Cholesterol metabolism has been linked to Alzheimer’s disease (AD) neuropathology, which is characterized by amyloid plaques, neurofibrillary tangles and neuroinflammation. Indeed, the use of statins, which inhibit cholesterol and isoprenoid biosynthesis, as potential AD therapeutics is under investigation. Whether statins offer benefit for AD will be determined by the outcome of large, placebo-controlled, randomized clinical trials. However, their use as pharmacological tools has delineated novel roles for isoprenoids in AD. Protein isoprenylation regulates multiple cellular and molecular events and here we review the complex roles of isoprenoids in AD-relevant processes and carefully evaluate isoprenoid pathways as potential AD therapeutic targets.

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APPs, C83, like C99, is a substrate for γ-secretase, which cleaves it to generate the non-amyloidogenic p3 fragment and AICD.

While mounting evidence suggests that Aβ plays a critical, early role in AD pathogenesis, the form (or forms) of Aβ responsible for brain damage observed during disease progression remains a strong focus for debate. Traditionally, AD pathophysiology is characterized by deposition of extracellular Aβ as cerebral amyloid plaques (Glenner and Wong, 1984; Masters et al., 1985). The highly amyloidogenic 42 amino acid form of the peptide (Aβ42) is the predominant Aβ species found in plaques and increased production of Aβ42 is associated with early onset familial AD (FAD). Furthermore, this species appears toxic to neurons both in vitro and in vivo (reviewed in Selkoe, 2001; Younkin, 1998).

In addition to extracellular amyloid, a role for intraneuronal Aβ as an early pathogenic event in AD has been recently proposed. Not only is intraneuronal Aβ detected at autopsy of patients with mild cognitive impairment (Gouras et al., 2000) but this form of Aβ marks the initial site of amyloid accumulation in Down’s syndrome patients, who invariably succumb to AD-like dementia (Busciglio et al., 2002; Cataldo et al., 2004; Mori et al., 2002). Furthermore, data from experimental systems indicate intraneuronal Aβ42 toxicity (Magrane et al., 2004; Skovronsky et al., 1998) and in an animal model of AD, intraneuronal Aβ appears to play a pathologic role in disruption of cognition (Billings et al., 2005). Finally, Aβ oligomers, soluble aggregates of Aβ, are potent neurotoxins that can acutely disrupt neuronal function in vivo (reviewed in Klein et al., 2004). Intriguingly, in addition to extracellular plaques and intracellular Aβ, Aβ oligomers accumulate in AD brain (Gong et al., 2003; Kuo et al., 1996).

**Cholesterol metabolism and AD**

Sporadic AD (SAD), which accounts for ~98% of all cases, and FAD share the same pathological lesions. In FAD, mutations in APP and key components of the γ-secretase complex, the presenilins (PS-1 and PS-2), characterize this disease and are highly penetrant (reviewed in Selkoe, 2001). Such mutations are absent in SAD cases and while the etiology of this disease is likely to involve Aβ42, its precise nature remains elusive.

However, risk factors associated with developing SAD have been identified and the continually evolving list of genetic and environmental factors that affect cholesterol metabolism and associate with AD is of particular interest.

There is a close relationship between AD and cardiovascular disease with coronary artery disease, hypertension, and hypercholesterolemia, amongst others, being significant risk factors in AD (Kivipelto et al., 2002; Skoog et al., 1996; Soneira and Scott, 1996; Sparks et al., 1990; reviewed in Skoog and Gustafson, 2002). Many such vascular-related AD risk factors have an established association with cerebral hypoperfusion. Indeed, accumulating experimental, neuropathological and clinical data strongly implicate the participation of brain vasculature in sporadic AD. Imaging studies have demonstrated widespread pathological perturbation in AD brain hemodynamics (Gonzalez et al., 1995; Harris et al., 1996), with significant hypoperfusion of frontal, temporal, parietal and cingulate regions of AD brain being observed (Alsp et al., 2000). Recently, Roher and co-workers proposed that extensive atherosclerotic occlusion of the arteries of the circle of Willis and convexities of the brain may play an important role in the hypoperfusion observed in a proportion of SAD cases (Kalbac et al., 2004). It is well established that hypercholesterolemia is a highly significant risk factor for the development of atherosclerosis (reviewed in Gylling, 2004). Furthermore, the homeostatic regulation of cholesterol metabolism may be altered in AD and several polymorphisms in genes involved in cholesterol transport and catabolism, such as LRP, Cyp46 and ApoE, are AD risk factors (Jarvik et al., 1995; Kolsch et al., 2003; Papassotiropoulos et al., 2003; Papassotiropoulos et al., 2005; reviewed in Wolozin, 2004).

Experimental evidence strengthens the putative association between cholesterol and AD. Animals maintained on cholesterol-rich diets demonstrated increased brain Aβ load that was reduced when the animals were returned to a regular chow diet (Sparks, 1996; Sparks et al., 1994). In addition, in vitro studies have revealed the cholesterol-sensitivity of APP metabolism, with increased cholesterol favoring the amyloidogenic processing of APP and facilitating Aβ production, whereas a reduction in cellular cholesterol levels leads to a corresponding decrease in Aβ secretion (Bodovitz and Klein, 1996; Ehehalt et al., 2003; Fassbender et al., 2001; Galbete et al., 2000; Kojro et al., 2001; Simons et al., 1998). Although these studies suggest that cellular cholesterol levels modulate APP processing, other reports indicate that cholesterol esters (rather than free cholesterol) affect the secretase activities such that low cholesterol ester levels decrease Aβ formation (Puglielli et al., 2001). Additionally, the subcellular distribution of cholesterol may also influence APP cleavage because mutations and pharmacological inhibitors of the Niemann–Pick complex cholesterol pathway alter the localization of the presenilin/γ-secretase complex and lead to elevated Aβ production (Burns et al., 2003; Jin et al., 2004; Runz et al., 2002; Yamazaki et al., 2001).

