Mol Neurobiol (2013) 48:438–451
DOI 10.1007/s12035-013-8435-5

# Peroxisome Proliferator-activated Receptors and Alzheimer's Disease: Hitting the Blood–Brain Barrier

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Received: 11 January 2013 / Accepted: 26 February 2013 / Published online: 14 March 2013


**Abstract** The blood–brain barrier (BBB) is often affected in several neurodegenerative disorders, such as Alzheimer's disease (AD). Integrity and proper functionality of the neurovascular unit are recognized to be critical for maintenance of the BBB. Research has traditionally focused on structural integrity more than functionality, and BBB alteration has usually been explained more as a consequence than a cause. However, ongoing evidence suggests that at the early stages, the BBB of a diseased brain often shows distinct expression patterns of specific carriers such as members of the ATP-binding cassette (ABC) transport protein family, which alter BBB traffic. In AD, amyloid-β (Aβ) deposits are a pathological hallmark and, as recently highlighted by Cramer et al. (2012), Aβ clearance is quite fundamental and is a less studied approach. Current knowledge suggests that BBB traffic plays a more important role than previously believed and that pharmacological modulation of the BBB may offer new therapeutic alternatives for AD. Recent investigations carried out in our laboratory indicate that peroxisome proliferator-activated receptor (PPAR) agonists are able to prevent Aβ-induced neurotoxicity in hippocampal neurons and cognitive impairment in a double transgenic mouse model of AD. However, even when enough literature about PPAR agonists and neurodegenerative disorders is available, the problem of how they exert their functions and help to prevent and rescue Aβ-induced neurotoxicity is poorly understood. In this review, along with highlighting the main features of the BBB and its role in AD, we will discuss information regarding the modulation of BBB components, including the possible role of PPAR agonists as BBB traffic modulators.

**Keywords** Blood–brain barrier · Oxidative stress · Alzheimer's disease · PPARs

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## General Considerations

The concept of a blood–brain barrier (BBB) was proposed by Lewandoswsky in 1900 [1] and originates from the observation of the lack of pharmacological activity of several compounds when administered systemically into the blood but with a critical impact when injected directly into the cerebrospinal fluid (CSF) [1, 2]. Further experimental evidence of the BBB's existence was provided a few years later by Goldman [3, 4] who demonstrated that Trypan Blue dye neither stained the brain nor the spinal cord when administered through the bloodstream; however, it stained these structures readily when administered into the CSF. No functional explanation was agreed and while some scientists proposed the transport across cell membranes, others suggested that the absence of Trypan Blue staining was due to the dye's inability to cross the permeable membrane of brain capillaries [5, 6]. The controversy was solved later by Reese and Karnovsky [7] and subsequently by Brightman and Reese [8] who, through the use of hydrophilic compounds, particularly horseradish peroxidase, demonstrated that polar solutes were unable to cross the BBB because of occluding tight junctions (TJ) established between adjacent endothelial brain cells.

Three barriers are recognized as part of the brain's isolating system that allows for maintenance of brain homeostasis:

(1) the BBB, (2) the blood–CSF barrier, and (3) the arachnoid epithelium [9, 10]. The BBB formation begins during embryonic development as a consequence of the close relationship established between blood vessels and neuroectodermal cells [11]. From the subarachnoid space, the pial arteries, the main blood vessels of the brain, project the intracerebral arteries to the brain, which in turn, ramify to ultimately form the brain capillaries [12]. The neurovascular unit is considered to be the minimal functional unit of the BBB, and three cellular subtypes can be identified: (1) vascular, composed of endothelial cells, pericytes, and vascular smooth muscular cells; (2) glial, composed of astrocytes, microglia, and oligodendroglia; and (3) neurons [11, 12] (Fig. 1). When mature, the BBB is a highly specialized structure where brain capillaries comprise a single endothelial cell connected with itself and with neighbor cells through occluding TJ and through nonoccluding adherens junctions (AJ) [13, 14], each of these comprised of a set of specific proteins. This primary structure is surrounded by pericytes, which are in direct contact with astrocyte end-feet [11, 14, 15]. The BBB functions not only as a barrier limiting the entrance of several substances into the brain [14] but also as a permeable structure that is able to ensure oxygen

