impact on the cholesterol composition of the nerve and glial cell plasmalemma, neurotransmitter receptor mobilization, myelination, dendritic arborization of neurons, synaptic vesicle release, and cell viability. Finally, the role of statins in disease is exemplified by Alzheimer and Parkinson diseases and some forms of epilepsy, both in animal models and in the human form of these pathologies.

1. Introduction

Statins are the most common form of therapeutic approach to reduce hyperlipidemia. Their introduction more than 30 years ago signified a remarkable breakthrough in the clinical management of cholesterol metabolism and transport and the risk of cardiovascular disease and stroke. Statins have a well-defined main target: the cholesterol biosynthetic machinery in the liver, the main organ in the organism involved in the endogenous production of cholesterol. Statin-mediated inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), the rate-limiting enzyme in the mevalonate-cholesterol biosynthetic pathway, upregulates the low-density lipoprotein (LDL) receptors and increases the clearance of LDL-cholesterol (LDL-C). In addition to the primary lipid-reducing target of the medication, statins exert multiple pleiotropic effects, some of which also impact on the cardiovascular system (reviewed in [1], see Fig. 1). Such additional effects are exerted through the inhibition of isoprenoid synthesis, a process necessary for the prenylation of proteins involved in the signaling pathways that regulate cellular growth and apoptotic death [2]. Statins hinder the post-translational prenylation of the small GTPases Rho and Rac, and their downstream effectors Rho kinase and NADPH oxidases.

In this review we examine the pleiotropic effect of statins on brain cells. One compelling reason to focus on brain is the likelihood that cholesterol homeostasis plays a complex role in brain, and that alterations in this homeostatic equilibrium can lead to disease conditions. An important group of diseases with possible links to dysfunctional cholesterol homeostasis are the dementias. Dementias are a health problem with a marked tendency to increase; ca. 115 million cases worldwide are expected for 2050 [3]. Potential risk factors for the various forms of dementia include cardiovascular risk, high levels of inflammation biomarkers, being an apolipoprotein E (*APOE*) ε4 allele carrier, depression symptoms [4] and, among the modifiable risk factors, lifestyle [5].

1.1. Brain cholesterol homeostasis

The cholesterol content in brain amounts to ~25% of the human body's total content of this neutral lipid. The central nervous system (CNS) has developed a local synthesis machinery to ensure physiological quantities of this lipid, due to the incapacity of the cholesterol-loaded blood lipoproteins to cross the blood-brain barrier (BBB) [7,8]. Myelin sheaths in the CNS are particularly rich in cholesterol, and this lipid accounts for ~25% of the total lipids in the plasmalemma of most cells, where it plays not only a critical structural role but also a

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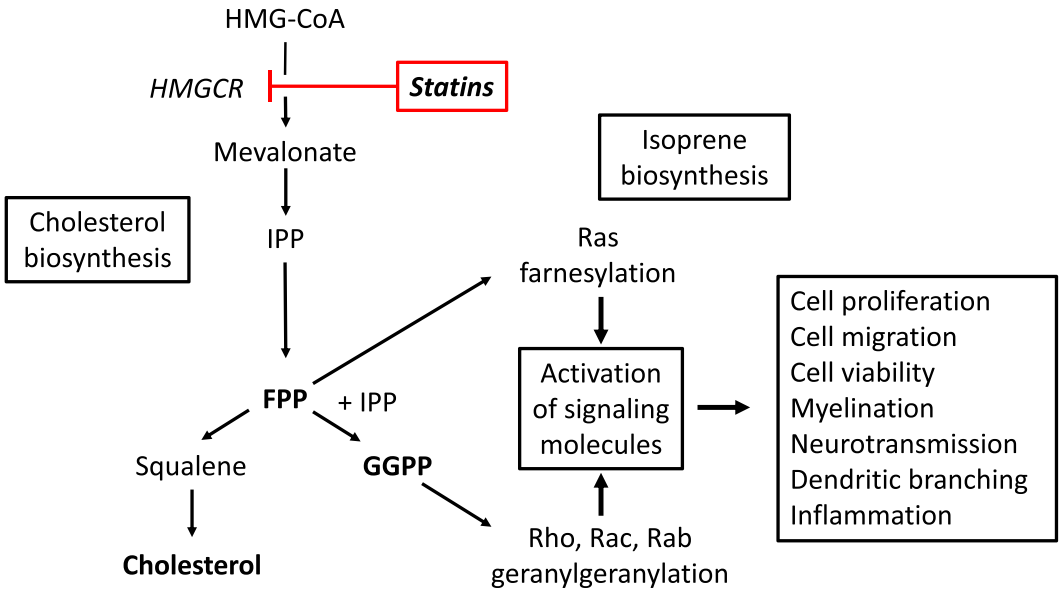

Fig. 1. Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) by statins interferes with cholesterol biosynthesis and also with the generation of isoprenoid intermediates such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), which contribute to cellular proliferation, migration and viability, myelination, dendritic branching, inflammation and neurotransmission. Inhibition of these isoprenoid intermediates may add to the pleiotropic effects of statins. Abbreviations: FFP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; IPP, isopentenyl pyrophosphate. Modified from Davignon & Leiter [6] to highlight the effects of statins on the brain.

regulatory role in membrane function, modulating signal transduction pathways that initiate at the plasma membrane by stimulating or dimerizing receptors [9], and the enzymatic processing of membrane proteins [10,11]. Importantly, alteration of cholesterol homeostasis is frequently associated with the etiology or pathophysiology of different neurological diseases such as Niemann-Pick [12], Alzheimer [13], Parkinson [14], Huntington [15], and epilepsy [16].

1.2. Cholesterol biosynthesis

Cholesterol biosynthesis involves several multimolecular reactions and has significant reduction power. Acetyl-CoA constitutes the master cholesterol precursor, stemming from different sources such as the catabolic fatty acid β-oxidation, ketogenic amino acid oxidation, and pyruvate dehydrogenase activity. Briefly, the energetically expensive biosynthesis proceeds in the cytoplasm in four consecutive stages: a) mevalonate synthesis from acetyl-CoA; b) conversion of mevalonate to two activated isoprenes; c) condensation of six activated five-carbon isoprenes to yield the 30-carbon squalene; and d) final conversion of squalene to the four-ring steroid nucleus.