Given these data, strategies aimed at the modulation of cholesterol metabolism, levels and distribution in the brain have received widespread attention for the prevention or treatment of AD. Although controversial, retrospective epidemiological studies have suggested that use of the cholesterol-lowering drugs, the statins, for the treatment of coronary artery disease, may reduce AD risk (Jick et al., 2000; S.L. Cole, R. Vassar / Neurobiology of Disease 22 (2006) 209 – 222
untreated individuals (Fassbender et al., 2002). Given the significant difference in cerebrospinal fluid Aβ42 levels following administration of low-dose statin. Furthermore, no in vitro findings indicating that statin administration may reduce Aβ42 levels was observed when statin-treated individuals were compared to untreated individuals (Fassbender et al., 2002). Given the significant hypoperfusion observed in some SAD cases, it should be considered that the putative protective effects of statins may be entirely independent of changes in amyloid load and could instead be a secondary effect due to an improvement in cardiovascular or cerebrovascular function (Fig. 3).

Secondly, if statin administration does indeed prove to be efficacious in lowering cerebral amyloid load then it may be either mediated via a cholesterol-reducing mechanism within the CNS or through a peripherally mediated effect. Due to lipophilic differences, statins differ in their central bioavailability. Interestingly, early retrospective clinical trial data indicated that both hydrophobic and hydrophilic statins might lower AD risk by up to 70% (Wolozin et al., 2000). Such data may support the hypothesis that any benefit statin therapy may offer to reduce AD risk may stem from a peripherally mediated mechanism (Fig. 3). It has been previously demonstrated that an equilibrium exists between CNS and plasma Aβ levels and that transport of Aβ between the CNS and plasma may regulate brain Aβ levels (DeMattos et al., 2001). Therefore, it may be hypothesized that any statin benefit in AD may result from a net efflux of Aβ from the CNS that occurs as a consequence of an initial statin-mediated reduction in peripheral Aβ production.

Alternatively, statins may indeed affect brain cholesterol metabolism, but rather than acting explicitly on neuronal populations, may do so indirectly, by acting at the level of the cerebral endothelium. Vega et al. demonstrated that both lipophilic and hydrophilic statins had similar effects on reducing the level of 24S-hydroxycholesterol, the plasma levels of which reflect brain cholesterol catabolism, while Eckert and colleagues have proposed that the regulation of brain cholesterol depends on the presence of ApoE (Eckert et al., 2001; Vega et al., 2003). Within the CNS,
astrocytes are the main source of ApoE and cross-talk between astrocytes and endothelial cells has been documented (reviewed in Ballabh et al., 2004). Thus, although a mechanism remains elusive, the presence of astrocytic end feet on endothelial cells raises the possibility that statins are sensed by astrocytes, which respond accordingly by reducing the secretion of apoE and cholesterol.

Cholesterol is critically important for the maintenance of physiological functions and cellular cholesterol homeostasis is tightly regulated. The majority of brain cholesterol is derived by de novo synthesis and the extent to which physiological statin concentrations will reduce brain cholesterol levels is likely to be small, if at all. Interestingly, a recent report using recombinant cells indicated that small reductions in membrane cholesterol actually raises the possibility that statins are sensed by astrocytes, which are the main source of ApoE and cross-talk between astrocytes and endothelial cells has been documented (reviewed in Ballabh et al., 2004). Thus, it appears likely then that APP processing is not simply modulated by membrane cholesterol levels but rather a more complex interplay between a number of lipid and protein components.

Isoprenylation

The observed clinical benefit with statin therapy is much greater than anticipated through the reduction of cholesterol levels alone. For example, statin therapy reduces the recurrence rate of cardiac events following acute coronary syndrome, with reduction in angina and new ischemic events from 4 and 16 weeks, respectively. While a reduction in serum cholesterol was observed, it appears unlikely that significant vascular remodeling could have occurred during such a short time period. Thus, the rapidity of statin action coupled with the efficacy of these drugs in patients without raised cholesterol levels suggests that other factors are at play (reviewed in Wierzbicki et al., 2003).

It is now well documented that statins are pleiotropic drugs that orchestrate a diverse array of cellular effects including improvement of endothelial function, antithrombotic actions and plaque stabilization, a reduction of vascular inflammatory processes and antioxidation effects. Importantly, in the periphery, many of these pleiotropic effects may be mediated though a statin-induced reduction in the synthesis of isoprenoid intermediates such as FPP and GGPP, and not through statin-mediated alterations in cellular cholesterol levels (reviewed in Liao and Laufs, 2005).

During isoprenylation, the attachment of FPP (farnesylation) or GGPP (geranylgeranylation), via the activities of farnesyl and geranylgeranyl transferases, is an important post-translational modification of a large number of proteins including nuclear lamins, the subunits of trimeric G proteins, protein kinases and Ras and Ras-related GTPases. Approximately, 0.5% of mammalian proteins undergo isoprenylation, and of the two types of prenylation, geranylgeranylation is the predominant modification (reviewed in Cox and Der, 1997; Zhang and Casey, 1996). The post-translational modification by isoprenoid lipids not only promotes the association of target proteins with plasma and intracellular membranes, but may critically influence the subcellular localization of a specific protein, in addition to its ability to interact with other proteins (reviewed in Cox and Der, 1997; Gibbs and Oliff, 1997; Sebti and Hamilton, 1997).