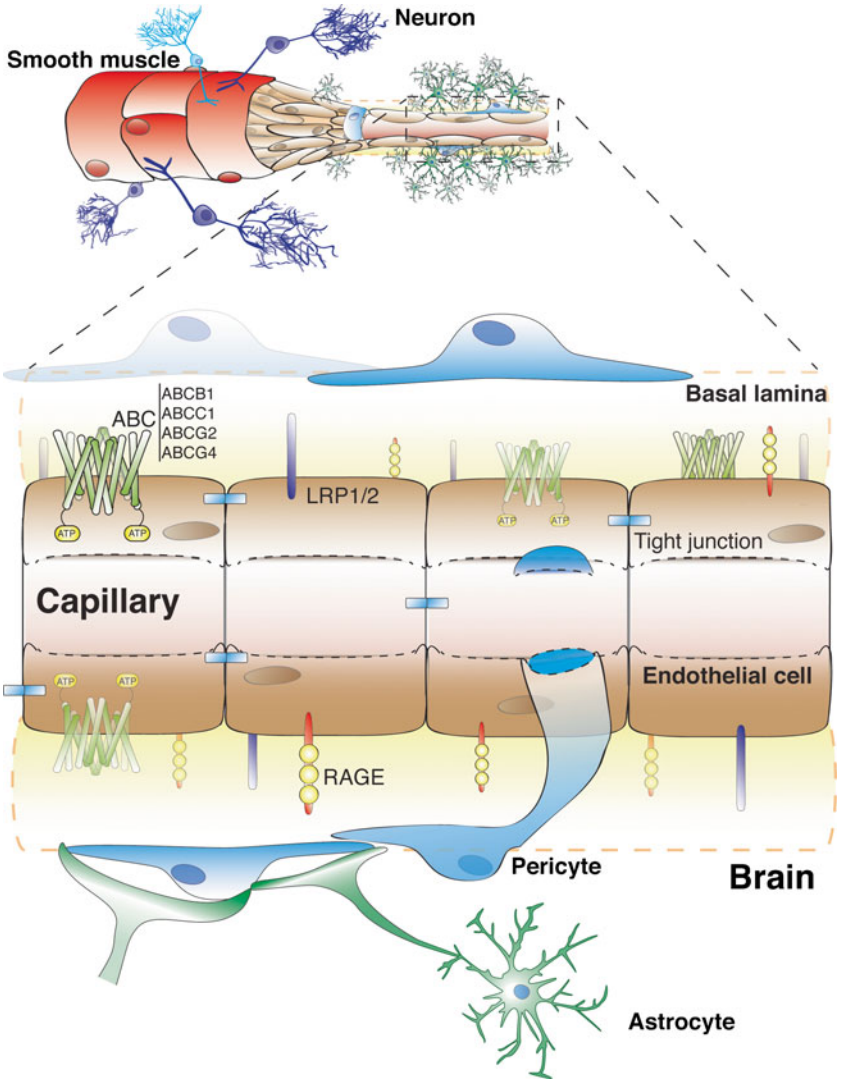


**Fig. 1** Main components of the BBB. The BBB is a complex structure composed of several cell types and characterized by distinct expression patterns of different transport and adhesion proteins. The combination of these components allows the BBB to exhibit low paracellular permeability and critically determinant cellular transport. The *diagram* represents a brief description of the structure of the BBB. At the brain capillaries, a monocellular layer of endothelial cells constitutes the basis of the barrier. Each endothelial cell encircles the lumen of the capillary and seals it, through establishment of TJ and AJ at the ends of the cell as well as with the adjacent cells. The endothelial wall is closely surrounded by pericytes, constituting an additional cellular layer of the BBB. Finally, astrocyte end-feet processes attach to the barrier forming an additional cellular envelope. Despite some of the most important BBB transport systems included in the diagram (*ABC* ATP-binding cassette, *LRP* low-density lipoprotein receptor-related protein, *RAGE* receptor for advanced glycation end products) shown only to be expressed by endothelial cells, several of these transporters are also expressed by the other cellular components of the BBB

and glucose delivery to brain tissue as well as removal of different metabolic end products, which helps to maintain brain homeostasis [16]. Additionally, correct functionality of the BBB is critical for neurons mainly because of the precise balance of ion gradients required to allow for electrical communication between them [11].

## Blood–Brain Barrier Carrier System

BBB traffic can be separated into a low permeable paracellular component, including the TJ and AJ, and a cellular component, composed mainly of endothelial cells expressing several specific carriers [17]. Only small lipid-soluble molecules are allowed to cross the BBB freely [15].

### Low Paracellular Permeability

The seal between endothelial cells depends on TJ and AJ, and forces molecules to undergo transcellular transport [16]. Physically, from the lumen of brain capillaries, TJ are the first intercellular seal followed by AJ, as a combined unit.

#### *Tight Junctions*

Molecular components of TJ (Table 1) can be divided into membrane and cytoplasmic proteins [16, 17]. Occludin [17, 18], claudins [17–19], the endothelial cell-selective adhesion molecules [20], and the junctional adhesion molecule-A [21, 22] constitutes the first group and are responsible for cell–cell anchorage. In the second group, acting as the scaffold proteins that link membrane proteins to the actin cytoskeleton [16, 17], we found the following: zonula occludens (ZO) protein-1, protein-2, and protein-3 [23–25], containing a PDZ domain; and non-PDZ proteins, such as cingulin [16] and the junction-associated coiled-coil protein (JACOP)/paracingulin [16, 26].

**Table 1** Main molecular components of tight and adherens junctions

| | |
|---|---|
| Tight junction | |
| | Claudins |
| | Occludins |
| | Junctional adhesion molecule A (JAM-A) |
| | Endothelial cell-selective adhesion molecule (ESMA) |
| | Zonula occludens |
| | Calcium-dependent serine protein kinase (CASK) |
| | Cingulin |
| | Multi-PDZ protein 1 (MUPP1) |
| | Membrane-associated guanylate kinase (MAGI) |
| Adherens junction | |
| | Vascular-endothelial cadherin (VE-cadherin) |
| | Platelet endothelial cell adhesion molecule (PECAM-1) |
| | Catenin (α, β, χ) |

#### *Adherens Junctions*

AJ are also responsible for cell–cell adhesion and critical functions have been described for it, such as contact inhibition during vascular growth and remodeling, cell polarity initiation, and being fundamental for TJ formation [16, 17]. The main proteins of AJ are VE-cadherin [27], an armadillo protein, and platelet endothelial cell adhesion molecule 1 (PECAM-1) and they are usually linked to the catenin protein family [17] (Table 1).