The committed step and major point of regulation of cholesterol biosynthesis involves the reduction of 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) to mevalonate, a reaction catalyzed by the HMGCR, an ER-resident enzyme. Two molecules of NADPH provide the reducing equivalents for this reaction. HMGCR enzymatic activity is regulated through different mechanisms. The main one is the control of the synthesis rate of the HMGCR reductase mRNA by a group of sterol-regulatory element-binding proteins (SREBPs). These are transcription factors belonging to the basic helix-loop-helix leucine zipper family of proteins that increase the expression of several genes involved in the biosynthesis and uptake of various lipids such as cholesterol, fatty acids, triacylglycerols and phospholipids, as well as in the biosynthesis of the required NADPH molecules involved in the biosynthesis of such lipids.

Three SREBP proteins are encoded by two different genes. The SREBP-1 gene gives rise to SREBP-1a and SREBP-1c, through the use of alternate promoters that yield transcripts in which different first exons are spliced to a common second exon. SREBP-2 is derived from a separate gene [17]. In most animal tissues, SREBP-1c is the predominant isoform [18], except in the brain, where SREBP-2 levels are higher and this isoform has been demonstrated to control cholesterol metabolism in astrocytes [19]. SREBP-1c predominately influences fatty acid synthesis, while SREBP1a can regulate the global lipid synthesis [18].

SREBPs augment HMGCR gene transcription by binding to the sterol-regulatory element (SRE). Once produced, SREBPs locate at the ER as integral proteins. Normally, when cholesterol levels suffice to maintain the cellular homeostasis, SREBPs remain bound to the SREBP cleavage-activating protein (SCAP) at the ER membrane. Upon reduction of cholesterol levels, cholesterol abandons the SCAP-binding site and the SREBP-SCAP complex migrates to the Golgi apparatus. At the Golgi, Site 1 and Site 2 proteases act enzymatically, releasing the NH-terminal domain of the transcription factor. The NH-portion is the active one that translocates to the nucleus to bind the SRE. In contrast, when the cytoplasmic concentration of cholesterol rises, cholesterol molecules bind to SCAP and prevent the transport of the complex to the Golgi apparatus, leading to an ensuing reduction in HMGCR transcription [17,20].

The HMGCR activity can be regulated through phosphorylation of this enzyme at the serine 871 by the AMP-activated protein kinase (AMPK), itself regulated by upstream kinases like liver kinase B1 (LKB1) [21–23]. Thus, the cholesterol synthesis rate is sensitive to the cellular AMP/ATP ratio, decreasing when the levels of ATP are low and increasing when the levels of this nucleotide are high [24].

1.3. Cholesterol transport

It is widely accepted that de novo cholesterol synthesis in adult brain neurons is very low. To satisfy the physiological cholesterol demand of neurons, an alternative mechanism has therefore evolved: the transport of this sterol from actively synthesizing astrocytes to “passive” receptive neurons. Astrocytes have the cellular machinery to secrete cholesterol-enriched apolipoproteins. In addition, neurons express LDL and LDL receptor-related protein (LRP) receptors, able to bind the incoming astrocyte-derived apolipoproteins loaded with cholesterol. The most abundant apolipoproteins in the brain are ApoE and ApoAI, the level of ApoE in the cerebrospinal fluid (CSF) being higher than that of ApoAI. ApoE, as other apolipoproteins synthesized in the liver, has the

ability to bind and transport cholesterol in aqueous media. Recent studies using human stem-cell derived astrocytes and neurons highlight the importance of the efficiency of this cholesterol transport mechanism between astrocytes and neurons in the etiology of sporadic Alzheimer disease [25].

Although astrocytes are responsible for most (approximately 80%) of the ApoE production in brain, healthy neurons synthesize minor amounts of ApoE that can eventually increase, particularly in response to injury or stress. Interestingly, simvastatin, a BBB-permeable statin, is able to reduce the expression of ApoE in human astrocytes in culture [26].

1.4. Cholesterol catabolism

It has been demonstrated that a small amount of cholesterol belonging to the metabolic pool effluxes the brain as 24S-hydroxycholesterol (24S-HC). The enzyme responsible for the cholesterol oxidation, CYP46A1, belongs to the cytochrome P450 family, and is selectively expressed in glutamatergic neurons of the hippocampus and cortex, Purkinje cells of the cerebellum, and GABAergic interneurons of the hippocampus and cerebellum [27]. Although CYP46A1 is not detected in glial cells under physiological conditions, brain injury can induce the expression of this enzyme in these cells [28,29]. The 24S-HC is not simply a by-product that diffuses out of the BBB to be further cleared by the liver; it is also a potent bioactive molecule able to affect different cellular processes in brain cells. The main brain-derived cholesterol metabolite has been shown to modulate cell survival [30–32], NMDA receptor activity [33,34], the exocytosis of synaptic vesicles [35], and the nuclear LXR-induced transcriptional activity [36]. In addition, the concentration of secreted 24S-HC in vitro is a direct indicator of the neuronal cholesterol loss that occurs after mobilization and activation of the enzyme CYP46A1 in pathological scenarios, e.g. excitotoxicity [37].

2. Statins

2.1. Structure and permeability

The chemical structure of statins is a fusion of the pharmacophore -a dihydroxyheptanoic acid segment- and a hydrophobic ring system with different substituents (Fig. 2A and B). The pharmacophore competitively and reversibly inhibits the HMGCR enzymatic activity in a dose-dependent manner. The chemical modifications in the ring system and the nature of the substituents generate the different statin structures. The hydrophobic ring system is covalently bound to the pharmacophore and plays a key role in the chemical interaction that leads to HMGCR inhibition.

