Review Article

Caffeine Protects Against Disruptions of the Blood-Brain Barrier in Animal Models of Alzheimer's and Parkinson's Diseases

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Abstract. Sporadic Alzheimer's disease (AD) and Parkinson's disease (PD) are two of the most common neurodegenerative diseases and as such they represent major public health problems. Finding effective treatments for AD and PD represents an unmet and elusive goal largely because these diseases are chronic and progressive, and have a complicated and ill-understood pathogenesis. Although the underlying mechanisms are not fully understood, caffeine, the most commonly ingested psychoactive drug in the world, has been shown in human and animal studies to be protective against AD and PD. One mechanism implicated in the pathogenesis of AD and PD is blood-brain barrier (BBB) dysfunction and we reported recently that caffeine exerts protective effects against AD and PD at least in part by keeping the BBB intact. The present review focuses on the role of BBB dysfunction in the pathogenesis of AD and PD, caffeine's protective effects against AD and PD, and potential mechanisms whereby caffeine protects against BBB leakage.

Keywords: Alzheimer's disease, blood-brain barrier, caffeine, Parkinson's disease

THE BLOOD-BRAIN BARRIER AND ITS PATHOPHYSIOLOGICAL IMPORTANCE

The blood-brain barrier (BBB), an exclusive component of the endothelium of brain capillaries where tight junctions are formed, is an important physical and metabolic barrier that helps keep the central nervous system separate from the systemic circulation [1–3] and that helps regulate and protect the microenvironment of the brain. The protection afforded by the BBB is essential for neuronal survival and proper central nervous system functioning [4], and once disrupted, synaptic and neuronal functions can be compromised [5].

The restrictive nature of the BBB is due mainly to tight junctions formed between adjacent endothelial cells and less so by the presence of an underlying continuous basement membrane [6]. Tight junctions restrict ion flux, paracellular diffusion and infiltration of peripheral inflammatory cells. Tight junctions consist of three transmembrane proteins, occludin, claudins, and junction adhesion molecules (JAM), as well as a number of membrane-associated and accessory proteins including zonula occludens (ZO-1, ZO-2, ZO-3) and cingulin (Fig. 1). One of the first transmembrane tight junction proteins described is occludin, a 65 kDa phosphoprotein [7,8]. Occludin has two extracellular loops, four transmembrane domains and three cytoplasmic domains; the cytoplasmic domain of occludin is directly associated with ZO proteins. Occludin is highly expressed in brain capillary endothelial cells and

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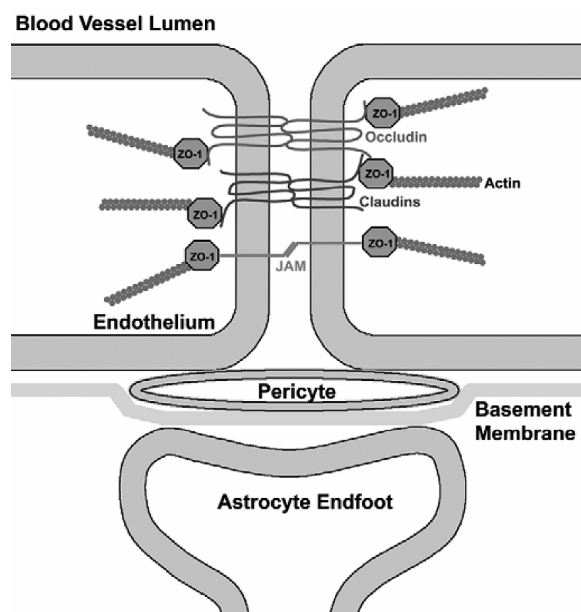
Fig. 1. A schematic diagram of endothelial cells that form the blood–brain barrier (BBB) and their associations with the lumen of a blood vessel, pericytes, basement membrane and endfeet of astrocytes. The restrictive nature of the BBB is due to tight junctions between adjacent endothelial cells at the apical membrane and a continuous basement membrane underlying the endothelium. Tight junctions consist of three transmembrane proteins: occludin, claudins, and junction adhesion molecules (JAM), as well as a number of membrane-associated and accessory proteins including zonula occludens (ZO-1).

