

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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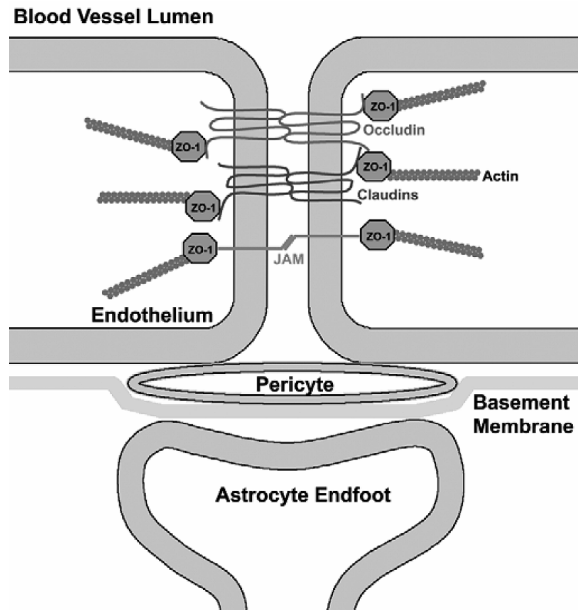


Fig. 1. A schematic diagram of endothelial cells that form the blood–brain barrier (BBB) and their associations with the lumen of a blood vessel, pericytes, basement membrane and endfeet of astrocytes. The restrictive nature of the BBB is due to tight junctions between adjacent endothelial cells at the 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 ($A\beta$) protein and intracellular neurofibrillary tangles composed of phosphorylated tau [39]. $A\beta$

is a series of proteolytic by-products of the amyloid- β protein precursor ($A\beta$ PP) that vary in length from 39 to 43 amino acids; $A\beta$ results from metabolism catalyzed sequentially by β - and γ -secretase enzymes. According to the “amyloid hypothesis”, increased levels of $A\beta$ occur in AD, and $A\beta$ leads to hyperphosphorylation of tau, synaptic dysfunction, neuronal cell death, and ultimately, impairment of higher cortical activity including memory and cognition. $A\beta$ (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 $A\beta$ PP, presenilin-1 and presenilin-2 genes; all lead to dramatic increases in amyloidogenic processing of $A\beta$ PP and $A\beta$ 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 $A\beta$ clearance and increasing $A\beta$ production. Clearance of $A\beta$ is controlled in part by an intact and functional BBB that transports soluble $A\beta$ 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 $A\beta$ chaperone proteins ApoE and apolipoprotein J (ApoJ) [60,61]. Thus, altered BBB function could

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

production. Subsequently, elevated levels of cholesterol were found to increase $A\beta$ generation [87–89], and lowering cholesterol levels decreased $A\beta$ 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 $A\beta$ 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 $A\beta$ in brain might involve increased $A\beta$ PP trafficking and increased amyloidogenic processing of $A\beta$ PP, and increasingly, endosomes/lysosomes have been implicated in amyloidogenesis. An involvement of endocytosis in amyloidogenic processing of $A\beta$ PP is suggested by findings of $A\beta$ PP and $A\beta$ PP cleavage products in clathrin-coated vesicles [97]. The involvement of endosomes/lysosomes was confirmed by findings that $A\beta$ production was decreased in cultured cells that were stably transfected with an $A\beta$ PP 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 $A\beta$ PP to generate $A\beta$, 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 $A\beta$ accumulates in neuronal endosomes/lysosomes of AD brain [104], abnormal endosomes occur before extracellular $A\beta$ is deposited [105], and intraneuronal deposition of $A\beta$ precedes extracellular deposition of $A\beta$ [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 $A\beta$ PP 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 $A\beta$ PP, increase $A\beta$ 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 $A\beta$ PP trafficking, amyloidogenic processing of $A\beta$ PP, and $A\beta$ 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 dysfunction 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 substantia 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 substantia 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 adenylyl cyclase by G_i and decreases intracellular levels of cAMP, whereas activation of A_{2A} and A_{2B} receptors stimulates adenylyl 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 cal-

cium 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 $A\beta$ 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 substantia 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