### Blood–Brain Barrier Transport

The transport across the BBB is highly specialized and depends on the expression of several transporters by the endothelial cells, which allow bidirectional traffic in order to maintain brain homeostasis (Table 2). Different authors have used diverse criteria to classify these transporters [16, 17, 28].

Glucose transporter 1 (GLUT1), monocarboxylate transporter 1 (MCT1), L1, and y+ amino acid transporters, excitatory acidic amino acid transporters (EAAT-1, EAAT-2, and EAAT-3), constitute the first group of transporters in charge of nutrients, several energy source molecules, lactate, and amino acids into and out of the brain [17, 28]. It is important to mention that EAATs determine the removal of glutamate from the brain, playing a critical role in the prevention of glutamate-induced excitotoxicity [29]. Another important group of BBB transporters is composed of the adenosine triphosphate (ATP)-binding cassette (ABC) efflux transporters, particularly ABCB1 (P-glycoprotein) [30]; the multidrug resistance proteins (MRP or ABCC), such as MRP-1, MRP-4, MRP-5, and MRP-6 [16]; and the breast cancer resistance protein (BCRP or ABCG2) [31]. Moreover, endothelial protein C receptor (EPCR), insulin receptor (IR), transferrin receptor (TFR), low-density lipoprotein receptor-related protein 1 (LRP1), peptide transport system (PTS-1, PTS-2, and PTS-3), and PTS4-vasopressin V1a receptor (V1AR) are specialized transporters for peptides [17, 28, 32, 33]. As mentioned, the electrical properties of brain networks are provided by a precise ion balance achieved and maintained through a whole range of ion transporters. The sodium pump ($Na^+/K^+$-ATPase), sodium–potassium–2 chloride ($Na^+/K^+/2Cl^-$), sodium–hydrogen exchanger ($Na^+/H^+$), sodium–calcium ($Na^+/Ca^{++}$), and chloride–bicarbonate exchanger ($Cl^-/HCO_3^-$) are the representative members of ion transporters present in the BBB [34–36].

Additionally, the BBB possesses enriched lipid microdomains (lipid rafts), composed of caveolae, which exert further

**Table 2** BBB transporter system

| | |
|---|---|
| GLUT1 | Glucose |
| MCT1 | Lactate |
| L1 | Essential amino acids |
| y+ | Cationic amino acids |
| XG− | Elimination of acidic amino acids |
| N | Elimination of nitrogen rich amino acids |
| ASC | Elimination of non-essential amio acids |
| LNAA | Elimination of essential amino acids |
| EAAT | Elimination of excitatory amino acids |
| N | Nitrogen-rich amino acids |
| Na+/K+/ATPase | Ions |
| Cl−/HCO3− | |
| Na+, K+/2Cl− | |
| H+/Na+ | |
| ATP-binding cassette (ABCB1, ABCC, ABCG2) | Peptides |
| Endothelial protein C receptor (EPCR) | |
| Insulin receptor (IR) | |
| Low-density lipoprotein receptor-related protein (LRP) | |
| Peptide transport system (PTS) | |

regulation of BBB traffic [37]. Furthermore, several receptors have been described to be associated with caveolar membranes, such as insulin and the receptor for advanced glycation end products (RAGE) [30]. Moreover, even when the BBB constitutes a physical barrier that allows for the exchange of a wide range of substances in and out of the brain through specific transporters, enzymatic activity has also been described for each cellular component of the BBB, offering additional metabolic protection against potentially neurotoxic compounds that could cross the BBB [32].

### Available Cellular Blood–Brain Barrier Models

Even when the main objective of the present review is not directly related with this particular issue, some words should be mentioned about this important matter. The current available BBB models can be divided into two groups: nonhuman and human-derived, and both can be further divided, according to the origin of the blood vessels, into noncerebral or cerebral endothelial models [16]. Despite several available nonhuman (noncerebral and cerebral) as well as human (noncerebral) cell models, such as MDCK, HUVEC, RBE4, GP8, GPNT, or primary cultures, it is important to mention that only a few of these retain the main characteristics of the BBB [16]. RBE4, GP8, GPNT, b.End3, and primary cultures of brain endothelial cells express most of the efflux/influx carriers as well as junctional proteins, but we must also remember in this case that these are murine models of the BBB and that, even by giving us an initial understanding of the complexity of the BBB, are quite far from indicating how the human BBB functions [16, 38–41]. Considering the critical role that the BBB carrier system plays in Alzheimer's disease (AD) pathogenesis, it is of most importance to have a more reliable model of the human BBB that expresses as many components as possible, to understand its main characteristics.

The hCMEC/D3 cells, described by Weksler et al. [42], are considered to be one of the most significant models for BBB studies, mainly because they correspond to a cerebral vessel human-derived BBB model that expresses the main characteristics of the human BBB [10, 42]. Additionally, considering the high complexity of the BBB, due to the interaction of several cell types, cocultures with glial cells and pericytes have emerged as more complete and complex models to study BBB properties [10, 16].

The selection of the appropriate model is critical when structural and physiological properties of the BBB are assessed, particularly in AD, not only because the integrity of the "barrier" is highly important but also because of the expression patterns of the several carriers involved in amyloid-β (Aβ) clearance.