appears to be a regulatory protein that can alter paracellular permeability [6,9]. Another group of transmembrane tight junction proteins are claudin proteins, 22 kDa phosphoproteins that contain four transmembrane domains. The carboxy terminal of claudin proteins binds to ZO proteins. Claudins form heterodimeric bridges with adjacent cells that block paracellular diffusion [3]. So far, more than 20 isoforms of claudins have been identified in humans and each of them shows a unique pattern of tissue expression [10]; claudin-3 and -5 and possibly -12 are expressed on brain endothelial cells. A third group of transmembrane tight junction proteins are junctional adhesion molecules (JAM) that have a molecular mass of 40 kDa and are separated into three subtypes, JAM-1, JAM-2 and JAM-3. JAM-1 and JAM-3 are expressed in the brain blood vessels [33]. However, the functions of JAM in the BBB are largely unknown.

Membrane-associated guanylate kinase-like proteins and other accessory proteins involved in tight junction formation include ZO proteins and cingulin [11,12]. Of the three membrane-associated guanylate kinase-like ZO proteins identified (ZO-1, ZO-2 and ZO-3), ZO-1 (220-kDa phosphoprotein), more so than ZO-2 (160-kDa phosphoprotein), links transmembrane tight junction proteins with actin cytoskeleton and stabilizes tight junctions to help maintain BBB integrity [13,14], while ZO-3 is not expressed at the BBB [15]. Other accessory proteins associated with the BBB include cingulin, AF-6, and 7H6, and they too appear to help regulate interactions between the cytoskeleton, tight junction proteins, and signaling events. It is through linkages between the transmembrane proteins and the actin cytoskeleton that occludin, claudins, and ZO proteins stabilize BBB tight junctions [6,16].

Tight junctions are dynamic structures that are highly-regulated by tight junction protein expression levels, post-translational modifications, protein-protein interactions, and multiple cell-signaling pathways. Most tight junction proteins are phosphoproteins with multiple sites for phosphorylation, and changes in phosphorylation status of tight junction proteins profoundly affect their respective expression levels, subcellular localization, protein-protein interactions, and the assembly of tight junction proteins at the BBB [17–22]. In addition, tight junction proteins are affected by intracellular signaling pathways including levels of intracellular calcium [23–25], vascular endothelial growth factor (VEGF) [26], small G-proteins of the Rho family [21,27–29], and cAMP which stabilizes the BBB [17,30–32]. Increasingly, BBB dysfunction has been implicated in the pathogenesis of a number of acute and chronic neurodegenerative disorders including brain trauma [33], stroke [34], multiple sclerosis [35], HIV-1 dementia [36], Alzheimer's disease (AD) [37], and Parkinson's disease (PD) [38]. Here we will focus on BBB dysfunction in the pathogenesis of AD and PD, the two most common neurodegenerative diseases, as well as a pharmacological strategy capable of affecting a wide variety of signaling molecules and stabilizing BBB integrity.

BBB DYSFUNCTION IN AD

AD, characterized clinically by progressive loss of memory and impaired cognition, is the most common form of irreversible dementia in people over the age of 60 years. Pathologically, AD is characterized by synaptic loss and neuronal cell death, as well as the presence of extracellular amyloid plaques composed of amyloid-β (Aβ) protein and intracellular neurofibrillary tangles composed of phosphorylated tau [39]. Aβ

is a series of proteolytic by-products of the amyloid-β protein precursor (AβPP) that vary in length from 39 to 43 amino acids; Aβ results from metabolism catalyzed sequentially by β- and γ-secretase enzymes. According to the "amyloid hypothesis", increased levels of Aβ occur in AD, and Aβ leads to hyperphosphorylation of tau, synaptic dysfunction, neuronal cell death, and ultimately, impairment of higher cortical activity including memory and cognition. Aβ (and tau) appear to play a critical role in the pathogenesis of AD, and compelling evidence supports the amyloid hypothesis of early-onset AD [40,41], which is caused by genetic mutations in the AβPP, presenilin-1 and presenilin-2 genes; all lead to dramatic increases in amyloidogenic processing of AβPP and Aβ production. However, early-onset AD represents less than 5% of all AD cases while the vast majority ($\sim$95%) of AD cases originate sporadically [42]. Although the etiology of sporadic AD is not known, several risk factors are implicated including the apolipoprotein allele E4 (ApoE4) genotype, previous head injury, and cardiovascular diseases such as atherosclerosis, stroke, and diabetes that could result in cerebrovascular dysfunction.