Complexity of GTPase biology

Isoprenylation affects a host of cellular processes and inhibition of specific GTPase isoprenylation has profound effects on cellular functions including dynamic cytoskeletal and morphological alterations and decreases in the efficiency of vesicular transport (Ridley, 2001; Vicent et al., 2000).

GTPase biology is highly complex (Fig. 4). The Ras GTPase superfamily is composed of over a 150 members, subdivided into five major branches, namely the small Arf/Sar and Ran families, and the larger Ras, Rho and Rab GTPase families (Rodriguez-Viciana et al., 2004; reviewed in Takai et al., 2001). Members of the Ras GTPase superfamily are integral components of complex signaling networks and control diverse cellular activities including intracellular vesicle transport, cytoskeletal organization, cell adhesion, smooth muscle contraction, endocytosis, receptor signaling, cell cycle progression and gene expression (reviewed in Takai et al., 2001).

GTPase isoprenylation

Multiple GTPases activate multiple downstream effector molecules (Fig. 4), and the functionality of GTPases is influenced by isoprenoid modification, although this remains incompletely understood.
Ras GTPases undergo farnesylation, whereas closely related proteins such as R-Ras1 are modified by GGPP. The Rho family is one of the largest members of the Ras GTPase superfamily, and the majority of Rho GTPases are geranylgeranylated (RhoA), although some are modified by a farnesyl group (RhoE), and others can be modified by both isoprenoids (RhoB; Adamson et al., 1992a,b; Baron et al., 2000; Foster et al., 1996; Solski et al., 2002).

The functions of particular Rho GTPases, such as RhoA, are critically dependent on prenylation, whereas for others, such as RhoB, function is observed in the absence of isoprenylation (Lebowitz et al., 1997). Furthermore, the function of specific Rho proteins is in part determined by the type of isoprenoid modification whereas with others, function, although fully dependent on isoprenylation, is independent of specific isoprenoid modification (Solski et al., 2002). For example, farnesylated RhoB exerts a growth-promoting function, whereas geranylgeranylated RhoB inhibits growth (Du et al., 1999; Liu et al., 2000; Solski et al., 2002). On the other hand, RhoA exhibited similar cellular functions regardless of whether it was modified by FPP or GGPP, indicating a certain degree of redundancy, under specific conditions (Solski et al., 2002). In addition, different GTPases have different intracellular localities and in some cases differential isoprenylation can affect GTPase subcellular distribution.

To understand the biological functions of different GTPases and their putative contribution to human diseases such as AD, it is also important to understand which effector pathways they regulate. In specific instances, a host of potential effectors have been identified for a single GTPase. For example, the effector molecules associated with RhoA include scaffold proteins such as rhophilin, Rhotekin, kinecin and diaphanous or serine/threonine protein kinases such as protein kinase C-related kinase (PRK1, also called protein kinase N or PKN), citron kinase and Rho-associated protein kinases (ROCKs; reviewed in Bishop and Hall, 2000; Kaibuchi et al., 1999). A similar situation is observed for members of the Ras GTPase family (Rodriguez-Viciana et al., 2004).

In addition to the competition between multiple effectors for GTPase binding, the recent identification of multiple contact sites on specific GTPases, such as RhoA, has indicated that a cooperative binding mechanism may exist for effector molecules such as ROCK (Blumenstein and Ahmadian, 2004). Furthermore, in the case of Ras GTPase effector molecules, many isoforms exist within a given effector family. It has been recently proposed that the specificity among Ras GTPases is not only achieved by the differential regulation of effector molecule combinations but also by the selective regulation of different isoforms within an effector family (Rodriguez-Viciana et al., 2004), thus adding another level of complexity towards comprehending the molecular mechanisms behind GTPase-mediated effector activation.

AD and GTPases

Several lines of evidence implicate the activities of specific GTPases in processes associated with AD pathophysiology. Failures of synaptic plasticity are thought to represent early events in AD course (reviewed in Arendt, 2004). Indeed, neuronal Ras, Rac1 and Rho-A appear to play a role in the plasticity of the adult mammalian brain, affecting neuronal architecture, synaptic connectivity and efficacy (Pilpel and Segal, 2004). Interestingly, Rac has been implicated as an essential component of the Aβ signaling cascade which leads to the generation of reactive oxygen species (Lee et al., 2002), and the relative levels of Ras and the presynaptic protein Rab3 are significantly reduced in AD brain (Reddy et al., 2005; Shimohama et al., 1999). In addition, APP metabolism has been linked to the activities of specific GTPases. Studies have demonstrated that geranylgeranylated G-proteins such as Rab1B and Rab6 play an important role in the trafficking and processing of APP. Indeed, the regulation of Rab6 membrane association appears dependent on PS1 (Scheper et al., 2004). Furthermore, a physical association between PS1 and Rab11 has been demonstrated (Dumanchin et al., 1999) and the levels of Rab8 are reduced in PC12D cells that express mutant, but not wild type, PS-1 (Kametani et al., 2004).

The functionality of specific GTPases is critically dependent on their prenylation and the role of lipid modification in neurodegenerative disorders is not without precedence. In addition to prenylation, protein palmitoylation involves covalently coupling a lipid moiety to a substrate protein. Interestingly, mutations in an enzyme involved in palmitoylation, namely palmitoyl-protein thioesterase (PPT), are associated with neurodegeneration and a role for PPT in the development and maintenance of cortical neurons has been established (Suopanki et al., 1999; Vesa et al., 1995). Intriguingly, in the context of AD, it is interesting to note that BACE1 undergoes palmitoylation, and it has been speculated that this modification serves primarily to direct the protein to discrete membrane microdomains (Benjannet et al., 2001). Furthermore, it has been reported that alterations in the level of specific isoprenoids are observed in AD brain compared to age-matched control brain (Edlund et al., 1994). Recently, while the combined data have led to somewhat confusing conclusions, a handful of reports have underscored an emerging role for the isoprenoid-mediated regulation of key processes implicated in AD pathogenesis, including APP metabolism, glial activation, tau phosphorylation and synaptic plasticity (Fig. 5).