The structure of the ring can adopt several forms: a reduced naphthalene moiety (as found in lovastatin, simvastatin and pravastatin), a pyrrole (in atorvastatin), an indole (in fluvastatin), a pyrimidine (in rosuvastatin), a pyridine (in cerivastatin), or a quinoline (in pitavastatin). The ring substituents play a major role in the solubility of the statins as well as in several of their pharmacological effects and pharmacodynamic properties. For instance, in simvastatin a 2,2-methylbutyrate ester is linked to the partially reduced naphthalene ring, thus substantially increasing the potency of the drug.

Statins are normally classified into two categories: statins of fungal origin (“natural statins”), also known as type-1 statins (lovastatin, simvastatin, pravastatin), and synthetic or type-2 statins [38]. The former were originally discovered as fungal secondary metabolites [39]. Among these, mevastatin was identified in *Penicillium citrinum* in 1976 [40]. The active form of this statin resembles HMG-CoA, one of the cholesterol precursors. Subsequently, a more active fungal metabolite, mevinolin (lovastatin), was isolated from *Aspergillus terreus* in 1980 [41]. It is important to stress that the main difference between the two types of statins lies in a) their capacity to bind and subsequently hinder HMGCR activity and b) their hydrophobicity. Type 2 statins such as atorvastatin and rosuvastatin are able to establish a larger number of interactions with HMGCR due to their higher hydrogen binding capacity [42].

Lovastatin, simvastatin, atorvastatin, fluvastatin, pitavastatin, and cerivastatin are more hydrophobic, whereas pravastatin and rosuvastatin are more hydrophilic. Except for pitavastatin, all statins have low systemic bioavailability owing to an extensive first-pass effect in the liver [43]. This could be advantageous, because liver is after all the main site of cholesterol biosynthesis, but due to their lipophilicity most statins can passively enter non-hepatic tissues, including brain, leading to side-effects. A recent study [44] assessed the effects of two short-acting statins with different hydrophobicities, showing that they produce different actions on skeletal, cardiac and vascular smooth muscle. Furthermore, hydrophilicity to a large extent determines the active transport mechanisms for entering the hepatocyte; due to their exclusion from non-hepatic tissues, statins with higher hydrophilicity turn out to be more hepato-selective. The equilibrium between favorable and undesired pharmacological actions of hydrophobic and hydrophilic statins is therefore still a matter of contention, an issue which is dealt with in this review. In summary, the main differences between the two classes of statin molecules is to be found in their differing chemical structures, the hydrophilicity/hydrophobicity ratio, their pharmacokinetic profiles, and their metabolic rate. These properties collectively contribute in a non-linear fashion to their pleiotropic effects.

2.2. Main pharmacological effects of statins

Statins bind to mammalian HMGCR in the nM range, efficaciously displacing HMG-CoA, the μM range-binding endogenous substrate [45]. Once the statin-HMGCR complex if formed, HMG-CoA is no longer converted to mevalonate, and the downstream cholesterol biosynthesis is halted. This is accompanied by a diminution in isoprenoid metabolite formation (e.g. geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP)). GGPP and FPP are lipid-attachment molecules involved in the isoprenylation of different proteins. The attachment of these lipids is fundamental for the activation of the small GTPase family members Ras, Rac, and Rho at the plasma membrane; these enzymes regulate multiple pathways in cell differentiation and proliferative mechanisms [46,47]. In neurons, it was demonstrated that atorvastatin requires GGTase-Iβ, which adds a geranylgeranyl group to certain proteins, and activation of Rac1 to induce neuroprotection and plasticity [48]. In astrocytes, Rac1 prenylation is catalyzed by GGTase-I, upregulating NF-κB expression and promoting neuronal apoptosis associated with hypoxic or ischemic damage [49]. It is clear from the important role played by these prenylated proteins that statin effects cover territories outside their canonical cholesterol reducing effects: by diminishing the levels of GGPP and FPP, statins show an example of their so-called pleiotropic effects.

2.3. Transcriptional effects of statins

Recently, the global impact of statins on brain gene expression patterns was analyzed by DNA microarray technology. Mice were treated with lovastatin, pravastatin or simvastatin for 21 days and their cortices were analyzed, after confirmation by liquid chromatography/tandem mass spectrometry of the statins' presence in brain tissue [50]. Fifteen genes involved in cell growth, signaling and trafficking were found to change to a similar extent upon treatment with the three drugs. Simvastatin had a stronger effect on expression, affecting 23 additional genes. There were important changes induced by the three statins on genes associated with cell growth: up-regulation of *Enc1*, *Cotl1* and *Arhu*, all related to actin function, and down-regulation of *Fin15*, a gene under the influence of the fibroblast growth factor. In addition, the expression of two genes related to glucose metabolism was differentially altered: *Igfbp3* and *Hk1* were increased by pravastatin and