BBB dysfunction is implicated in the pathogenesis of AD, and cerebrovascular dysfunction risk factors including atherosclerosis, stroke, and diabetes can lead to BBB dysfunction [43–46]. The first indication of BBB disruption in AD brain came from the observation that IgG and complement proteins aggregate near plaques, indicating focal or subtle changes in BBB permeability [47]. Subsequent studies confirmed the notion that BBB disruption is a pathological characteristic of AD as evidenced by increased leakage of serum proteins into AD brain parenchyma [48–50], increased cerebrospinal fluid (CSF):serum albumin ratios in AD patients [51–54], and pathological changes in the microvasculature of AD brain [37,55]. Currently, BBB dysfunction is considered to be one of the earliest pathological events underlying AD [56].

Although the exact mechanisms whereby BBB dysfunction contributes to the pathogenesis of AD are not fully understood, BBB dysfunction could affect AD pathogenesis by decreasing Aβ clearance and increasing Aβ production. Clearance of Aβ is controlled in part by an intact and functional BBB that transports soluble Aβ from blood to brain mainly via the Receptor for Advanced Glycation End-products (RAGE), and from brain to blood via the low-density lipoprotein receptor-related protein (LRP-1) [57–59], and depends on the Aβ chaperone proteins ApoE and apolipoprotein J (ApoJ) [60,61]. Thus, altered BBB function could

lead to accumulation of Aβ within brain because of inadequate Aβ efflux (due to decreased expression of LRP-1), and increased Aβ influx (due to increased expression of RAGE) [5,59,62–64]. ApoE4 allele, the only known genetic risk factor of sporadic AD, slows Aβ clearance from brain in an isoform-specific manner [61]. Alternatively, significant amounts of Aβ are produced in the periphery [65–67]. In addition, Aβ can be produced locally in and around the BBB; AβPP is expressed in endothelial cells and pericytes, and Aβ production has been demonstrated in isolated brain microvessels [68,69]. Under conditions of increased BBB permeability, which has been shown to occur in AD, Aβ from peripheral sources including blood, platelets, and skeletal muscle could flood into brain parenchyma. Increased Aβ production can also occur as a consequence of BBB disruption and increased entry of blood-borne pathogens, substances, drugs, and peripheral inflammatory cells [70]. Conversely, the presence of Aβ can adversely affect brain endothelial cells and can disrupt the BBB [71–75]. Therefore, BBB disruption by Aβ can potentiate further increases in Aβ accumulation in brain, thus creating a vicious cycle.

THE LINK BETWEEN HIGH LEVELS OF CHOLESTEROL AND DECREASED BBB INTEGRITY IN AD

One extrinsic factor that contributes to increased Aβ production and possibly the pathogenesis of sporadic AD is increased levels of cholesterol; high levels during mid-life increases the risk of developing AD later in life [76–78]. Under physiological conditions, brain is a net exporter of cholesterol and brain levels of cholesterol are mainly dependent on *in situ* synthesis and not dietary uptake [79,80]. However, under conditions associated with AD pathogenesis the situation may be quite different. For example, with increased BBB leakiness cholesterol in the blood could enter brain and disturb brain cholesterol homeostasis. Furthermore, ApoE4, the major genetic risk factor of sporadic AD, is critical for transportation of cholesterol between cells in brain [81], and is associated with elevated cholesterol levels and an increased risk of developing AD [82–85]. Thus, evidence for an important role of cholesterol in the pathogenesis of AD comes from a variety of experimental approaches including environmental, genetic, and epidemiological.

Experimentally, Sparks and co-workers [86] first reported an association between cholesterol and Aβ

production. Subsequently, elevated levels of cholesterol were found to increase Aβ generation [87–89], and lowering cholesterol levels decreased Aβ production [89–91]. Rabbits fed a diet enriched in cholesterol have for years been used as a model for cardiovascular disorders, especially atherosclerosis. Rabbits fed cholesterol-enriched diets are now known to exhibit neurovascular and other disorders, and we have shown that such diets induce pathological features of AD such as learning deficits, increased Aβ plaque formation, and hyperphosphorylation of tau [86,92–95]. Moreover, we reported recently that rabbits fed a cholesterol-enriched diet exhibit pathological features of AD, including increased BBB leakage and disrupted integrity of the BBB [96]. Thus, elevated levels of cholesterol could contribute to the pathogenesis of AD, at least in part, because the integrity of the BBB is compromised.