## Blood–Brain Barrier and Neurodegenerative Disorders

Despite the known existence of the BBB for more than a century, only in the last few decades have significant efforts been made in order to understand the real impact of the role of the BBB on several neurodegenerative disorders. Of course, each of these disorders has its own etiology and particular hallmarks; however, the health or integrity of the BBB has often been shown to be compromised, and the

severity of changes observed usually relates to the progression of the disease [17, 30, 43].

Several authors have analyzed the particularities of the BBB in different disorders, such as epilepsy [44, 45], multiple sclerosis [46], AD, Parkinson's disease, and Huntington's disease [17, 30, 31], among others, and have found an altered function of TJs, AJs, or in the carrier transport system that controls the BBB traffic, such as occludins, claudins, cadherins, EAAT, MCT1, and GLUT1.

## Blood–Brain Barrier and Alzheimer's Disease

AD is an age-associated neurodegenerative disorder characterized by progressive memory and cognitive impairment that eventually leads to death [47, 48]. Clinically, AD progression reflects gradual neurodegeneration with a compromise of short-term memory at the beginning of the disease followed by long-term memory loss [49]. Brain atrophy and gradual loss of neurons, mainly in the hippocampus (HC), frontal cortex (FC) and limbic areas, together with extracellular accumulation of Aβ plaques and intraneuronal formation of neurofibrillary tangles (NFT), composed of hyperphosphorylated aggregates of microtubule-associated protein *tau*, are pathologic hallmarks of AD [48–50]. In AD patients, whether in the familial or in the sporadic form, increased levels of Aβ are usual and considered to be the basis of the pathologic changes observed during AD progression [51]. When Aβ accumulates around blood vessels, it leads to neurovascular dysfunction and cerebral amyloid angiopathy [17]. Indeed, several changes take place in the cerebral blood vessels of AD patients, including loss of vascular density, decreased luminal diameter of vessels and capillaries, and thickness of vessels walls [52]. However, even when the relationship between Aβ accumulation and BBB damage seems evident, it is important to consider that increased levels of Aβ in the brain interstitial fluid depends not only on the production rate but also on the clearance rate from the brain. In fact, the recently published work of Cramer et al. [53] suggested the critical role of Aβ clearance in AD and the importance of considering Aβ-related transporters as targets in future AD therapies.

### Compromised Transporters in Alzheimer's Disease

As a key hallmark of AD, the proper excretion of Aβ from the brain, preventing its neurotoxic accumulation, depends on an appropriate transport through the BBB (Fig. 1).

#### *LRP1 and LRP2*

LRP are widely expressed by several cell types, including neurons, and constitute the main Aβ clearance system of the brain [54, 55]. LRP1-associated Aβ clearance requires Aβ binding to specific proteins, such as apolipoprotein E (ApoE), apolipoprotein J (ApoJ), and α2-macroglobulin. ApoE, the main apolipoprotein of the brain, binds to Aβ forming a complex, which is the substrate of LRP1 [56–60]. In the same way, LRP2 needs the clusterin (or ApoJ)–Aβ complex in order to remove Aβ [56, 61, 62]. In fact, several studies have evaluated how decreased gene expression of LRP1 and/or LRP2 leads to an increased risk of AD [59, 61, 63, 64]. Moreover, it has been demonstrated that LRP as well as neprilysin, the main brain Aβ-degrading enzyme, are target genes of the Aβ precursor protein (APP) intracellular domain (AICD), a small peptide derived from APP γ-secretase processing [65, 66].

#### *ApoE*

Several authors have reported the critical role of isoform variations of ApoE or ApoJ on Aβ clearance and BBB integrity [58, 61, 62, 67]. Furthermore, specific ApoE isoform 4 (ApoE4) is related with decreased Aβ clearance from the brain and constitutes a recognized genetic risk factor for AD development. On the other hand, ApoE isoform 2 (ApoE2) has shown to act as a protective factor, reducing the risk of developing AD [54, 68–70]. This point suggests that further therapies based on increased expression of chaperone proteins, such as ApoE, should be carefully studied and the genetic pull of each single patient must be considered in order to properly offer low-risk therapies.

#### *ABC*

ABCB1 (P-gp) is one of the most important members of the ABC transporters and its expression is often altered in AD [60, 71]. Mainly related with drug transport across the BBB [16], it is also related to Aβ clearance. Indeed, it has been observed that ABCB1 polymorphisms are associated with increased Aβ levels [71]. Additionally, it has been demonstrated that neuroinflammation, often present in several neurodegenerative disorders, also interferes with Aβ traffic through mechanisms that involve main carrier systems found in the BBB, such as ABCB1 [72, 73]. Despite some doubts regarding the real impact of altered ABCB1 function in AD pathogenesis [74], different studies have focused on the identification of different compounds that are able to rescue or enhance BBB traffic through ABCB1 modulation [60, 75]. Additional members of the ABC family have also been described as being related to Aβ efflux across the BBB, such as ABCC1 [76], ABCG2 (BCRP), and ABCG4 [31].