Isoprenoids and APP metabolism

Isoprenylated Rho GTPases exert many cellular effects via activation of protein kinases such as ROCK. Interestingly, Zhou et al. (2003) have implicated the Rho/ROCK pathway in the modulation of APP metabolism. Addition of exogenous GGPP or FPP specifically increases the functional activity of small GTPases and it was demonstrated that stimulation of the RhoA/ROCK pathway following GGPP supplementation, but not FPP addition, facilitated a specific increase in the secretion of Aβ42. Conversely, inhibition of RhoA/ROCK signaling using ROCK inhibitor Y-27632 was coupled to a specific decrease in Aβ42 levels. Importantly, in vivo, Y-27632 preferentially lowered cortical Aβ42 load by 33% and reduced the Aβ42/Aβ total ratio. Thus, the Rho/ROCK pathway is involved in the regulation of APP metabolism. Furthermore, it was proposed that the observed effects of the Rho/ROCK signaling pathway on Aβ42 secretion were mediated by modification of γ-secretase cleavage specificity.

Reports have recently suggested that γ-secretase activity is predominantly localized to lipid rafts, membrane microdomains rich in sphingolipids and cholesterol (Urano et al., 2005; Vetrivel et al., 2004, 2005). While the cellular mechanisms underlying γ-secretase cleavage modulation by the Rho/ROCK pathway remain to be determined, it is interesting to note that a very recent report implicated a role for GGPP in the association of the active γ-secretase complex with lipid rafts (Urano et al., 2005).
In many instances, an increase in non-amyloidogenic APP metabolism is coupled to a reciprocal decrease in the amyloidogenic processing pathway, as the \( \alpha \)- and \( \beta \)-secretase moieties compete for APP substrate (Skovronsky et al., 2000; Vassar et al., 1999). Indeed, in vitro reports suggest that the moderate reduction in cholesterol levels following statin treatment facilitates increased expression of the \( \alpha \)-secretase, ADAM10, thus leading to enhanced APP\( \alpha \)-secretase release and decreased APP\( \beta \) secretion (Cole et al., 2005; Kojro et al., 2001).

While stimulation and inhibition of the Rho/ROCK pathway specifically modulated cleavage by \( \gamma \)-secretase and thus increased and decreased APP\( \beta \)-42 secretion, respectively, the secretion of APP was unchanged following treatment with either GGPP or Y-27632 (Zhou et al., 2003). However, Pedrini et al. (2005) have recently reported that statin-induced inhibition of the Rho/ROCK pathway facilitates dose-dependent increases in APP\( \alpha \)-secretion. In addition to supporting the initial observation that the Rho/ROCK pathway modulates APP metabolism, this report also indicates that, at least in vitro, statins may mediate non-amyloidogenic APP processing through mechanisms other than those dependent on reductions in cellular cholesterol.

Using mouse neuroblastoma N2a cells expressing APP carrying the FAD Swedish mutation (APP\( \text{sw} \)), Pedrini and colleagues proposed that statin-induced APP\( \alpha \)-secretion involves inhibition of an isoprenoid-mediated protein phosphorylation event. Activation of ROCK by isoprenylated Rho proteins leads to the phosphorylation of specific target proteins. Consequently, statin-induced inhibition of isoprenoid synthesis can potentially modulate ROCK1 activity via alterations in the amount of membrane-associated, activated Rho. Consistent with this notion, a statin-induced dose-dependent increase in APP\( \alpha \)-secretion was observed, that appeared dependent on inhibition of isoprenoid synthesis. To examine the potential involvement of changes in ROCK1 activity following statin treatment, the effects of dominant negative (DN) and constitutively active (CA) ROCK1 mutant expression on APP\( \alpha \)-secretion were examined. Indeed, both DN ROCK1, and, in a separate study, an inhibitor of farnesyl transferase, the enzyme mediating Rho modification by FPP, increased APP\( \alpha \)-secretion. Conversely, CA ROCK1 was able to reduce statin-induced APP\( \alpha \)-secretion. Hence, it was suggested that a reciprocal relationship exists between isoprenoid-mediated Rho/ROCK signaling and APP\( \alpha \)-secretion, where ROCK1 activation blocks APP\( \alpha \)-secretion and conversely, ROCK1 inhibition enhances APP\( \alpha \)-secretion (Pedrini et al., 2005).

These data delineate a relationship between Rho/ROCK signaling and statin effects on \( \alpha \)-secretase-mediated APP metabolism. While the use of standard experimental dose statin reduces cholesterol levels by up to 67% in N2a cells, the effects of cholesterol modulation in mediating the statin-induced increase in APP\( \alpha \)-secretion were not closely examined in this cell line (Pedrini et al., 2005). In fact, during overexpression of CA ROCK, which in accordance with the above proposal, should negate statin-induced APP\( \alpha \)-secretion, statin treatment still elevated APP\( \alpha \)-secretase levels above those observed in control cells, suggestive that at least part of the observed statin-mediated increase in APP\( \alpha \)-secretion was related to factors other than ROCK inhibition. In addition, any inhibition of the isoprenoid pathway that facilitates enhanced \( \alpha \)-secretase APP processing may favor a beneficial decrease in APP\( \beta \) production either by directing APP from the \( \beta \)- to the \( \alpha \)-processing pathway or by a direct effect on \( \beta \)-secretase. While the effects of farnesylation and ROCK activity on APP\( \beta \) levels and amyloidogenic APP processing in N2a cells remain to be established, data from our laboratory indicate a role for isoprenoids in mediating this amyloidogenic cleavage event (Cole et al., 2005).