Cholesterol-induced increases in levels of Aβ in brain might involve increased AβPP trafficking and increased amyloidogenic processing of AβPP, and increasingly, endosomes/lysosomes have been implicated in amyloidogenesis. An involvement of endocytosis in amyloidogenic processing of AβPP is suggested by findings of AβPP and AβPP cleavage products in clathrin-coated vesicles [97]. The involvement of endosomes/lysosomes was confirmed by findings that Aβ production was decreased in cultured cells that were stably transfected with an AβPP construct where the C-terminal endocytic targeting signal was removed [98, 99], and when cells were transfected with a dominant-negative form of dynamin [100]. Furthermore, beta-site amyloid precursor protein-cleaving enzyme-1 (BACE-1), the major β-secretase to cleave the AβPP to generate Aβ, is localized in endosomes and its activity is optimal under acidic conditions [101–103]. Even more direct evidence for the involvement of endosomes/lysosomes in amyloidosis and AD pathogenesis comes from findings that Aβ accumulates in neuronal endosomes/lysosomes of AD brain [104], abnormal endosomes occur before extracellular Aβ is deposited [105], and intraneuronal deposition of Aβ precedes extracellular deposition of Aβ [106].

Cholesterol in most types of cells comes largely from lipoproteins up-taken through receptor-mediated endocytosis. Following binding of lipoproteins to its receptors, the receptor-lipoprotein complex is internalized and transported to endosomes/lysosomes where cholesterol esters are hydrolyzed to free cholesterol, which is then transported to various cellular compartments, including plasma membrane and endoplasmic reticulum [80,107]. Although brain cholesterol is large-ly derived from de novo synthesis within the brain, lipoprotein transport across the BBB can play a role in delivering essential lipids including cholesterol to brain cells [108,109]. In brain, ApoE is the major endogenous lipoprotein that transports cholesterol from astrocytes to neurons, and neurons express low density lipoprotein (LDL) receptors, including low density lipoprotein receptors (LDLR), very low density lipoprotein receptors(VLDLR), and LRP-1 [110–112]. It has been shown that receptor-mediated endocytosis of cholesterol promotes AβPP internalization and processing [81,113–118]. Under conditions when circulating LDL cholesterol levels are high, high levels of LDL cholesterol would be expected to enhance receptor-mediated endocytosis of cholesterol at the BBB and thereby impair endothelial barrier function [119,120]. Alternatively, high levels of LDL cholesterol could promote amyloidogenic processing of AβPP, increase Aβ production, and in so doing impair the BBB function. Once the BBB is disrupted, it is expected that increased levels of LDL cholesterol coming from the periphery could increase brain levels of cholesterol, enhance neuronal uptake of cholesterol, and increase AβPP trafficking, amyloidogenic processing of AβPP, and Aβ generation.

BBB DYSFUNCTION IN PD

PD is a chronic neurodegenerative disease characterized clinically by tremor, bradykinesia, rigidity, and postural instability, and pathologically by loss of dopaminergic neurons mainly in the substantia nigra pars compacta [38]. Although the etiology of PD is not known, the observation that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a parkinsonian syndrome similar to PD has led to the hypothesis that environmental toxins similar to MPTP could play an important role in the pathogenesis of PD [121]. The cellular and molecular mechanisms underlying the pathogenesis of PD are unclear at present, but PD has been linked increasingly to neuroinflammation and oxidative stress [122–125].

Similar to AD, BBB disruption may play an important role in the pathogenesis of PD. Increasingly, BBB dysfunction has been reported to contribute to PD progression [38,126,127], and it has been found that pesticides disrupt BBB permeability [128], that neuroinflammation and oxidative stress compromises BBB [129–133], that pathological alterations in endothelial cells within the substantial nigra are noted in

patients with PD [134] and BBB dysfunction is present in PD patients [38], that BBB disruption occurs in PD animal models including MPTP-treated mice [133] and 6-hydroxydopamine (6-OHDA)-treated rats [135], and that BBB disruption precedes dopaminergic neuronal loss in substantial nigra [136]. Thus, BBB dysfunction appears to contribute, at least in part, to the pathogenesis of PD and may help explain why even short exposures to MPTP could result in the development of a progressive parkinsonian disorder.