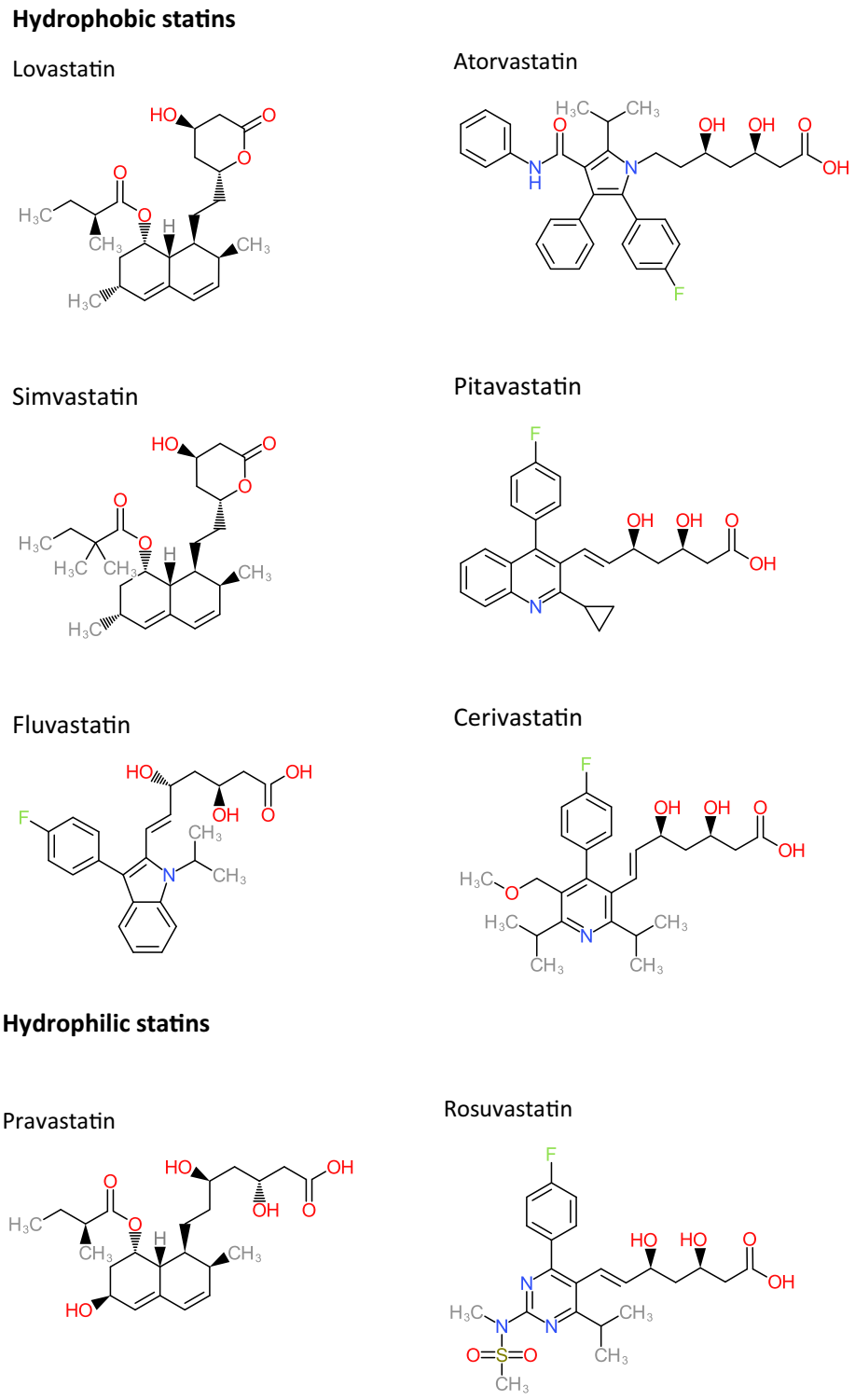

Fig. 2. Chemical structure of statins, subdivided into A) hydrophobic and B) hydrophilic statins, according to their n-octanol/water partition coefficient (logP): atorvastatin, 1.11; cerivastatin, 1.69; fluvastatin, 1.27; lovastatin, 1.70; pitavastatin, 1.49; simvastatin, 1.60; pravastatin, −0.84; and rosuvastatin, −0.33.

simvastatin but not by lovastatin. The *Igfbp3* gene encodes a carrier for the insulin-like growth factors 1 and 2 (IGF-1 and -2) which are expressed in neurons and astrocytes. High levels of IGFBP3 have been reported in AD brains [51], but whether the elevation of this protein is associated with the neurodegenerative process or is part of a protective response remains elusive. *Hk1* encodes for the key glucose-phosphorylating enzyme hexokinase 1, and its increased expression after pravastatin and simvastatin treatments would indicate a stimulated glycolysis. In relation to neurotransmission, lovastatin and pravastatin increased the expression of *Npy1r*, the gene that encodes neuropeptide Y receptor 1. The expression levels of genes involved in apoptosis were primarily affected by simvastatin. An important antiapoptotic gene upregulated by simvastatin was Bcl2, which has been demonstrated to be essential for neuronal survival [52]. In the study analyzing the impact of the 3 statins, the reductions in cortical cholesterol were minimal (8, 11 and 12% for lovastatin, pravastatin and simvastatin, respectively)

and the expression of genes directly involved in cholesterol biosynthesis remained unaffected, suggesting isoprenoid-independent pleiotropic effects. Surprisingly, detectable levels of the hydrophilic statin pravastatin were found in mouse cortices. The authors argue that pravastatin could have crossed the blood-brain barrier thanks to the activity of the monocarboxylic acid transporter 2 (MCT2) or organic anion transporters (OATs).

In another study, the administration of atorvastatin for 4 weeks to Wistar-Kyoto rats, at a dose that did not change the concentration of total cholesterol in plasma, affected the gene expression patterns in brain, heart and kidney [53]. Although the most significant changes in gene expression occurred in heart, 21 genes were up-regulated and only 3 were down-regulated in the brain. Ten of the upregulated genes participate in cell signaling/communication (neuropeptide signaling pathway and synaptic transmission), cell structure/motility, cell division, protein catabolism, gene/protein expression, and neuronal function (learning and memory, feeding behavior). The remaining down-regulated genes are involved in cell division, cell signaling/communication, and gene/protein expression. Much more work is needed to understand the complex actions of statins on the different gene expression patterns and their contribution to the pleiotropic effects.