Previous studies have indicated that statin-induced blockade of isoprenoid-synthesis may be abrogated by adding low concentrations of either mevalonate or a specific isoprenoid to the culture medium during statin treatment (Brown and Goldstein, 1980; Fassbender et al., 2001; Goldstein and Brown, 1990; Keller and Simons, 1998; Simons et al., 1998). Under these conditions, cholesterol production remains low, whereas isoprenoid function is rescued. By exploiting this, total cellular cholesterol and isoprenoid levels were manipulated independently of one another during exposure of HEK293-APP\( \text{sw} \) cells to statin. Consequently, it was demonstrated that low cholesterol and low isoprenoid levels have opposing effects on APP metabolism and APP\( \beta \) genesis. In addition, evidence was provided for the existence of two cellular APP\( \beta \) pools that behave independently of one another, an intracellular pool regulated by isoprenoids and a secreted pool influenced by cholesterol (Cole et al., 2005).

In agreement with Pedrini and co-workers, a statin-mediated increase in APP\( \alpha \) and C83 levels was observed. However, these non-amyloidogenic statin effects were consistent with earlier studies, being mediated by low cellular cholesterol levels that favored the \( \alpha \)-secretase pathway and decreased APP\( \beta \) secretion within the endocytic pathway (Fassbender et al., 2001; Kojro et al., 2001; Simons et al., 1998). Conversely, in this system, low isoprenoid levels did not appear to affect either APP\( \alpha \) or APP\( \beta \) secretion.
secretion, as may have been predicted from the prior studies, but rather resulted in the accumulation of Aβ, likely within biosynthetic compartments (Cole et al., 2005). Importantly, these results provide the first evidence that isoprenylation is involved in determining levels of intracellular Aβ.

A current question in AD concerns the mechanism and extent to which intracellular versus extracellular Aβ contributes to Aβ. While the relationship of intracellular Aβ to AD remains uncertain, there is growing evidence to suggest that this form of Aβ is involved in some of the early stages of AD development (Billings et al., 2005; Busciglio et al., 2002; Cataldo et al., 2004; Gouras et al., 2000; LaFerla et al., 1995; Magrane et al., 2004; Mori et al., 2002; Skovronsny et al., 1998; Takahashi et al., 2002, 2004).

The accumulation of intracellular Aβ under low isoprenoid conditions was associated with a corresponding increase in intracellular APP holoprotein and β-secretase cleavage products, APPβ and C99. Furthermore, the levels of BACE1 protein increased in neuronal cultures following statin treatment. The dependence of APP and amyloidogenic fragment accumulation on inhibition of isoprenoid synthesis was established by the demonstration that these effects were fully rescued with either mevalonate or GGPP supplementation, suggesting the involvement of geranylgeranylated target proteins. Our results indicated that low isoprenoid levels inhibit the trafficking of APP through the secretory pathway (Cole et al., 2005). While further studies are required, we speculate that, given the relationship that exists between APP metabolism and small GTPases, the statin-induced inhibition of prenylation could reduce APP trafficking and intracellular localization of APP. Under specific conditions, reduced APP transport through the secretory pathway would lead to elevated levels of APP in biosynthetic compartments such as the endoplasmic reticulum and trans-Golgi network (TGN) and could account for our observation that immature APP levels were increased following statin exposure. The TGN is a major site of BACE1 intracellular localization and accumulation of APP and BACE1 in the TGN would raise rates of enzyme-substrate interaction and subsequent β-secretase APP cleavage, thus increasing APPβ and C99 levels. Furthermore, the localization of the γ-secretase complex to the TGN could lead to increased conversion of C99 into Aβ and thus the accumulation of intracellular Aβ. Given the observed increases in APP holoprotein and corresponding increase in intracellular Aβ levels following statin treatment, it is interesting to note that a statin-induced two-fold increase in APP holoprotein was observed by Pedrini et al. However, whether the observed increase in APPβ secretion is separable from altered Aβ production in the N2a cells remains to be determined.

These recent studies underscore a putative role for prenylation in the regulation of APP metabolism and, under specific circumstances, alterations in isoprenoid levels may differentially affect the activities of all three secretases. On the one hand, low FPP levels appear to enhance α-secretase APP cleavage events and elevate APPα secretion (Pedrini et al., 2005), whereas on the other hand, low GGPP levels facilitate an increase in intracellular Aβ via a β-secretase-mediated mechanism (Cole et al., 2005) and affect the specificity of γ-secretase cleavage to modulate Aβ42 secretion (Zhou et al., 2003).

Geranylgeranylation and ApoE

In addition to the putative direct role protein prenylation may play in APP metabolism, this post-translational modification may also affect Aβ-associated pathology indirectly, through an association with ApoE. Polymorphisms in ApoE are well-described risk factors in SAD. At a molecular level, the association between ApoE and increased AD risk remains to be explicitly determined although it has been reported that ApoE is essential for two centrally important factors in plaque formation, namely Aβ fibrilization and deposition (Bales et al., 1999; Dolev and Michaelson, 2004). Interestingly, Naidu and co-workers reported that statin treatment of primary mixed glial cultures and organotypic hippocampal slices in vitro resulted in decreased ApoE secretion. Specifically, the use of protein prenylation inhibitors indicated that ApoE secretion requires protein geranylgeranylation (Naidu et al., 2002).