CAFFEINE AND ITS MECHANISM(S) OF ACTIONS

Caffeine, the main topic of these reviews, is widely consumed [137,138] and has diverse pharmacological actions [138]. Of relevance to the control of BBB integrity, caffeine's actions have been shown to be mediated through blocking cell surface adenosine receptors, through inhibition of cAMP phosphodiesterase (PDE) activity, and by affecting the release of calcium from intracellular stores. Each of these three mechanisms has been implicated in modulating BBB functions, and the effects of caffeine on these mechanisms are clearly concentration- and dose-dependent.

Caffeine at low concentrations (μM range) can block all four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B} and A_3), with most of its actions being mediated through inhibition of the high-affinity A_1 and A_{2A} receptors and, to a lesser extent, the low-affinity A_{2B} and the high-affinity low-density A_3 receptors [137]. These receptors are G protein-coupled receptors that affect many cell-signaling mechanisms including cAMP. Activation of A_1 and A_3 receptors leads to the inhibition of adenylate cyclase by G_i and decreases intracellular levels of cAMP, whereas activation of A_{2A} and A_{2B} receptors stimulates adenylate cyclase through G_s and increases intracellular levels of cAMP [139]. Because caffeine is commonly ingested chronically, it is important to note that long-term exposure to adenosine receptor antagonists like caffeine can have effects that resemble the acute effects of adenosine receptor agonists [140], due likely to up-regulation of adenosine receptors (A_1 and A_{2A}) and adaptive changes leading to adenosine receptor sensitization [140–142]. At higher concentrations, caffeine and its intermediate metabolites such as theophylline, theobromine, and paraxanthine can elevate intracellular cAMP levels by inhibiting cAMP PDE activity [137]. At high, possibly toxic concentrations (mM range), caffeine can mobilize calcium from endoplasmic reticulum stores through actions of IP_3 and ryanodine receptors [137,143]. Caffeine is also known to affect a number of other cell signaling molecules and physiological functions, the composite of which may affect the pathogenesis of AD and PD as well as BBB structure and function.

THE ROLE OF THE BBB IN THE PROTECTIVE EFFECTS OF CAFFEINE IN ANIMAL MODELS OF AD AND PD

Recent epidemiological and experimental studies indicate that caffeine, when administered chronically, has beneficial effects against a number of acute and chronic neurological disorders including stroke, AD, and PD [144–156]. For AD, the protective effects of caffeine have been observed in humans as well as in animal models of this neurodegenerative disorder. Epidemiologically, chronic ingestion of caffeine conferred protective effects against AD [157,158], and a retrospective study showed that caffeine intake is associated with a significantly lower risk for AD [150]. Prospective studies confirmed the above findings by showing that chronic caffeine intake improved memory and cognitive function in normal aged individuals as well as in AD patients [148,151,159–162]. Subsequently, experimental studies conducted using animal models of AD noted that caffeine improved cognitive abilities [149, 163], reduced Aβ production [149,163,164], and stabilized BBB integrity [96]. Thus, caffeine and drugs like caffeine might be part of any regimen intended to prevent, delay, and/or treat AD.

For PD, it was reported some 35 years ago that caffeine through blocking adenosine receptor activation could ameliorate parkinsonian symptoms [165]. Subsequent retrospective, prospective, and epidemiologic studies demonstrated that caffeine, when administered chronically, decreased the risk of developing PD [152–155]. Experimental studies confirmed and extended the epidemiological findings by showing that caffeine was neuroprotective against the loss of dopaminergic neurons that occurs in the substantial nigra of MPTP-treated mice [166]. Thus, current evidence strongly suggests that caffeine is a promising agent in the prevention and/or treatment of PD [167]. Although the mechanisms whereby caffeine exerts its protective effects on PD are not fully understood, much work has been conducted on the involvement of adenosine receptors in the pathogenesis and possible treatment of PD [168]. Of the identified adenosine receptor

