Opinion

What are lipoproteins doing in the brain?

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Lipoproteins in plasma transport lipids between tissues, however, only high-density lipoproteins (HDL) appear to traverse the blood–brain barrier (BBB); thus, lipoproteins found in the brain must be produced within the central nervous system. Apolipoproteins E (ApoE) and ApoJ are the most abundant apolipoproteins in the brain, are mostly synthesized by astrocytes, and are found on HDL. In the hippocampus and other brain regions, lipoproteins help to regulate neurobehavioral functions by processes that are lipoprotein receptor-mediated. Moreover, lipoproteins and their receptors also have roles in the regulation of body weight and energy balance, acting through lipoprotein lipase (LPL) and the low-density lipoprotein (LDL) receptor-related protein (LRP). Thus, understanding lipoproteins and their metabolism in the brain provides a new opportunity with potential therapeutic relevance.

Lipoprotein metabolism in the systemic circulation

Plasma lipoproteins contain lipids and proteins, and transport lipids in a polar environment. Four major classes of lipids are found in lipoproteins: triacylglycerols (TGs), unesterified cholesterol, cholesteryl esters (CE), and phospholipids. Being the most polar, phospholipids are associated with apolipoproteins and surround the less polar lipids. Apolipoproteins serve as enzyme cofactors and receptor ligands. Lipid delivery by lipoproteins and their processing are regulated by lipoprotein receptors located on cell surfaces such as the LDL receptor (LDLR) and LRP, as well as other proteins associating with lipoproteins, for example lipoprotein-associated phospholipase A2 ($LpPLA_2$) and serum amyloid A (SAA). Recent evidence also suggests that circulating extracellular microRNAs (miRNAs) are associated with lipoproteins for their transfer between cells [1].

Lipoproteins are traditionally classified by density. The larger lipoprotein particles – chylomicrons and very low density lipoproteins (VLDL) are the TG-rich lipoproteins produced by the intestine and liver. LDLs are predominantly formed by the hydrolysis of TG-rich lipoproteins by LPL. HDLs are also secreted by the intestine and liver as nascent ApoA-I/phospholipid discs which incorporate cholesterol through the ATP-binding cassette transporter proteins. HDL increases in lipid content and size when TG-rich lipoproteins are processed by LPL, and facilitates reverse cholesterol transport for biliary excretion. Lipoprotein metabolism has been well characterized in plasma, but much less is known about lipoproteins in the brain.

Major differences between lipoproteins in plasma versus in the brain

Early studies of lipoproteins in brain focused on particles in the cerebrospinal fluid (CSF) [2–4]. Using gel electrophoresis and electron microscopy, CSF lipoproteins were shown to be mostly spherical, resembling the size and density of plasma HDL [4]. Unlike plasma, the most abundant apolipoprotein in CSF lipoproteins is ApoE, which is usually localized to the largest particles [3]. ApoA-I and ApoA-II are present on smaller particles, and ApoJ is distributed across the particle size-range. Other apolipoproteins such as ApoA-IV, ApoD, and ApoH are also found in the CSF. In addition, some CSF lipoproteins are found to be associated with amyloid beta (Aβ, suggesting a role for lipoproteins in Aβ polymerization, transport, and clearance [2]. Another source of lipoproteins in the CSF is the choroid plexus. For example, ApoB-containing lipoproteins, which can be identified in CSF, are found at a concentration consistent with porous diffusion enhanced by CSF secretion [5,6].

Despite the existence of the BBB, some of the smaller circulating HDL lipoproteins can enter the brain [2,4]. However, when mice are injected with adenovirus expressing human ApoE isoform 3 (ApoE3), ApoE3 protein is found in plasma lipoproteins at high levels but remains undetectable in CSF [7]. In brain, ApoE is expressed predominantly by astrocytes and microglia, and in reduced quantity in neurons, whereas ApoJ is expressed in astrocytes, neurons, and the ependymal cells lining the ventricle [8]. The majority of the ApoE- and ApoJ-containing lipoproteins found in CSF are thought to originate from surrounding astrocytes. Cholesterol is the most-studied lipid in the brain. In the brain, the BBB necessitates that cholesterol homeostasis be maintained by local synthesis, and this process has been studied in detail in mice [9]. Cholesterol transporter Niemann–Pick type C protein 1 and cholesterol 24(S)-hydroxylase are essential for cholesterol metabolism in the brain, although changes in the plasma cholesterol concentration or loss of function of ATP-binding cassette AI transporter (ABCA1), scavenger receptor class B, type I (SR-B1, also known as Scarb1), LDLR or ApoE, or ApoA-I have no effect

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on sterol turnover in the brain [9]. Overall, data strongly suggest that during early development, cholesterol originates entirely from local synthesis, but in the adult there is a constant excretion of sterol from the brain into the plasma [9].