However, considering that the above-mentioned transporters work as required to remove Aβ from the brain once it reaches the luminal space of the brain microvessels, the

Aβ must be eliminated in order to prevent influx to the brain. In fact, it has been demonstrated that peripheral injections of Aβ leads to increased Aβ brain levels and to the amyloid-associated pathology. Moreover, the link between hepatic failure and increased Aβ brain levels has also been established suggesting that a poor systemic excretion of Aβ contributes to brain amyloidosis [77–80]. In fact, it has recently been demonstrated that increasing liver LRP receptor expression is a valid strategy in order to favor circulating Aβ elimination and that it is possible to target distant organs in order to promote brain and systemic Aβ clearance [80]. RAGE has been described as the main carrier related to Aβ brain influx and this association, RAGE–Aβ, leads to several pathologic changes not only in the brain but also at the BBB affecting its permeability through several mechanisms that also include TJ alterations [81, 82] (Table 3).

## Peroxisome Proliferator-activated Receptors

Despite the fact that peroxisome proliferator-activated receptors (PPARs) have been known for a long time [83], the recent work of Cramer et al. [53] has redirected the attention to this nuclear receptor subgroup as a key target in AD therapy. Indeed, PPARs have already been suggested as potential targets for AD therapies [84–88].

Nuclear receptors are a class of transcription factors that sense both the extra- and the intracellular environment [89, 90]. PPARs correspond to a type 2 nuclear receptor characterized by the formation of heterodimers with the retinoid X receptor (RXR) [89]. The PPAR-RXR receptor, when inactivated, forms complexes with corepressor proteins and its activation induces transcriptional regulation of target genes through direct binding to the DNA peroxisome proliferator response elements (PPREs) [90, 91]. Additionally, it has been described that PPAR-RXR activation leads to interactions with different cell signaling transduction pathways, such as the MAPK, PI3K/Akt, and Wnt pathways, inducing posttranslational events [89]. However, the mechanisms of action as well as the interactions with different cell signaling pathways remain to be fully elucidated [92].

Three different mammalian PPARs have been identified: PPARα, PPARβ/δ, and PPARγ with dissimilar distribution among different tissues [84, 91]. "PPARα is highly expressed in several tissues. PPARβ/δ is an APC-regulated target of nonsteroidal antiinflammatory drugs, and PPARγ participates in biological pathways of intense basic and clinical interest" [84]. Although all PPARs have been described in the adult and developing brain [93], PPARγ is the most studied isoform and has showed the most promising neuroprotective effects in various models of neurodegenerative disorders [84, 91, 92].

**Table 3** Compromised BBB transporters in AD

| | |
|---|---|
| LRP 1 | Reduced expression |
| ApoE allele ε4 | Alters Aβ clearance by LRP1 |
| LRP2 | Reduced expression |
| ABC transporters | |
| ABCB1<br>ABCC1 | Polymorphisms and reduced expression |
| ABCG2 | |
| ABCG4 | |
| Receptor for advanced glycation end products (RAGE) | Increased influx of Aβ into the brain |

## Peroxisome Proliferator-activated Receptors and Alzheimer's Disease

Experimental data have pointed out that insulin-sensitizing thiazolidinedione (TZD) drugs, such as troglitazone (TGZ) and rosiglitazone (RGZ), which are known PPARγ agonists and primarily used to treat type II diabetes, are able to delay Alzheimer's development and promote cell survival through PPARγ activation [84, 94]. PPARγ activity related to oxidative stress response is well documented and direct prooxidant as well as antioxidant activity have been described [50, 95]. However, interaction with several antioxidant and antiinflammatory regulatory pathways, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), nuclear factor erythroid 2-related factor (NRF2), or the Wnt/β-catenin pathway, have also been noted [96]. In the same way, Fuenzalida et al. [86] have proposed that PPARγ also upregulates Bcl-2, an antiapoptotic protein and a Wnt target gene [97], in addition to traditional survival pathways, such as MAPK or Akt, preventing neural degeneration and increasing mitochondrial stability.

Recently, Cramer et al. [53] demonstrated that Aβ clearance can be enhanced through ApoE increased expression as well as its transporter proteins, ABCA1 and ABCG1, by the activation with the RXR agonist, bexarotene. Moreover, the bexarotene treatment was able to reverse the Aβ-induced neurotoxicity, improving mice behavior. Despite the impact derived from Cramer's work and the expectation of a successful therapy against AD, there are some questions that need to be answered. Although this finding offers a reliable mechanism of increased Aβ clearance from the brain mediated by ApoE expression modulation [98], it poorly explains all the benefits observed in the treated mice. Furthermore, the behavioral improvement suggests that additional underlying mechanisms, including the potential interactions of

PPARγ:RXR and LXR:RXR heterodimers with cell signaling pathways related to neuron and synaptic recovery, such as Wnt, might play a critical role in the effects observed with the RXR agonist. Even when some authors have tried to explain the full range of effects observed by a stimulation of nuclear receptor agonist treatments, they are focused on the reduction in Aβ levels due to increased glial activity or increased chaperone protein expression [99, 100] and as of yet nothing has been described to explain the potential effects of PPARs on the Aβ toxicity-derived effects or on the BBB. Indeed, as mentioned previously and as has been pointed out by other authors, the effects observed in Cramer's research suggest the involvement of the BBB Aβ–efflux system [76].