subtypes, the main focus continues to be on A_{2A} receptors [169], and preclinical studies suggest strongly that A_{2A} receptor antagonists are protective against PD [170–174]. However, clinical trials with an A_{2A} receptor antagonist were rather disappointing in that only minimal improvements in PD symptomatology were noted in the PD patients [175,176]. Nevertheless, A_{2A} receptor antagonists may still prove to be effective against PD, because those clinical trials were designed to determine only the extent to which they might show a therapeutic effect in patients already living with PD. Results from animal studies and epidemiological studies have demonstrated repeatedly that caffeine and blockade of adenosine A_{2A} receptors afford prophylactic protection and not therapeutic rescue of endpoints related to PD. Furthermore, it remains a possibility that an inadequate dose of the A_{2A} receptor antagonist was tested in the clinical trials.

BBB dysfunction has been implicated in the pathogenesis of AD [48–50], and although more controversial, PD as well [38,126,127]. Thus, caffeine might exert its protective effect against AD and PD by virtue of its action on the BBB. Indeed, we reported recently that chronic ingestion of caffeine protected against BBB dysfunction in both a rabbit model of sporadic AD [96] and a mouse model of PD [177]. In a rabbit model of sporadic AD, we demonstrated that caffeine (3 mg/day, a human equivalent of ∼1 cup of coffee per day) blocked cholesterol-enriched diet-induced increases in leakage of Evan's blue dye (Fig. 2A), and decreases in levels of the tight junction proteins occludin (Fig. 2B, C) and ZO-1. In a MPTP neurotoxin model of PD, we demonstrated that caffeine (10 mg/kg, a human equivalent of ∼5 cup of coffee per day) blocked MPTP-induced increases in leakage of Evan's blue dye (Fig. 2D), and decreases in levels of the tight junction proteins occludin (Fig. 2E, F) and ZO-1 specifically in striatum. Although the molecular mechanisms by which caffeine protects against BBB dysfunction remain unclear, the mechanisms to consider include blockade of adenosine receptors, inhibition of cAMP PDE activity, or mobilization of intracellular calcium from endoplasmic reticulum stores. Because the doses needed to stabilize the BBB in animal models of AD and PD were in the pharmacologically relevant range, the protective effects of caffeine on the BBB are most likely achieved through blockade of adenosine receptors. Of the four subtypes of adenosine receptors, A_{2A} and A_{2B} receptors are most prominently expressed on brain endothelial cells [178,179], and their activation elevates intracellular levels of cAMP, which is well

known to stabilize the BBB [17,30–32]. That this is an underlying mechanism for the action of caffeine is supported more directly by findings that activation of A_{2B} receptors protects against vascular leakage [180]. However, to invoke such a mechanism it is important to note that, although the direct effect of acutely administered caffeine blocks adenosine receptors (A_{2A} and A_{2B}), thus decreasing intracellular levels of cAMP, chronic ingestion of caffeine as occurs typically in humans can result in up-regulation of adenosine receptors (A_{2A} and A_{2B}) on brain endothelial cells and/or increased sensitivity of adenosine receptors (A_{2A} and A_{2B}) to their endogenous ligand, adenosine. Thus, under conditions when and where brain levels of adenosine are high [181–184], the activation of already sensitized and/or up-regulated adenosine receptors on brain endothelial cells, especially during the discontinuous presence of caffeine, could lead to greater elevation of intracellular levels of cAMP, thus protecting the BBB against disruption. Our observation that caffeine specifically affects the striatum in the MPTP neurotoxin model of PD could be related to the very high density of adenosine A_{2A} receptors found normally in that brain region [137,166]. It is also possible that caffeine could stabilize the BBB by increasing intracellular levels of cAMP via inhibition of cAMP PDE activity in brain endothelial cells [137,185], but this is not so likely because cAMP PDE has an approximately 1-2 order of magnitude less sensitivity to caffeine compared to adenosine A_1, A_{2A}, and A_{2B} (but not A_3) receptors. In addition, the observed protective effects of caffeine on endothelial cells of the BBB in our animal models, especially the rabbit AD model, might have been due to changes in lipids and/or cholesterol metabolism [186–188], inhibiting endocytosis [189,190], and/or affecting lysosomal pH and trafficking [191,192].