3. Cellular effects of statins

3.1. Cholesterol composition of the plasma membrane in relation to AD

Cholesterol is known to be a key molecule in plasma membrane integrity, and consequently of the physiological status of brain cells. Hydrophobic statins can passively enter the cells of extrahepatic organs like the brain, surpassing the BBB. In relation to AD, cholesterol has been found to modulate the enzymatic processing of the transmembrane APP protein and thereby modify the production of the Aβ peptide. In hippocampal neurons, cholesterol depletion with lovastatin and methyl-β-cyclodextrin completely inhibits the formation of Aβ; cholesterol re-addition fully rescues the levels of this pathogenic peptide [54]. In addition to this in vitro study clearly showing how cholesterol synthesis inhibition affects the cleavage of membrane-bound proteins, alterations induced by hydrophobic statins in brain cholesterol levels were also studied in C57BL6 mice (young and middle-aged animals; 1 and 12 months, respectively) subjected to lovastatin treatment (100 mg/kg) for 3 weeks. This statin treatment produced a reduction in the amount of cholesterol in brain membranes at both ages. This was not the case in hypercholesterolemic ApoE KO mice at 12 months of age [55]. In another work, the effects of two hydrophobic and one hydrophilic statin were studied in C57BL6 mice specifically at the synaptic plasma membrane. Whereas application of simvastatin (50 mg/kg) or lovastatin 100 (mg/kg) for 23 days reduced the amount of un-esterified cholesterol at the synaptic plasma membrane, 100 mg/kg of pravastatin did not induce changes in the level of this lipid. Interestingly, total cholesterol at the synaptic plasma membrane remained unaffected under these treatments, while cholesterol esters slightly increased with simvastatin and lovastatin administration, implying a redistribution of this sterol into different cellular pools. Curiously, none of the statin treatments affected the serum total cholesterol levels. Furthermore, whereas hydrophilic pravastatin only affected the outer leaflet of the plasmalemma, hydrophobic simvastatin and lovastatin modulated both the inner and outer leaflets of the membrane [56]. In another interesting study, the impact of 5 months of atorvastatin treatment on hippocampal CA1 neurons was analyzed. This lipophilic statin reduced the cellular content of these cells and increased the inhibitory activity of the G protein-gated inwardly rectifying potassium (GIRK) channels, without modifying the total, LDL or HDL cholesterol in serum [57]. Altogether, these results demonstrate the independent regulation of central and peripheral cholesterol homeostasis, as evidenced by the fact that changes in brain membranes are not paralleled by changes in serum total cholesterol.

3.2. Neurotransmitter receptor activity and trafficking

It has been demonstrated that the hydrophobic statin simvastatin can affect the activity of the glutamate NMDA receptor, a key player in the regulation of the excitatory synaptic strength. Interestingly, both the in vivo administration of this statin in mice (20 mg/kg/day for 5 days) and the ex vivo treatment of mouse hippocampal slices (10 μM for 4 h) increased the density of the NMDA-induced inward currents (iNMDA) in CA1 pyramidal cells of the hippocampus. This augmented response was blocked by farnesol (FOH) but not geranylgeraniol (GGOH). The higher activity of the NMDA receptor in response to simvastatin in the live animal or in hippocampal slices was paralleled by increased phosphorylation of the receptor subunits GluN2B and GluN2A and the Src kinase, also FOH-dependent. PP2, a Src blocker, inhibited the simvastatin-enhanced phosphorylation of GluN2B and GluN2A and simvastatin-augmented iNMDA. The amounts of GluN2B mRNA and protein were elevated in simvastatin-treated mice, a change that was abolished by FOH. Furthermore, simvastatin also acted at the transcriptional level, affecting the acetylation of the histones H3K9 and H3K27 of the GluN2B gene in mice, an effect that was suppressed by FOH. The reduction of FPP by a farnesyl transferase inhibitor increased the levels of GluN2B expression, histone H3K9 and H3K27 acetylation and GluN2B, GluN2A and Src phosphorylation. Altogether, these results led the authors to propose a molecular explanation for the simvastatin-induced activation of NMDA receptors, where this statin enhances GluN2B expression and GluN2B and GluN2A phosphorylation, increases the NMDA receptor currents through the reduction of FPP, and augments histone acetylation of GluN2B and Src signaling [58].

Another reported effect of simvastatin is the enhancement of glutamate signaling and synaptic plasticity in the CA1 region of the hippocampus following stimulation of the α7 nicotinic acetylcholine receptor (α7nAChR). Simvastatin treatment of hippocampal slices (0.1–20 μM for 2 h) evoked an increase in the amplitude of cholinergic inward currents (iACh) and the amount of α7nAChR protein on the neuronal membrane, without producing changes in the degree of phosphorylation of the α7nAChR. The administration of FOH, which converts to FPP, abrogated these simvastatin-induced changes. In addition, simvastatin enhanced the phosphorylation of CaMKII and PKC. Thus, these molecular changes suggest that acute simvastatin treatment enhances trafficking and activity of the α7nAChR by augmenting PKC phosphorylation and diminishing FPP, leading to CaMKII activation triggered by NMDA receptors [59].

Unpublished results from our laboratory (M.V. Borroni & F.J. Barrantes) show that hippocampal neurons grown for 14 days in a medium supplemented with a low concentration of lovastatin (50 nM) exhibit higher surface levels of the α7- and α4-subunit nAChRs, measured by radioactive ligand binding assays and fluorescence microscopy. The increased surface levels of α7nAChR and α4nAChR are paralleled by higher expression levels of these receptors as measured by western blotting.

3.3. Synaptic vesicle release

The treatment of cultured hippocampal neurons with a low concentration of lovastatin (0.25 μM) for 7–14 days in vitro impairs synaptic vesicle release and reduces synapse density [60]. In the same vein it was demonstrated that cholesterol reduction with different strategies, including mevastatin (4 μM), impairs synaptic vesicle exocytosis in cultured hippocampal neurons. It was suggested that this effect could be a consequence of the modulation of the membrane curvature by cholesterol reduction, which favors a “negative curvature” [61].

3.4. Neurotransmitter levels

The above findings of compromised synaptic vesicle release under

conditions of low cholesterol lead one to think in terms of reduced synaptic activity due to lower amounts of released neurotransmitters in general, irrespective of their type. Curiously, mice that received 40 mg/kg/day of simvastatin for 7 days showed a significant reduction in the striatal levels of dopamine [62], but whether this effect is due to the death of striatal neurons or a consequence of the reduced synaptic vesicle release induced by low cholesterol, remains unanswered. Therefore, the chronic use of statins may have a negative impact on affective disorders, which are hypothesized to be mainly triggered by a drop in the synaptic levels of biogenic amines (dopamine, noradrenaline and serotonin).