## Peroxisome Proliferator-activated Receptors and the Blood–Brain Barrier

Our laboratory has been working with PPAR agonists for several years [84, 86, 97] and more recently, we have published a study using two different PPAR agonists: 4-phenylbutyric acid (4-PB), a PPARγ agonist, and WY 14,643 (WY), a PPARα agonist, assessing the effects of these drugs in a double transgenic mouse model of AD [88]. We have found that both drugs are able to improve the cognitive impairment and alleviate the main pathological changes observed in this murine model, even when it has been suggested that WY cannot cross the BBB [101]. This result has prompted us to consider the possibility that part of the effects observed with the PPAR agonists are due to a direct effect of the drug on one or more components of the neurovascular unit, which leads to an increased Aβ clearance, and that its activation serves to alleviate or prevent the cerebral amyloid angiopathy. To our knowledge, all the information available regarding AD and PPAR effects are centered on neurons and astrocytes and not even one article was found directed at assessing the implications of PPAR activation on the BBB. The following lines constitute an attempt to relate what is known about the PPARs mechanisms of action and how their activation could induce a wide range of effects on the BBB, acting through the different cellular components of the neurovascular unit.

### Peroxisome Proliferator-activated Receptors and Blood–Brain Barrier Amyloid-β Clearance

We have previously mentioned that the level of amyloidosis depends on the balance between production and excretion of Aβ from the brain and how the excretion also depends on the binding of Aβ with additional proteins, such as ApoE, which will serve as a substrate of BBB transporters for the final elimination of Aβ from the brain. The studies of Cramer et al. [53] and Mandrekar-Colucci et al. [100] have highlighted the importance of the Aβ clearance in AD, including binding proteins and the contribution of the glial components to this process. However, this explains only one-half of the problem and does not consider the role of the neurovascular unit in the Aβ clearance process. Even more, it does not take into account the rescue function that must take place in order to induce the cognitive and behavioral improvements observed.

Several authors have indicated that PPAR activation (α, β/δ, γ) is able to induce changes in the BBB, protecting the brain as well as the BBB itself under different negative stimuli. PPARα activation has been related to an increased expression of ABCG2 [102] and with protection against deprivation stimuli in BBB models [103]. On the other hand, the PPARβ/δ effects on the BBB are poorly studied but its overexpression has been related to increased protection during cerebral ischemia [104] and also with Aβ burden decrease in AD murine models [105]. PPARγ are the most studied subgroup of PPARs but only a few studies have focused on BBB changes due to PPARγ activation [106–110]. However, even when it is possible to infer that PPARγ activation leads to increased ABCA1 and ABCG1 levels [53], no studies have examined the changes in the expression of BBB transporters after PPARγ activation.

From our point of view, the increased Aβ clearance must also be due to an increased expression of specialized transporters at the endothelial level. However, the absence of information regarding this issue induces the underestimation of PPAR agonist effects on the BBB and AD.

### Peroxisome Proliferator-activated Receptors and Blood–Brain Barrier Protection and Stabilization

It has been well noted that one of the mechanisms involved in Aβ neurotoxicity is mediated by oxidative stress [50, 111–113] and through induction of mitochondrial dysfunction that further leads to oxidative damage by an increased production of reactive oxygen species (ROS) [114–116]. Indeed, from these observations, it has been proposed that enhancing the cellular antioxidant mechanism could prevent neurodegeneration [50, 86, 117, 118]. Considering the structure of the neurovascular unit, the increased Aβ-induced oxidative stress, and the concomitant mitochondrial dysfunction that enhance the production of ROS, it might be that the basis of the cerebral amyloid angiopathy and capillary disruption is due to the inability of endothelial cells, pericytes, and/or astrocytes to properly respond to the increasing levels of ROS, leading to oxidative damage of the BBB (Fig. 2).

Additionally, the tasks carried out at the BBB are high-energy demanding [11] and the Aβ-induced mitochondrial dysfunction might also have an impact on the energy