Lipoprotein synthesis, assembly, and metabolism in astrocytes and other cell types in the brain

Astrocytes are specialized star-shaped glial cells that surround and support neurons to provide essential nutrients for neuronal growth and function. Astrocytes are also considered to be the major sites for synthesis of lipoprotein constituents and lipoprotein assembly in the brain. However, recent evidence suggests that, at least for cholesterol, astrocytes and neurons cooperate in the regulation of its synthesis and redistribution in the brain [10]. Specifically, selected enzymatic steps and precursors in the biosynthesis of cholesterol differ in cultured astrocytes versus neurons. In addition, different mechanisms appear to regulate cholesterol efflux from neurons and astrocytes, reflecting the different roles these cell types play in brain cholesterol homeostasis. For example, astrocytes produce and release ApoE, whereas neurons metabolize cholesterol to 24(S)-hydroxycholesterol. Cholesterol efflux from astrocytes is facilitated by apolipoproteins alone or lipoprotein particles, whereas cholesterol removal from neurons is triggered only by lipoprotein particles. ABCA1- and ABCG1-regulated cholesterol efflux occurs only in astrocytes whereas ABCG4-mediated cholesterol efflux takes place only in neurons [11]. Furthermore, the newly synthesized cholesterol is rarely converted to CE, and is quickly redistributed among various cell types within the brain (reviewed in [10]). Moreover, the half-life of brain-derived cholesterol is much longer (up to 5 years) compared to that of days in the periphery, with extensive redistribution and transportation via ATP-binding cassette transporters and HDL-like lipoproteins respectively, as the main mechanism to maintain homeostasis.

Apolipoproteins are expressed at higher levels in astrocytes than the rest of the brain. The mRNA and protein levels of ApoE and ApoJ are age-dependent – with ApoJ increasing ~5–10-fold and ApoE levels dropping with aging [12]. ApoJ is a ubiquitous multifunctional glycoprotein and its expression in the brain is upregulated in response to neuronal damage, brain injury and other stress; and ApoJ has been proposed to play a role in Aβ clearing [13]. ApoJ-containing lipoprotein particles usually contain the least amount of lipid, whereas ApoE-containing lipoproteins carry the most lipid and are the largest in size [14].

The role of ApoE-containing lipoproteins in the brain has been extensively studied and reviewed. In brief, three major functions have been suggested for astrocyte-derived ApoE-containing lipoproteins: (i) the transfer of phospholipids and cholesterol via ATP-binding cassette (ABC) transporters such as ABCA1 and ABCG1 [8]; (ii) interaction with the LDLR superfamily of proteins located on the surface of neurons to facilitate axonal growth and neuronal survival [15]; and (iii) interaction with the LRP1-dependent cellular uptake pathway in the deposition of amyloid plaques [16,17]. Although there is minimal direct interaction between ApoE and soluble Aβ in CSF [18], ApoE isoforms in ApoE-containing lipoprotein complexes can regulate the metabolism of soluble Aβ by competing for the binding of LRP1 with Aβ in astrocytes [18,19].

ApoE knockout mice placed on a diet enriched in homocysteine to induce oxidative stress, show impaired learning and memory [20]. Of the three major isoforms of ApoE – ApoE2, ApoE3, and ApoE4 – ApoE4 confers the major risk for Alzheimer's disease (AD). The expression of the ApoE4 allele usually results in increased expression of ApoC1 [21]. Mice overexpressing human ApoC1 also display impaired learning and memory [22]. Interestingly $Apoc1^{-/-}$ mice also show impaired hippocampal-dependent memory with no gross changes in brain morphology or brain cholesterol levels, but increased expression of the proinflammatory marker tumor necrosis factor-α [23].

With all the evidence discussed above several major questions remain: (i) what is the role of neurons in the synthesis and regulation of lipoprotein metabolism? (ii) Can different lipoprotein particles enter neurons or be recognized by surface markers on neurons? (iii) What are the major functions of lipoproteins in the brain – is it simply lipid delivery and/or are they carriers for various biological molecules? Some of the answers to these questions may relate to the existence and properties of specific lipid structures in the brain, and the cell type- and region-specific expression of lipoprotein receptors in the brain.

Lipid rafts and neuronal porosomes

Lipid rafts are cholesterol-enriched domains in biomembranes that serve as the preferential clustering site of membrane signaling proteins. Lipid rafts exist in both neurons and astrocytes [24]. In addition to cholesterol and sphingolipids, saturated fatty acids are enriched in lipid rafts. In systemic circulation, lipid rafts serve as a signaling platform linking lipoprotein metabolism to atherosclerosis [25]. In neurodegenerative diseases, lipid raft disarrangement may be an early marker for diagnosis [26]. Size-exclusion chromatography and electron microscopy have been used to study the lipid composition of nascent HDL formed by ABCA1. The proportions of free cholesterol, glycerophosphocholine, and sphingomyelin are similar between nascent HDL and lipid rafts [27], suggesting a possible role of lipid rafts in lipoprotein assembly in addition to their role in facilitating lipoprotein-mediated lipid exchange.

Porosomes are universal secretory portals at the plasma membrane that facilitate the transient docking and fusion of membrane-bound secretory vesicles to exchange intravesicular contents to the outside [28]. In neurons, 12–17 nm cup-shaped porosome structures are present at the presynaptic membrane where 40–50 nm synaptic vesicles transiently dock and fuse to release neurotransmitters [29]. The neuronal porosome complex has been isolated, its composition determined, and it has been structurally and functionally reconstituted in artificial lipid membranes [30,31]. Cholesterol was found to be an integral component of the neuronal porosome complex, and crucial to the stability of the porosome/fusion pore [32,33]. Porosomes are also found in astrocytes and have a similar structure but are smaller in size (10–15 nm) [34,35]. Proteomic analysis of neuronal porosomes revealed the absence of