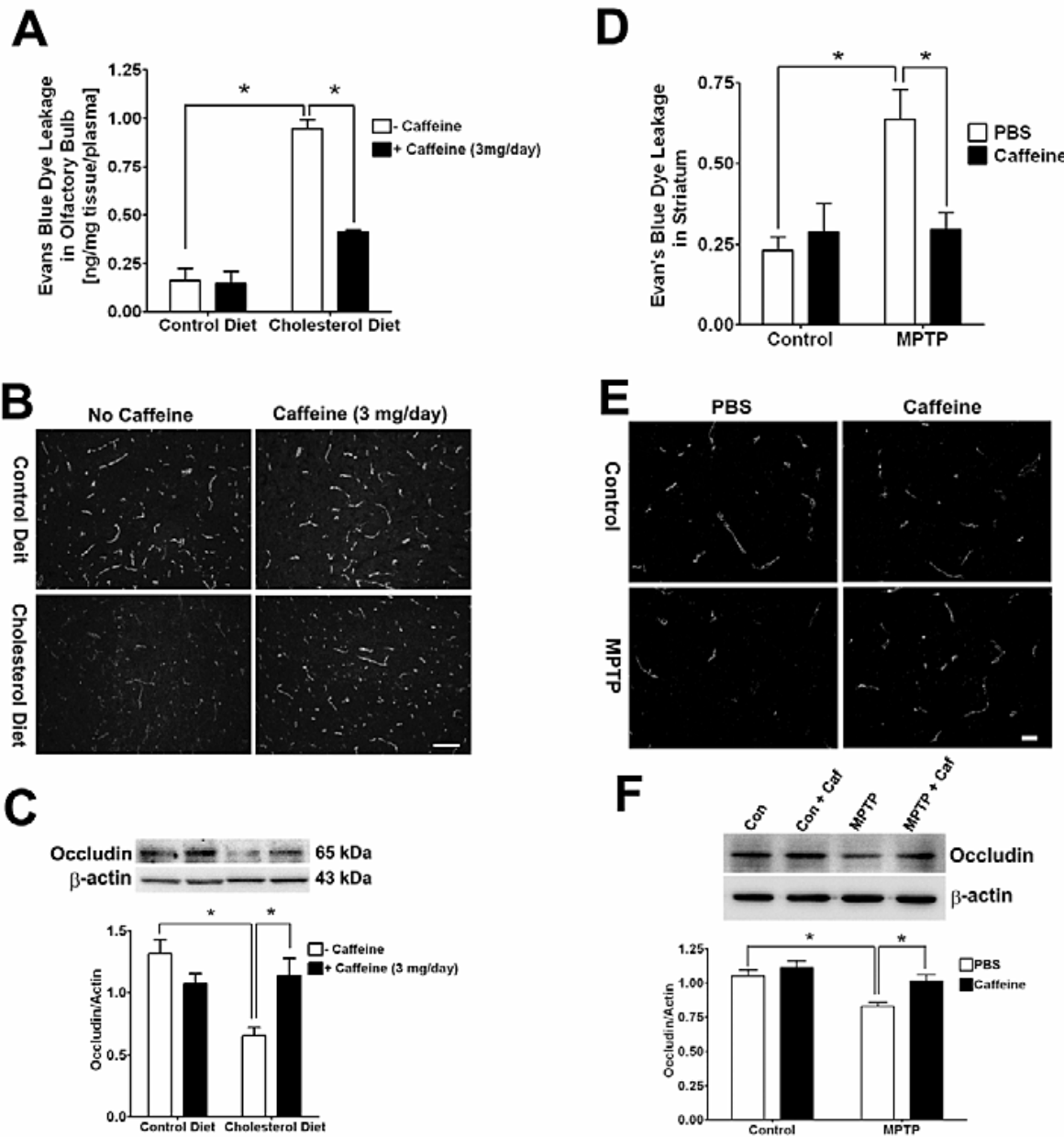


Fig. 2. In a rabbit model of sporadic AD, caffeine at a dose of 3 mg/day blocked cholesterol-enriched diet-induced increases in leakage of Evan's blue dye (A) and decreases in occludin immunostaining (B) and protein levels (C) in olfactory bulb (Bar = 100 μm). Modified with permission from [96]. In a 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 increasingly 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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REFERENCES