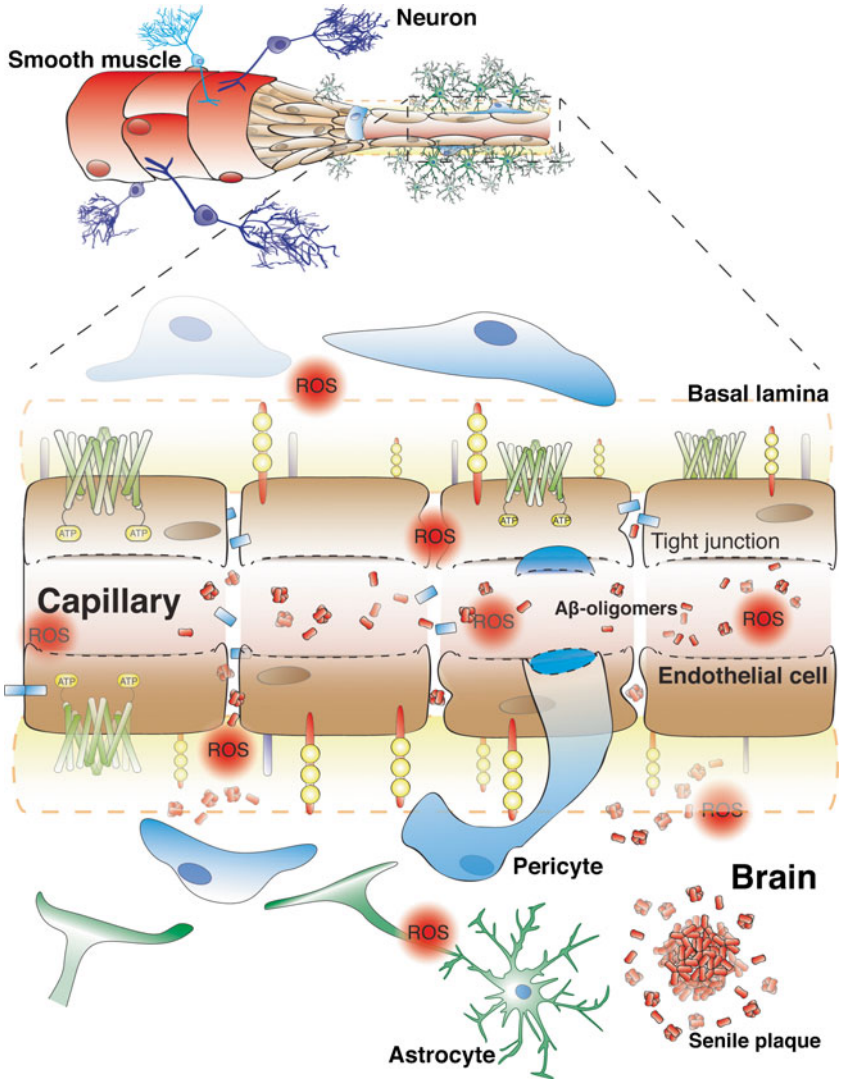


**Fig. 2** Aβ/ROS-based BBB failure. It is well known that one of the main mechanisms involved in the Aβ-induced neurotoxicity is through increased oxidative stress derived by an increased production of reactive oxygen species (ROS). The present model suggests that an increased level of ROS might exert the same toxic effects at the BBB, disrupting the normal structure of the barrier, and altering the functionality of this critical structure. Moreover, it is well recognized that this event happens early in the development of AD and suggests that the altered function of the BBB could accelerate the progress of the pathology and might contribute to Aβ decreased clearance

balance of the different components of the neurovascular unit, altering the traffic across the BBB and perhaps also affecting the ability to maintain the brain environment, necessary to ensure electrical communication between neurons. PPAR activation by several PPAR agonists has proven to reduce oxidative damage through the reduction of ROS production in several tissues, including the brain, liver, and blood vessels, among others, due to the interaction of PPARs with antiinflammatory pathways such as NF-κB and NRF2, the reduction of COX-2 expression or the interaction with antiapoptotic pathways such as Bcl-2 [85, 86, 96, 119–122]. Additionally, we have recently found that PPAR agonist treatment also increases catalase activity in the brain of an AD mouse model [88], suggesting an enhancement of the antioxidant capacity mediated by PPAR activation. It is possible to hypothesize that astrocytes, pericytes, and endothelial cells are able to respond in a similar way as has been observed for other cell types, including highly specialized cells such as neurons, controlling the surrounding environment and limiting the oxidative damage. Additionally, if the oxidative insult is controlled, functional rescue occurs as observed in Cramer's work [53] as well as in our research [88]. Also, in the latter case, the available information seems to suggest a ROS-mediated crosstalk between PPARs and proliferative/prosurvival pathways, such as the Wnt signaling pathway [123]. In fact, several authors have proposed that β-catenin-Tcf/Lef binding, a key step of the Wnt signaling pathway, can be modulated under oxidative stress and redirected to FoxO, inducing cell senescence and the release of proapoptotic signals [124, 125].

On the other hand, it has been described that targeting PPARs leads to the activation of the PPARγ coactivator 1-α (PGC1α) transcription factor, which has been related with several proteins linked to mitochondrial biogenesis and respiration, leading to higher mitochondrial density in neurons as well as increased activity of several

antioxidant enzymes, such as SOD-1, SOD-2, CAT, and GPx [126, 127]. Moreover, PGC1α has become an interesting target in Huntington's disease due to the impact that this cofactor has on mitochondrial stabilization [128, 129]. Furthermore, our recent studies [88, 130] offer further support to the importance of Aβ peripheral clearance, as proposed by Nishitsuji et al. [58] and Sutcliffe et al. [79], due to the increased peroxisomal activity reported in the liver of 4-PB- and WY-treated mice and also allow us to hypothesize that PPAR activation, through different agonists, may lead to PGC1α-increased expression at the BBB. In fact, according to our results [88], part of the benefits observed after PPAR agonist (4-PB and WY) treatment should be related to a direct effect on the BBB components. Moreover, WY is a PPARα agonist that seems unable to cross the BBB. If we consider that PGC1α can also be activated as a consequence of the PPARα activation [131], this hypothesis could explain an additional mechanism that accounts for the wide range of effects observed after PPAR agonist treatment (Fig. 3).