However, the relationship between ApoE and AD pathophysiology appears complex. Other than amyloid pathology, AD brain exhibits several characteristic hallmarks, including glial activation together with classic features of immune response including increases in pro-inflammatory cytokines. Both ApoE and activated glia are found associated with amyloid plaques in AD brain (Arelin et al., 2002) and recently, a dual role for ApoE in neuro-inflammation has been reported. In vitro, Aβ stimulation of glial apoe appears to limit neuroinflammation but overproduction of apoe by activated glia was associated with exacerbated inflammation (Guo et al., 2004). Therefore, whether decreased protein prenylation could reduce ApoE secretion in vivo and whether such an event would have positive, negative or incidental effects on AD progression remain to be seen.

Isoprenoids and glia

In addition to the amyloid studies, several studies have used statins to investigate the effect of altering protein prenylation on other processes involved in AD pathophysiology. Chronically activated glia associated with amyloid plaques in vivo may contribute to neuronal dysfunction via the generation of inflammatory mediators. Indeed, Aβ is sufficient to induce glial activation and promote the generation of inflammatory mediators. Consistent with the concept that neuroinflammation is detrimental in AD is the observation that epidemiological studies indicate that some non-steroidal anti-inflammatory drugs (NSAID) may reduce AD risk and slow progression (Anthony et al., 2000; in ‘t Veld et al., 1998; reviewed in Schenk and Yednock, 2002). However, whether the potential therapeutic benefit is associated with NSAID anti-inflammatory effects or, as recently reported, is due to their ability to reduce Aβ42 levels in the brain, remains to be determined. Indeed, Zhou et al. (2003) demonstrated that a subset of NSAIDs can reduce Aβ42 levels via inhibition of Rho activity. Conversely, accumulating evidence suggests that microglial activation may enhance amyloid removal and thus retard the development of AD symptomatology (Brazil et al., 2000; reviewed in Schenk and Yednock, 2002). Therefore, the specific question as to whether activated microglia make positive or negative contributions to disease progression remains unresolved.

Recently, a putative role for protein prenylation in glial activation has been reported although divergent data make for another confusing situation. Bi et al. (2004) reported that statin treatment of hippocampal slice preparations elicited microglial activation through suppression of the mevalonate pathway and inhibition of target protein geranylgeranylation. This activation was associated with both morphological- and gene-related
changes. Statin treatment caused Rho to dissociate from membranes in this preparation and it was hypothesized that disruption of tonically active pathways driven by Rho proteins is the most likely route whereby statin inhibition of the mevalonate pathway results in microglial activation. Interestingly, statin treatment, under conditions that led to the accumulation of APP holoprotein and amyloidogenic fragments, was dose-dependently coupled to significant alterations in astrocyte morphology, leading to an activated-appearing phenotype (Cole et al., 2005). Although the activation status of the astrocyte population was not examined closely, it was speculated that the observed effects were dependent on reductions in Rho GTPase activity, due to statin-induced decreases in isoprenoid availability, and that Rho GTPases may play a role in the cytoarchitectural changes that occur upon astrocyte activation. Indeed, a role for isoprenoids in the production of inflammatory mediators is not without precedent. A previous study indicated that statin treatment stimulates the production of TNF-α in macrophages via the blockage of GGPP and Rho GTPase membrane attachment (Monick et al., 2003).

Conversely, Cordle and Landreth indicated that statin treatment of microglia and monocyte cultures suppressed the Aβ-stimulated expression of interleukin1-β and inducible nitric oxide synthase and reduced the production of nitric oxide. These anti-inflammatory statin actions were attenuated by addition of either mevalonate or GGPP and were similar in nature to effects observed following inhibition of Rho family function (Cordle and Landreth, 2005). Indeed, Cordle and colleagues recently proposed that statin-induced isoprenoid inhibition prevents both the translocation of Rho GTPases to the plasma membrane and their interaction with the negative regulator, Rho-GDI. These effects culminate in the functional inhibition of Rho-family GTPases and thus a mechanism by which statins attenuate Aβ-stimulated inflammation was proposed (Cordle et al., 2005). Thus, under specific experimental conditions, a statin-mediated reduction in the availability of GGPP and a decrease in the amount of available, active Rho is associated with both pro- and anti-inflammatory effects.

Isoprenoids and tau

Currently, little is known about the existence of any relationship between tau pathology and protein prenylation. However, in a single report, Meske et al. (2003) recently demonstrated that statin-induced inhibition of GGPP and inhibition of Rho-GTPase modification by GGPP in cultured neurons evoked changes in tau phosphorylation that were characteristic of those observed in the preclinical stages of AD. Indeed, following statin treatment, the neuritic network was affected and eventually destroyed in a process marked by alterations in the microfilament and microtubule system that was independent of the execution phase of apoptosis, although prolonged suppression of GGPP synthesis under experimental conditions eventually induced neuronal death. Thus, in addition to the putative role of protein prenylation in APP metabolism and glial activation, this post-translational modification may also be involved in mediating the state of tau phosphorylation, at least in vitro.

Isoprenylation and synaptic plasticity

As previously indicated, specific GTPases play a role in synaptic plasticity and alterations in this plasticity are early events in AD pathophysiology (reviewed in Arendt, 2004). Given the fact that activation of specific GTPases depends somewhat on their prenylation status, it appears likely that protein prenylation is, at least, indirectly involved in mediating synaptic plasticity, although reports describing this are somewhat lacking from the literature. However, a molecular study of the neuromuscular junction (NMJ) by Luo et al. (2003) has provided evidence for an important role of protein prenylation in regulating synapse formation and/or maintenance, both in vitro and in vivo.