- [1] Gloor SM, Wachtel M, Bolliger MF, Ishihara H, Landmann R, Frei K (2001) Molecular and cellular permeability control at the blood-brain barrier. *Brain Res Brain Res Rev* **36**, 258-264.
- [2] Reese TS, Karnovsky MJ (1967) Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J Cell Biol* **34**, 207-217.
- [3] Turksen K, Troy TC (2004) Barriers built on claudins. *J Cell Sci* **117**, 2435-2447.
- [4] Abbott NJ (2005) Dynamics of CNS barriers: evolution, differentiation, and modulation. *Cell Mol Neurobiol* **25**, 5-23.
- [5] Zlokovic BV (2008) The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* **57**, 178-201.
- [6] Vorbrodt AW, Dobrogowska DH (2003) Molecular anatomy of intercellular junctions in brain endothelial and epithelial barriers: electron microscopist's view. *Brain Res Brain Res Rev* **42**, 221-242.
- [7] Ando-Akatsuka Y, Saitou M, Hirase T, Kishi M, Sakakibara A, Itoh M, Yonemura S, Furuse M, Tsukita S (1996) Interspecies diversity of the occludin sequence: cDNA cloning of human, mouse, dog, and rat-kangaroo homologues. *J Cell Biol* **133**, 43-47.
- [8] Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S (1993) Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol* **123**, 1777-1788.
- [9] Hirase T, Staddon JM, Saitou M, Ando-Akatsuka Y, Itoh M, Furuse M, Fujimoto K, Tsukita S, Rubin LL (1997) Occludin as a possible determinant of tight junction permeability in endothelial cells. *J Cell Sci* **110**(Pt 14), 1603-1613.