In addition to its actions on endothelial cells, caffeine might exert its protective effects on the BBB indirectly through modulation of other cell types in brain including astrocytes, microglia, and neurons. Activation of astrocytes and microglia are major components of neuroinflammation, which is implicated in the pathogenesis of both AD and PD [193,194]. Reactive gliosis can release a cascade of proinflammatory and neurotoxic factors including TNF-α, IL-1 and reactive oxygen species [193,195–198], all of which can disrupt the BBB [24]. The leaky BBB could further potentiate neuroinflammatory responses by allowing peripheral inflammatory cells to infiltrate into brain parenchyma, creating a vicious cycle. Several lines of evidence indicate that caffeine and the adenosine receptors it is
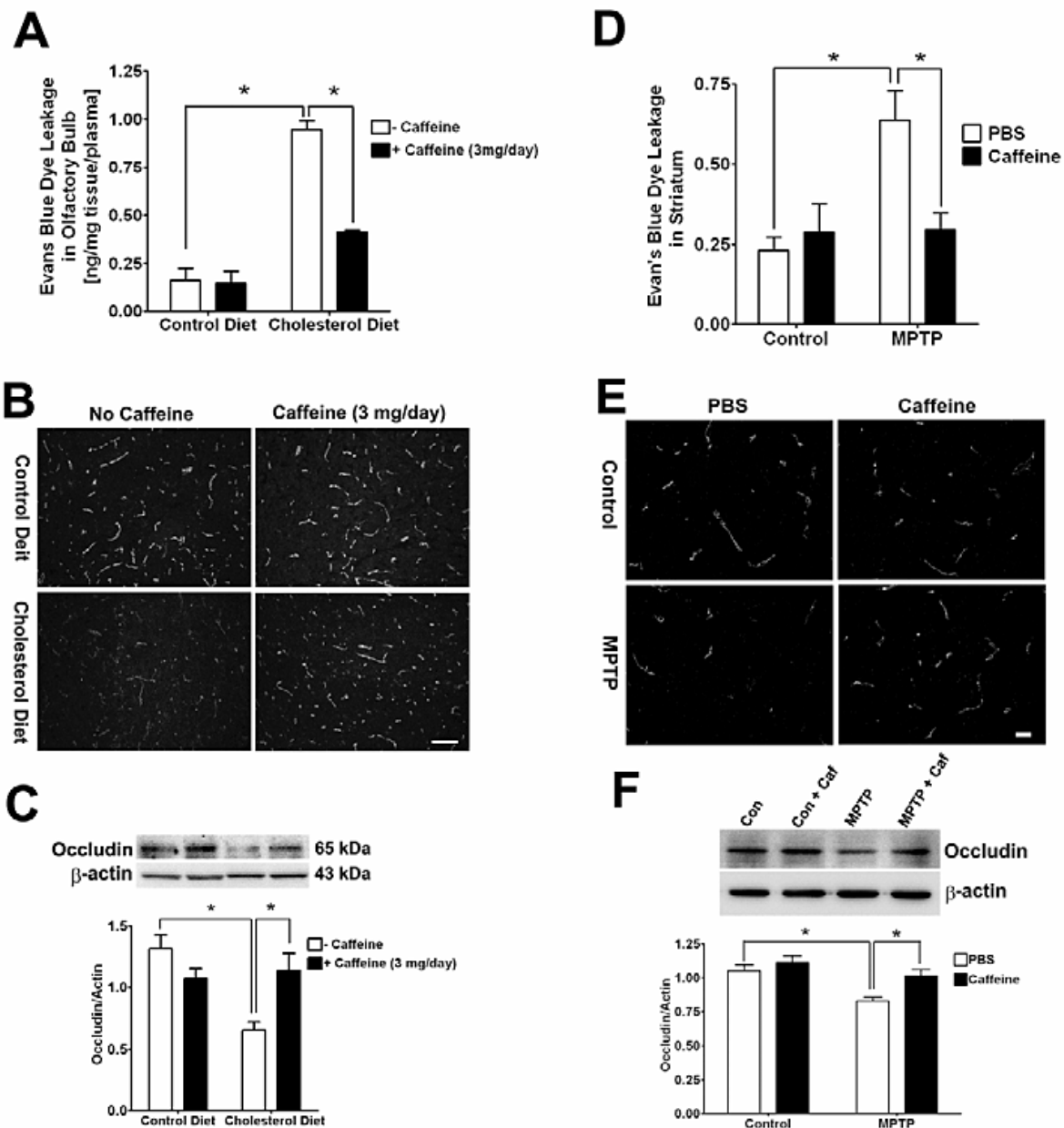
Fig. 2. In a rabbit model of sporadic AD, caffeine at a dose of 3 mg/day blocked cholesterol-enriched diet-induced increases in leakage of Evan's blue dye (A) and decreases in occludin immunostaining (B) and protein levels (C) in olfactory bulb (Bar = 100 μm). Modified with permission from [96]. In a MPTP neurotoxin model of PD, caffeine at the dose of 10 mg/kg/day blocked MPTP-induced increases in leakage of Evan's blue dye (D) and decreases in occludin immunostaining (E) and protein levels (F) in striatum (Bar = 20 μm). Modified with permission from [177].