Efficient neuronal communication requires the enrichment of synaptic proteins at synapses and during synapse formation, neurotransmitter receptors cluster at the postsynaptic membrane. Agrin, a molecule released from the terminals of motor neurons, induces acetylcholine receptor (AchR) clustering. Along with a number of other molecules, Rho GTPase activation is required for this clustering at the NMJ and inhibition of Cdc42 or Rac1 blocks AchR clustering. Luo et al. reported that the activity of geranylgeranyltransferase I (GGT), the enzyme that catalyzes the geranylgeranylation of specific Rho GTPases, is modulated via Agrin stimulation. In response to Agrin, the phosphorylation and activation of GGT increase. Indeed, inhibition of GGT expression or function reduced Agrin-Rho GTPase activation, and prevented AchR clustering and the formation of the NMJ in vitro. This relationship was delineated further, with the observation that transgenic mice, impaired in GGT activity, demonstrated neuromuscular defects. These results suggested that the activity of Rho GTPase is regulated by GGT upon Agrin stimulation. While the importance of prenylation in synaptic function was underscored in this study, the role of prenylation and Rho GTPase regulation in other aspects of neural development and maintenance, including in the diseased state, remain the subject of further investigation.

Statins as pharmacological tools

Several of the reports discussed above originated from examining the effect of statin treatment on key cellular events implicated in AD pathophysiology. However, it is of critical importance to consider that these studies likely do not provide insights into the cellular mechanisms underlying the putative clinical benefits of this drug class.

Detectable statin concentrations in the cerebrospinal fluid are significantly lower than those detected in the plasma (0.9–1.4 ng/ml; Botti et al., 1991) and such findings suggest that only a partial inhibition of HMG CoA reductase could occur within neurons (IC50 for hepatocytes 2.7 ng/ml; Haley and Dietschy, 2000). According to the flux diversion hypothesis, following a decrease in the activity of HMG CoA reductase, the enzymes regulating the penultimate step in cholesterol biosynthesis remain saturated with substrate (Faust et al., 1980; Grabowska et al., 1998; reviewed in Scheffler, 1999). Thus, in the presence of low statin concentrations, such as those detected in the cerebrospinal fluid, the levels of the isoprenoid intermediates remain unchanged although cholesterol levels are reduced. Therefore, while it is plausible that statin levels within the brain could influence cerebral cholesterol levels to a small extent, it is highly unlikely that they are sufficient to inhibit isoprenoid synthesis within the CNS.

Nevertheless, the use of statins as pharmacological tools in such studies has proved invaluable for implicating a novel role for protein prenylation in several AD-related processes, including APP
metabolism (Cole et al., 2005; Pedrini et al., 2005; Zhou et al., 2003), glial activation (Bi et al., 2004; Cordle and Landreth, 2005; Cordle et al., 2005) and tau phosphorylation (Meske et al., 2003). However, investigating isoprenoid function via statin treatment in an experimental setting is both complex to perform and analyze.

In addition to affecting cellular cholesterol and isoprenoid levels, statins exert multiple other cellular effects including alterations in intracellular cholesterol distribution, modulation of gene expression and changes in proteasomal activity (Kumar and Reynolds, 2005; Kumar et al., 2002; Rao et al., 1999). Thus, careful experimental design is required to examine isoprenoid-specific effects independently from other statin-mediated effects. Furthermore, the nature of the interwoven feedback pathways that exist within the mevalonate pathway, such as the fine-tuning of the isoprenoid-target protein(s) exerting the observed effects in all of the GTPase composition, may account for some of these conflicting reports. Indeed, during certain cellular events, the requirement, regulation and activation of certain GTPases is cell type-specific and effector molecules specific to particular cell types have recently been identified (Collier et al., 2004). It can be speculated that the selective interactions of GTPases with various effectors may have important biological consequences depending on the specific properties and functions of the different effectors as well as the patterns of expression of various GTPases and effectors (and associated isoforms) in different cell types.

Factors that affect the extent of cholesterol and isoprenoid synthesis inhibition, such as statin type, concentration and exposure time, and differences in cell type, that may exhibit differences not only in the proteolytic processing of APP but also in GTPase composition, may account for some of these conflicting reports. Indeed, during certain cellular events, the requirement, regulation and activation of certain GTPases is cell type-specific and effector molecules specific to particular cell types have recently been identified (Collier et al., 2004). It can be speculated that the selective interactions of GTPases with various effectors may have important biological consequences depending on the specific properties and functions of the different effectors as well as the patterns of expression of various GTPases and effectors (and associated isoforms) in different cell types.

Cross-comparison of published data sets is complicated by the fact that studies, specifically those examining APP metabolism, have ultimately either focused on different secretase activities or different Aβ forms. Indeed, some of the data generated from several studies are discordant. Under specific experimental conditions, a reduction in isoprenoid levels appears to mediate divergent effects on both the non-amyloidogenic and amyloidogenic pathways (Cole et al., 2005; Pedrini et al., 2005), in addition to affecting both pro- and anti-inflammatory states (Bi et al., 2004; Cordle and Landreth, 2005). Some of these disparities may have arisen from differences in experimental design.

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These preliminary studies serve to outline the novel relationship that exists between the isoprenoids and markers of AD pathophysiology, and while Pedrini and colleagues identified ROCK1 as a potential downstream target of statins in mediating the increase in non-amyloidogenic APP processing, the precise primary isoprenylated target protein(s) exerting the observed effects in all of the statin-studies remain to be identified. Given the complexity of GTPase biology, the task of identifying such a target may not be a simple one. For example, the increase in APPs hiding following statin treatment in N2a cells was apparently mediated by both isoprenoid moieties, although it is predominately farnesylated in vivo (Solski et al., 2002). However, it has been reported that farnesylated RhoB is principally localized to endosomal compartments (Adamson et al., 1992a,b). Thus, the farnesylated Rho protein responsible for mediating the Rho/ROCK-dependent increase in APPs h secretion remains elusive.