In an interesting study designed to evaluate the neurobiological basis of aggressive behavior, it was demonstrated that in *Nile tilapia*, a fish with a belligerent behavior, atorvastatin treatment reduces plasma cholesterol and the telencephalic ratio between the main serotonin metabolite, 5-HIAA, and serotonin, and increases aggressive behavior compared to control fish [63]. These observations suggest that modification of plasma cholesterol may impact on the neurochemical mechanisms responsible for the belligerent *tilapia* behavior, pointing to the possibility that this mechanism is conserved in the phylogeny of vertebrates. In a randomized clinical controlled trial on humans, the effects of 20 mg of simvastatin or 40 mg of pravastatin per day were carefully evaluated [64]. The authors of this study concluded that statin effects on aggression differed by sex and age. Statin administration decreased aggressive behavior in men and increased it in women, and induced a greater aggression decline in individuals younger than 40 years. Therefore, it can be postulated that the cholesterol reduction induced by statins may lead to depressed activity of the serotonergic neurons with a resulting decrease in the synaptic concentration of serotonin, this in turn leading to augmented aggressive behavior.

3.5. Myelination

In vitro, simvastatin hampers the myelination process in mature oligodendrocytes through a reduction in the association of the molecules p21/Ras and Rho-A to the plasma membrane, and lower Erk1/2 activity. In vivo, simvastatin delays the re-myelination in a mouse model of cuprizone-induced re-myelination [65]. Another report concluded that simvastatin impedes post-cuprizone remyelination in mice [66].

3.6. Dendritic branching

Treatment of adult male rats with atorvastatin (20 mg/kg/day) for 7 days reduced dendritic branching in vivo in sympathetic ganglia. Similarly, when sympathetic neurons in culture were treated with statins, dendrites retracted and bone morphogenetic protein (BMP)-induced dendritic growth was blocked in a reversible manner, without affecting cell survival or axonal growth. Supplementation with mevalonate or isoprenoids, but not cholesterol, reduced the detrimental effects of statins on dendritic development. The blockage of isoprenoid biosynthesis resulted in similar effects. The mechanism underlying the atorvastatin effect is the blockage of RhoA translocation to the plasma membrane, a phenomenon that requires isoprenylation [67].

Another piece of evidence in favor of the view that statins interfere with the cellular cytoskeleton derives from studies carried out on the OLN-93 cell line and rat oligodendrocytes in culture [68]. In these systems, simvastatin interfered with the outgrowth and branching of the oligodendrocytes by reducing the expression and membrane-bound amounts of tubulin and 2,3-cyclic nucleotide-3-phosphodiesterase (CNP).

3.7. Cell viability

More than two decades ago, it was reported that long-term exposure of human fetal brain cells to lovastatin (100 ng/ml) produced deleterious ultrastructural changes in neuronal and glial cells that eventually resulted in cell death [69]. Lovastatin (0.01–1000 ng/ml) was found to block cholesterol biosynthesis in primary and immortalized astrocytes and in glial-neuronal reaggregated cultures. Primary astrocytes were more sensitive to the lowest lovastatin concentrations than the immortalized cells and glial-neuronal aggregates. Proliferation of immortalized astrocytes was totally inhibited by lovastatin at a concentration of 100 ng/ml but not at 5 ng/ml. Altogether, these data indicate that lovastatin behaves as a neurotoxin for developing brain cells. After this initial report, a good number of studies in different cellular models confirmed the adverse effect of statins on cell viability. Indeed, it was observed that statins can induce apoptosis in rat brain neuroblasts [70], human and rat malignant glioma cell lines [71] and rat primary neurons [72]. In addition, statins trigger differentiation and cell death in neurons and astroglia derived from newborn rats [73].

Simvastatin (0.1 μM for 6 days) triggers cell death in a mouse cerebellar slice culture model of developmental myelination. This statin drastically hindered the lifetime of Purkinje neurons and oligodendrocytes at the early stage of myelination. Oligodendrocytes were less affected than Purkinje cells, and the simvastatin effect was completely reversed by mevalonate but not by the isoprenoids FPP or GGPP, which could only partially reverse the deleterious statin effect [74].

In another report, it was shown that atorvastatin (0.1 to 20 μM), does not alter the viability of cortical astrocytes in culture. However, in glioma cells, this statin showed cytotoxic effects at concentrations of 10 and 20 μM [75].

In mouse cortical neurons in culture, chronic treatment with mevastatin impairs the expression of synaptic proteins, reduces NMDA receptor-mediated currents and accelerates the neuronal death associated with aging. The mevastatin-induced decrease in neuronal protein expression is additive with the typical aging-related decline in culture and affects synaptic function [76].

In a recent study [77] it was demonstrated that a concentration of 10 μM of simvastatin or atorvastatin, applied for 3 days in vitro, produced a drastic reduction in the viability of human iPSC-derived astrocytes. Interestingly, iPSC-derived neurons under the same treatment conditions showed no alterations in their viability in response to these two statins.

4. Effects of statins on animal models of disease

4.1. Alzheimer disease (AD)

The impact of different statins on learning and memory capabilities has been assessed using behavioral tests in different mouse models of AD. In general, the findings of these studies have rendered conflictive conclusions, often due to their inability to provide simultaneous information on behavioral responses at specific ages and changes in evolution markers of the disease. Another conflictive point was the use of cholesterol determinations in plasma as a basis for the claim regarding the cholesterol-lowering actions of statins in the brain. Few studies measure the amount of brain cholesterol after statin treatment, and no study evaluated the statin concentration in the brain. So far, only two works have confirmed FPP-dependent effects of statins in mouse brain, using farnesol addition as the experimental tool [78,79]. The administration of simvastatin (40 mg/kg/day) for 11 days following the intra-cerebroventricular (i.c.v.) injection of Aβ25-35 ameliorated the impaired performance induced by this peptide in spatial memory tasks. This memory improvement was sensitive to farnesol, indicating that simvastatin mediates its effects via a reduction in the levels of FPP [78]. In addition to cognitive deficits, these mice i.c.v. injected with Aβ25-35 developed a diminution of the LTP phenomenon in the hippocampal CA1 region. The intra-gastric administration of atorvastatin (5 mg/kg/day) was able to ameliorate both deficits [80]. Furthermore, the Aβ25-35 peptide induced a rise in the number of Iba-1 positive microglial cells and inflammatory components that diminished

after atorvastatin treatment; these effects were FPP-dependent [79]. Importantly, the administration of Aβ25-35 to hippocampal slice cultures triggered neurotoxicity and different concentrations of atorvastatin (0.5, 1, 2.5 μM) prevented cell injury in a dose-dependent manner [79].