known to block can inhibit neuroinflammation in *in vitro* models devoid of BBB [199–201]. Therefore, the protective effects of caffeine against BBB leakage might result from its ability to inhibit neuroinflammation. The anti-inflammatory actions of caffeine may also involve blockade of adenosine A_{2A} receptors out-

side of the brain [202], which could limit brain cytokine up-regulation (eg. interleukin-1) known to accompany brain insults, including AD [203]. In addition, caffeine has been shown to have direct neuroprotective effects and it remains a possibility that caffeine's protective effects against BBB leakage are secondary to its neu-

roprotective effects. However, we do favor the notion that neuroprotection is secondary to the BBB effects because of our findings in a rabbit model of sporadic AD that caffeine can protect against increased BBB leakage in the absence of apparent neuronal loss by the cholesterol-enriched diet [96].

It is becoming increasing clear that synaptic dysfunction is the key event in pathogenesis of both AD and PD. In AD patients, it is the loss of synapses rather than neurons that effectively correlates with dementia [204,205]. In AD animal models, there is hardly any neuronal loss, but synaptic loss and dendritic spine abnormalities have been demonstrated in several transgenic mouse models of AD [206–208]. Altered synaptic plasticity has also been demonstrated in PD patients [209] and in PD animal models [210–213]. As mentioned earlier, the BBB limits the entry of blood-borne pathogens, substances, drugs, and cells into brain parenchyma and helps regulate and protect the microenvironment of the brain such that once the BBB is disrupted, synaptic and neuronal functions are compromised [5]. In the cholesterol-fed rabbit model of AD, both BBB disruption and learning deficit have been demonstrated [95,96]. Furthermore, our observation that caffeine protects against BBB disruption is consistent with the findings that caffeine intake protects against memory loss in aging and in AD [150, 151]. Thus, synaptic dysfunction and loss of synaptic markers should be a focus in future studies using the rabbit model of AD.

CONCLUSIONS

Sporadic AD and PD are the two most common neurodegenerative diseases, with unknown etiologies and limited available therapeutic interventions. Recent epidemiological and experimental studies suggest strongly that caffeine, the most commonly ingested psychoactive drug in the world, is protective against these degenerative diseases. Furthermore, elucidating underlying mechanisms whereby caffeine protects against AD and PD will undoubtedly lead to new therapeutic strategies that could prevent, delay, and/or treat these deleterious diseases.

Emerging evidence suggests that BBB dysfunction plays an important role in the pathogenesis of both AD and PD. Our recent findings that caffeine, a safe and readily available drug, can stabilize BBB, have important implications for therapeutic interventions against these neurological disorders. Nevertheless, further

detailed studies are now warranted to determine first the temporal and spatial sequence of caffeine's effects on BBB leakage, neuroinflammation, synaptic dysfunction, and neuroprotection, and second the detailed molecular mechanism(s) whereby caffeine protects against BBB disruption. However, because caffeine can prevent the opening of the BBB in two very different models of neurodegenerative disorders and in two different species and therefore may be of generalized importance, it might be important to limit ingestion of caffeine by patients undergoing therapeutic interventions wherein controlled opening of the BBB is integral to the desired clinical endpoints.

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