A final point worthy of consideration is the maturity of the cell population being analyzed. It has been recently established that the intracellular distribution of Rho GTPases shifts during hippocampal neuron development. During the time of axon and dendrite sprouting, RhoA, Rac1 and cdc42 appear to be evenly distributed whereas in the mature neuron, distribution is more polarized with Rac1 in axons, RhoA enriching in dendrites and cdc42 being equally abundant in both domains (Santos Da Silva et al., 2004). Given that the amyloidogenic and non-amyloidogenic APP cleavage events occur in specific cellular localities, changes in the subcellular distribution of any molecule involved in these events could potentially have an impact on the outcome of APP metabolism.

**GTPase-independent isoprenoid effects**

In the studies described above, the identified actions of FPP or GGPP have been initially attributed to their role in prenylation, where changes in small GTPase farnesylation or geranylgeranylation have been linked to alterations in APP metabolism, glia activation, tau phosphorylation and synaptic plasticity. However, analysis of isoprenoid-mediated effects may be more complex than initially suspected.

In agreement with the findings of Zhou et al., Kukar et al. (2005) have recently reported that GGPP and FPP can increase the levels of secreted Aβ42 and decrease Aβ38 levels, indicating that they may have physiological relevance. However, most importantly, this study excluded protein prenylation as a mechanism, indicating instead that these endogenous isoprenoids can act directly on the γ-secretase complex. Whether similar isoprenoid-like compounds exert similar effects remains a possibility that requires further investigation.

To date, the physiological relevance of APP metabolism and Aβ production is unknown, and it may be speculated that different Aβ isoforms have specific biological functions. Therefore, naturally occurring isoprenoids may act as endogenous modulators of Aβ isoforms by acting on the γ-secretase complex to adjust the Aβ population to suit different physiological requirements. Whether GGPP and/or FPP exert GTPase independent effects on α- or β-secretase remains to be determined.

The cellular effects of the isoprenoids are obviously highly complex. One layer of complexity stems from the functioning of different GTPases in diverging cellular processes, as discussed previously. Indeed, in the case of glial activation, Bi et al. (2004) speculated that different members of the Rho GTPase family initiated different signaling cascades, with pathways leading to cytoskeletal versus gene regulation, diverging at the initial part of the isoprenylation step.

Further complexity is added to the situation as it appears highly likely that the effects of APP and Aβ generation are multi-faceted. In contrast to the effects of low isoprenoid concentrations, which appear to facilitate intracellular Aβ accumulation possibly via reduced GTPase geranylgeranylation, Kukar et al. (2005) reported that increasing cellular isoprenoid levels modulated the γ-secretase complex and were associated with increased Aβ42 secretion. Therefore, it may be hypothesized that isoprenoids might function in separate pathways, in a concentration-dependent manner: at low concentrations, isoprenoids may primarily affect the activity of...
small GTPases that in turn influence Aβ trafficking, while at high concentrations, they may directly modulate the γ-secretase complex to alter Aβ isomeric balance.

Concluding remarks

Whether or not statins will offer therapeutic benefit for AD remains to be determined by the outcome of large-scale, long-term, placebo-controlled, randomized clinical trials. However, their use as pharmacological tools in vitro has recently proved invaluable in delineating a novel role for isoprenoid moieties in the regulation of key processes central to AD neuropathology. These studies not only offer additional insight into AD pathogenesis but may also provide the basis for further research into potential new drug targets.

The studies reviewed here have indicated a wide range of cellular isoprenoid activities that appear relevant to AD pathogenesis. Not only do isoprenoid lipids appear to regulate the activities of α-, β-, and γ-secretases during APP metabolism, but isoprenoid-dependent roles have also been proposed in the modulation of glial activation, tau phosphorylation and synaptic plasticity. Furthermore, alterations in cell cycle control have been linked with the etiology of AD and oxidative stress is a key factor involved in AD development. Intriguingly, it is noteworthy that isoprenoids not only exert control over the cell cycle (Fuse et al., 2004; Nishimura et al., 1999; Nishimura et al., 1997; Terano et al., 1998) but a recent, novel finding has indicated the inhibition of oxidative DNA damage by GGPP (Ling et al., 2004). These isoprenoid-mediated effects remain to be examined in the context of AD pathogenesis.

With the exception of Zhou and colleagues, the relationship between isoprenoid-mediated pathways and AD-related cellular events was delineated in vitro, and discrepancies between data currently make for a confusing situation. Under specific conditions, high isoprenoid levels have been implicated in contributing to AD pathogenesis, whereas other reports indicate that low isoprenoid concentrations may be involved in processes with uncertain relationships to AD pathogenesis, such as the intracellular accumulation of Aβ and microglial activation. In the absence of more detailed studies, the cellular mechanisms underlying these putative effects remain ambiguous although it is clear that the biological functions of isoprenoid moieties extend beyond their role in protein prenylation. Clearly, there is a demand for further experimental studies to confirm these original observations and to determine the precise cellular mechanisms underlying the observed effects.

To date, our knowledge of isoprenoid biology in the CNS is scarce and the relative concentrations of FPP and GGPP within the brain remain unknown. While in vitro studies provide the preliminary delineation of a putative relationship between isoprenoids and AD, the extent to which these initial findings represent true in vivo events remains largely undefined.

Regardless of the cellular mechanism, the fundamental question of whether changes in isoprenoid levels could impact central APP processing, Aβ synthesis and AD neuropathology should be addressed. Thus, carefully designed in vivo experiments involving the supplementation of diet with either dietary isoprenoids or inhibitors of isoprenoid activities, for prolonged periods should prove to be most informative. Analysis of the effect of such dietary supplementation on brain neuropathology will provide a more accurate analysis of the putative role of isoprenoids in contributing to the pathogenesis of AD.


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