In female Tg2576 mice that overexpress human APP carrying the double Swedish mutation (K670M/M671L), the addition of simvastatin in the solid diet (~50 mg/kg) from 11 to 14 months of age reversed the typical learning and memory deficits of these animals. However, this effect was not specific, because the same treatment also enhanced learning and memory in control non-transgenic mice. Intriguingly, the memory changes in transgenic and non-transgenic animals involved a simvastatin-induced reduction in the total cholesterol concentration in plasma without affecting the amount of brain cholesterol [81]. In the same AD mouse model, the effects of atorvastatin (30 mg/kg/day) and pitavastatin (3 mg/kg/day) on cognitive dysfunction, amyloid plaque deposition and levels of tau phosphorylation were monitored between 5 and 20 months of age. The positive effects of these statins on the tested hallmarks of the disease were evident after 10 months of administration [82]. In another study, fluvastatin (5 mg/kg/day) administration before the i.c.v. injection of the Aβ25-35 peptide prevented the expected amyloid-induced memory impairment, an effect which was not observed when fluvastatin was administered just after Aβ25-35 exposure [83].

Simvastatin treatment (40 mg/kg/day) for 3 to 6 months led to full recovery of the short- and long-term memory deficits in 6-month-old, but not in 12-month-old AD mice that overexpressed the Swedish and Indiana mutations of the human APP protein. Curiously, these advantageous effects on memory occurred without reductions in soluble amyloid levels or amyloid plaque load. The protein expression of the memory-associated immediate-early genes c-Fos and Egr-1 were found to reach normal or up-regulated levels in the CA1 neurons of the hippocampus of AD mice exhibiting memory recovery, indicating that simvastatin can improve neuronal function [84]. Contrasting results were found in another rodent model, the bi-transgenic mouse that overexpresses a mutant of the human APP (Swedish, Indiana) together with a constitutively-active form of TGF-β1, leading to both amyloid and cerebrovascular pathology. In this bi-transgenic model simvastatin (40 mg/kg/day) did not ameliorate the deficient spatial learning and memory nor did it improve the reduced expression of the memory-associated protein Egr-1 in the hippocampal CA1 region [85].

A comparative study carried out in male and female Tg2576 mice treated with lovastatin at a dose able to reduce plasma cholesterol level in males and females, showed that this drug enhances the amount of Aβ in female mice only. Similarly, lovastatin increases the amount of plaques in the hippocampal and cerebral cortex of female animals, without variations in the amount of full-length APP, enzymatically cleaved APP, or PS1 in either sex. The observation that lovastatin reduces plasma cholesterol in both male and female animals but increases Aβ production and plaque formation only in female Tg2576 animals, led the authors to conclude that reduced plasma cholesterol levels could in fact constitute an AD risk factor in females [86].

APP/PS1 mice, the most widely employed murine model of AD, exhibit a marked increase in the levels of water-soluble Aβ between 6 and 8 months of age. These levels remain stably high until 18 months. In contrast, detergent-soluble and formic acid-soluble Aβ species normally increase across the lifespan, indicating that while amyloid deposition continues, the levels of water-soluble Aβ remain relatively constant. In these transgenic AD mice the LTP phenomenon was normal at 6 months, but markedly reduced at 8 and 18 months. A diet containing 0.04% simvastatin administered for one month (between 7 and 8 months) led to recovery of synaptic plasticity in the APP/PS1 animals, with a reduction in plasma cholesterol and without changes in the levels of the three forms of Aβ. Notably, the phosphorylation of Akt and GSK-3β augmented with the simvastatin-supplemented diet [87].

Aβ-immunization has been proposed as a strategy to fight AD and is currently under deep scrutiny. In AD mice of 22 months of age, an adenovirus vector coding for 11 tandem repeats of Aβ1-6 was employed for this purpose. The virus-induced antibody response was low, but the simultaneous administration of simvastatin potentiated the concentration of antibodies against Aβ. Immunization per se in absence of simvastatin did not reduce Aβ deposits in the brains of these mice, but increased soluble Aβ. The immunization process enhanced the hippocampal amyloid-induced vascular pathology and augmented leukocyte invasion, an effect abrogated by simvastatin, indicating that the statin adjuvates to enhance soluble Aβ levels and diminish the vaccination-associated inflammation [88].

The cerebro-microvascular endothelial cells of 3xTgAD mice carrying mutations on APP, MAPT and PS1, have also been addressed experimentally. Elevated expression levels of ApoJ (clusterin) and LRP1 were measured in these cells compared to those of non-transgenic animals. Simvastatin treatment of these endothelial cells increased the intracellular and secreted ApoJ levels, augmented the amount of secreted Aβ oligomers and reduced the Aβ uptake and cell-associated Aβ oligomers. Importantly, these simvastatin effects on ApoJ, APP processing, and LRP1 expression in endothelial cells were also evident in vivo [89].

4.2. Parkinson disease (PD)

The putative protective effect of simvastatin against nigrostriatal degeneration after MPTP intoxication in mice was investigated. In mouse microglial cells in culture, the toxin MPP+ activated p21 and nuclear factor-kappa beta (NF-κβ), and simvastatin attenuated the activation of both mediators. A fast activation of p21 in vivo in the substantia nigra pars compacta of MPTP-intoxicated mice was also consistently found in this animal model. Orally-administered simvastatin reached the substantia nigra and produced beneficial effects, reducing the activation of p21 and attenuating the activation of NF-κβ. Simvastatin also abrogated the expression of proinflammatory molecules and blocked the activation of glial cells. In parallel, the striatal concentration of dopamine reached normal levels with recovery of motor functions in MPTP-treated mice. Another statin, pravastatin, was found to block microglial inflammatory responses in mice treated with MPTP and exerted a protective effect on dopaminergic neurons. When administered 48 h after commencement of the experimentally-induced disease, both statins prevented the death of dopaminergic neurons and the associated depression of neurotransmitter levels, indicating that statin treatment slows down neuronal loss in MPTP-treated mice [62].