## Final Considerations

Even when the importance of the BBB is out of discussion, there is an absence of information on the impact that several potential drugs may exert on the BBB. Recently, the work of Cramer et al. [53] has astonished the scientific community because of the possibility of having a novel and effective therapy against AD. However, some voices have called for calm and have reminded us that in the past, several therapies have promised a lot but when transferred to real patients, have failed [98, 132]. Indeed, potent PPARγ agonists, such as RGZ and pioglitazone [122], have shown impressive results in different AD models, but when transferred to patients have proven to be little effective. Well, maybe part of the problem involves the BBB and perhaps the lack of knowledge about what roles are played by the BBB in neurodegenerative disorder therapies have lead us once again to failure. Nuclear receptors, and particularly the PPARs family, are a quite complex group of receptors involved in several cellular physiological mechanisms and

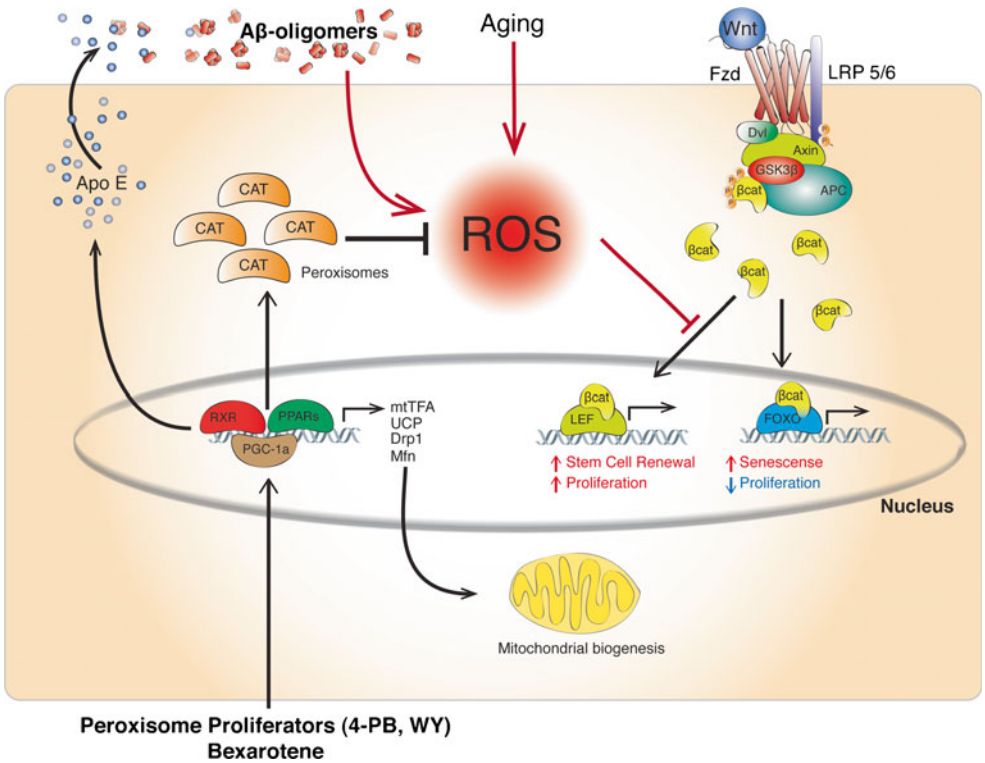


**Fig. 3** RXR/PPAR-based therapy model. According to current information as well as our recent results, it is possible to hypothesize a more complex mechanism of action of an RXR/PPAR-based therapy. Beyond the ApoE increased expression due to pharmacologic RXR:PPARγ dimer stimulation, additional underlying effects might be occurring. It is well noticed that PPAR agonist enhances antioxidant defenses through increased expression of several antioxidant enzymes, such as catalase (CAT), superoxide dysmutase (SOD), and glutation peroxidase (GPx). Despite the contribution to attenuate the oxidative status of the cell, it is important to highlight the effects on cell signaling derived from ROS levels. The Wnt signaling pathway, a proliferative prosurvival pathway, is inhibited in the presence of high levels of ROS by diverting β-catenin from TCF/Lef to the FoxO pathway. Moreover, PPARs have been described to be able to activate PPARγ coactivator 1α (PGC-1α), a transcription coactivator related to mitochondrial dynamics and biogenesis, which could further account for mitochondrial stabilization enhancing efficient ROS management as well as reducing mitochondrial ROS production

which effects are quite far to be fully addressed [122]. Perhaps the systemic administration of PPARs agonists leads to the BBB partial recovery and to an improved BBB trafficking, which in turn, might reduce the ability of the agonists to enter into the brain. Unfortunately, there is a lack of studies focused on BBB recovery related with PPARs agonists administration. We believe that the knowledge derived from this kind of studies will help to understand why several drugs fail when transferred from in vitro or in vivo neurodegenerative disease models to real patients. Scientific progress has allowed us to develop more complex BBB models than before [133], offering the possibility to answer some of the questions regarding the structure and function of the BBB. Moreover, new technologies, such as nanotechnology, have already shown promising results regarding drug delivery to the brain, suggesting that in the future, critical advances will be made in this field [134].

Nuclear receptors and particularly PPARs have become promising targets in several neurodegenerative disorders. The expectation for a novel AD therapy is higher than ever, but several questions about the mechanism of action and the potentialities of such a treatment must be answered in order to be certain of risks and benefits derived from PPAR activation. Some of these questions certainly involve the BBB structure and function. We have tried to briefly introduce, considering the available information about the mechanisms of action of PPARs, how its activation could also induce beneficial effects in the BBB and explain the improvements observed after PPAR agonist treatments in AD mouse models.

**Acknowledgments** This work was supported by grants from the Basal Center of Excellence in Aging and Regeneration (CONICYT-PFB 12/2007) and FONDECYT (No. 1120156) to N.C.I. Graphic work was carried out by Graphique-Science (http://graphique-science.blogspot.com).

**Conflicts of interest** None

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