In two different transgenic mouse models that overexpress human α-synuclein in neurons, lovastatin treatment reduced levels of plasma cholesterol and oxidized cholesterol metabolites in the brain. Neuronal α-synuclein aggregates were shown to decrease, as were the levels of α-synuclein immune-reactivity in the temporal cortex neuropil. Furthermore, immunoblots of brain homogenates showed a diminution of total and oxidized α-synuclein in lovastatin-treated α-synuclein transgenic mice, coincident with the retraction of the neuronal pathology [90].

In another model of PD, the LRRK2-G2019S knock-in mice, and in a human dopaminergic clonal cell line, lovastatin was found to abrogate neurite degeneration in a dose-dependent manner. The effect of lovastatin was found to be due to the stimulation of the anti-apoptotic Akt/Nrf cascade and the diminution of caspase 3 levels. Furthermore, lovastatin inhibited GSK-3β, a kinase downstream of Akt, through the induced increase of GSK3β phosphorylation. GSK-3β blockage led in turn to a reduction in tau phosphorylation, a condition which renders the neuronal cytoskeleton unstable. These findings demonstrate that lovastatin can suppress neurite degeneration by stimulating the Akt/NRF2 pathway and hindering GSK3β action, which decreases phosphorylated tau levels [91].

4.3. Epileptic seizures

The effects of the combined administration of antiepileptics and statins were monitored using an experimental model of generalized tonic-clonic seizures, the DBA/2 mouse strain. Simvastatin, fluvastatin, lovastatin and atorvastatin were shown to exhibit an additive anticonvulsant effect when administered together with antiepileptic drugs like carbamazepine, diazepam, felbamate, lamotrigine, topiramate and valproate. This implies that certain hydrophobic statins can easily cross the BBB and affect the activity of brain regions implicated in the generation of or the susceptibility to seizures [92].

5. Effects of statins on human brain

The US Food and Drug Administration (FDA) warns on statin labels that some people under statin treatment may develop memory loss or confusion [93–95]. These side effects reverse once the medication is stopped. Though there is still too little evidence to prove a cause-effect relationship, these off-target effects make it clearly apparent that statins reach the brain and affect its function. So far, evidence that statins can hinder or improve the memory deficits of AD patients [96] is scanty and controversial, since some reports show no beneficial effects at all [97,98]. The impact of statins on the brain is currently under scrutiny in different experimental models of AD (discussed above), although there have been and still are few clinical trials tackling the question of whether these cholesterol/isoprenoid-lowering drugs affect particular brain functions.

The lack of clarity of some clinical trials derives from several factors not taken into consideration in their design. First, although it is widely recognized that there is a spectrum of BBB permeabilities relating to the hydrophobicity of statins, most of the studies did not include a hydrophilic statin for comparative purposes. Secondly, the central and peripheral effects on cholesterol and other disease biomarkers were not evaluated in parallel in the same trial, making it difficult to interpret the statin-induced brain changes. Thirdly, the trials were more focused on checking whether statins had some beneficial effects on AD patients at advanced stages, instead of exploring the possible capacity of statins to reduce the risk of dementia at earlier symptomatic stages of the disease (i.e. mild cognitive impairment (MCI) or mild AD). In this regard, there is currently one active trial, not recruiting yet (https://www.clinicaltrials.gov/ct2/show/NCT00842920?term=statins&cond=MCI&draw=2&rank=1), in which the effects of 20 and 60 mg of the hydrophobic simvastatin will be evaluated in amnestic MCI patients.

It is thought that the potential reduction in AD risk associated with statins could arise from their pleiotropic action, most likely due to a reduction in the accumulation of soluble Aβ species mediated by low levels of FPP and GGPP that affect the isoprenylation of different proteins, some of them present at the synapse.

6. Conclusions and perspectives

Since the introduction of the first commercially available drug of its kind -lovastatin, in 1987- statins have undoubtedly had a tremendous impact on the prophylaxis and therapeutics of cardiovascular disease mortality, cardiac and cerebrovascular events. The metabolism of these chemicals, their mechanisms of action on LDL-cholesterol, and the impact of these changes in cholesterol levels on the vascular system, including the coronary vessels, are relatively well understood. However, brain poses a singular problem for the delivery of substances because of its unique BBB properties which impede the crossing of this multicellular fence to a great variety of chemicals. Simplistically, the barrier could be envisaged as a blocking mechanism to the passage of macromolecules, imposing a “size criterion”, i.e. basically excluding protein molecules. This is the case, for instance, of man-tailored proteins such as bevacizumab, an antibody molecule currently used for the treatment of brain cancers and whose mechanism of action relies on the sequestration of vascular endothelial growth factor (VEGF) within the vascular lumen in the tumor proper, but which is unable to permeate the BBB [99]. Because of their small size and hydrophobicity, molecules as small as cholesterol (MW 386.7) could be assumed to be able to cross the barrier. However, 98% of all small drugs and 100% of large molecules do not cross the barrier [100]: cholesterol is transported in blood bound to relatively large lipoproteins, thus precluding its ability to cross the BBB. This trans-vascular “insulation” has probably led to the evolutionarily acquired self-sufficiency of the brain in terms of cholesterol biosynthesis, conducted mainly by glial cells under physiological conditions.

As we have analyzed in this review, statins are relatively small organic compounds (MW 390.5–558.6) of natural or synthetic origin, having variable degrees of BBB crossing ability. Once they penetrate the brain parenchyma, they not only affect cholesterol biosynthesis but also impact on neuronal and glial cells, affecting neurotransmitter levels, neurotransmitter receptors in the synapse, cellular viability, arborization of neuronal dendrites, oligodendrocyte-mediated myelination, etc., *selective* manifestations in the brain of the *pleiotropic* effects observed in other organs and tissues. This wide spectrum of multifaceted effects in brain reflects the complexity of this organ compared to others in the body's economy. It is expected that the many gaps remaining in our knowledge of these pleiotropic effects will be filled in as our understanding of brain physiology progresses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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