- [10] Morita K, Sasaki H, Furuse M, Tsukita S (1999) Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells. *J Cell Biol* **147**, 185-194.
- [11] Citi S, Sabanay H, Jakes R, Geiger B, Kendrick-Jones J (1988) Cingulin, a new peripheral component of tight junctions. *Nature* **333**, 272-276.
- [12] Stevenson BR, Heintzelman MB, Anderson JM, Citi S, Mooseker MS (1989) ZO-1 and cingulin: tight junction proteins with distinct identities and localizations. *Am J Physiol* **257**, C621-628.
- [13] Fischer S, Wobben M, Marti HH, Renz D, Schaper W (2002) Hypoxia-induced hyperpermeability in brain microvessel endothelial cells involves VEGF-mediated changes in the expression of zonula occludens-1. *Microvasc Res* **63**, 70-80.
- [14] Abbruscato TJ, Lopez SP, Mark KS, Hawkins BT, Davis TP (2002) Nicotine and cotinine modulate cerebral microvascular permeability and protein expression of ZO-1 through nicotinic acetylcholine receptors expressed on brain endothelial cells. *J Pharm Sci* **91**, 2525-2538.
- [15] Inoko A, Itoh M, Tamura A, Matsuda M, Furuse M, Tsukita S (2003) Expression and distribution of ZO-3, a tight junction MAGUK protein, in mouse tissues. *Genes Cells* **8**, 837-845.
- [16] Wolburg H, Lippoldt A (2002) Tight junctions of the blood-brain barrier: development, composition and regulation. *Vascul Pharmacol* **38**, 323-337.
- [17] Ishizaki T, Chiba H, Kojima T, Fujibe M, Soma T, Miyajima H, Nagasawa K, Wada I, Sawada N (2003) Cyclic AMP induces phosphorylation of claudin-5 immunoprecipitates and expression of claudin-5 gene in blood-brain-barrier endothelial cells via protein kinase A-dependent and -independent pathways. *Exp Cell Res* **290**, 275-288.
- [18] Andreeva AY, Krause E, Muller EC, Blasig IE, Utepergenov DI (2001) Protein kinase C regulates the phosphorylation and cellular localization of occludin. *J Biol Chem* **276**, 38480-38486.
- [19] Farshori P, Kachar B (1999) Redistribution and phosphorylation of occludin during opening and resealing of tight junctions in cultured epithelial cells. *J Membr Biol* **170**, 147-156.
- [20] Sakakibara A, Furuse M, Saitou M, Ando-Akatsuka Y, Tsukita S (1997) Possible involvement of phosphorylation of occludin in tight junction formation. *J Cell Biol* **137**, 1393-1401.
- [21] Yamamoto M, Ramirez SH, Sato S, Kiyota T, Cerny RL, Kaibuchi K, Persidsky Y, Ikezu T (2008) Phosphorylation of claudin-5 and occludin by rho kinase in brain endothelial cells. *Am J Pathol* **172**, 521-533.
- [22] Hirase T, Kawashima S, Wong EY, Ueyama T, Rikitake Y, Tsukita S, Yokoyama M, Staddon JM (2001) Regulation of tight junction permeability and occludin phosphorylation by RhoA-p160ROCK-dependent and -independent mechanisms. *J Biol Chem* **276**, 10423-10431.
- [23] Nagy Z, Goehrlert UG, Wolfe LS, Huttner I (1985) Ca²⁺-depletion-induced disconnection of tight junctions in isolated rat brain microvessels. *Acta Neuropathol* **68**, 48-52.
- [24] Abbott NJ (2000) Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell Mol Neurobiol* **20**, 131-147.
- [25] Abbott NJ, Revest PA (1991) Control of brain endothelial permeability. *Cerebrovasc Brain Metab Rev* **3**, 39-72.
- [26] Antonetti DA, Barber AJ, Hollinger LA, Wolpert EB, Gardner TW (1999) Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors. *J Biol Chem* **274**, 23463-23467.
- [27] Schreiber G, Kooij G, Reijkerker A, van Doorn R, Gringhuis SI, van der Pol S, Weksler BB, Romero IA, Couraud PO, Piontek J, Blasig IE, Dijkstra CD, Ronken E, de Vries H (2007) Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3 kinase, and PKB signaling. *FASEB J* **21**, 3666-3676.
- [28] Jou TS, Schneeberger EE, Nelson WJ (1998) Structural and functional regulation of tight junctions by RhoA and Rac1 small GTPases. *J Cell Biol* **142**, 101-115.
- [29] Hopkins AM, Li D, Mrsny RJ, Walsh SV, Nusrat A (2000) Modulation of tight junction function by G protein-coupled events. *Adv Drug Deliv Rev* **41**, 329-340.
- [30] Stelzner TJ, Weil JV, O'Brien RF (1989) Role of cyclic adenosine monophosphate in the induction of endothelial barrier properties. *J Cell Physiol* **139**, 157-166.
- [31] Rubin LL, Hall DE, Porter S, Barbu K, Cannon C, Horner HC, Janatpour M, Liaw CW, Manning K, Morales J (1991) A cell culture model of the blood-brain barrier. *J Cell Biol* **115**, 1725-1735.
- [32] Wolburg H, Neuhaus J, Kniesel U, Krauss B, Schmid EM, Ocalan M, Farrell C, Risau W (1994) Modulation of tight junction structure in blood-brain barrier endothelial cells. Effects of tissue culture, second messengers and cocultured astrocytes. *J Cell Sci* **107**(Pt 5), 1347-1357.
- [33] Hoane MR, Kaplan SA, Ellis AL (2006) The effects of nicotine on apoptosis and blood-brain barrier breakdown following traumatic brain injury. *Brain Res* **1125**, 185-193.
- [34] McGill JK, Gallagher L, Carswell HV, Irving EA, Dominczak AF, Macrae IM (2005) Impaired functional recovery after stroke in the stroke-prone spontaneously hypertensive rat. *Stroke* **36**, 135-141.
- [35] Minagar A, Alexander JS (2003) Blood-brain barrier disruption in multiple sclerosis. *Mult Scler* **9**, 540-549.
- [36] Petitto CK, Cash KS (1992) Blood-brain barrier abnormalities in the acquired immunodeficiency syndrome: immunohistochemical localization of serum proteins in postmortem brain. *Ann Neurol* **32**, 658-666.
- [37] Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Hulette CM, Vitek MP, Hovanesian V, Stopa EG (2007) Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. *Neurobiol Aging* **28**, 977-986.
- [38] Kortekaas R, Leenders KL, van Oostrom JC, Vaalburg W, Bart J, Willemsen AT, Hendrikse NH (2005) Blood-brain barrier dysfunction in parkinsonian midbrain *in vivo*. *Ann Neurol* **57**, 176-179.
- [39] Wenk GL (2003) Neuropathologic changes in Alzheimer's disease. *J Clin Psychiatry* **64**(Suppl 9), 7-10.
- [40] Hardy J, Allsop D (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol Sci* **12**, 383-388.
- [41] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353-356.
- [42] Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer's disease. *Lancet* **368**, 387-403.
- [43] Knottnerus IL, Ten Cate H, Lodder J, Kessels F, van Oostenbrugge RJ (2009) Endothelial dysfunction in lacunar stroke: a systematic review. *Cerebrovasc Dis* **27**, 519-526.
- [44] Taguchi A (2009) Vascular factors in diabetes and Alzheimer's disease. *J Alzheimers Dis* **16**, 859-864.
- [45] Huber JD (2008) Diabetes, cognitive function, and the blood-brain barrier. *Curr Pharm Des* **14**, 1594-1600.

- [46] Rapp JH, Pan XM, Neumann M, Hong M, Hollenbeck K, Liu J (2008) Microemboli composed of cholesterol crystals disrupt the blood-brain barrier and reduce cognition. *Stroke* **39**, 2354-2361.
- [47] Alafuzoff I, Adolfsson R, Grundke-Iqbal I, Winblad B (1987) Blood-brain barrier in Alzheimer dementia and in non-demented elderly. An immunocytochemical study. *Acta Neuropathol* **73**, 160-166.
- [48] Kalaria RN (1999) The blood-brain barrier and cerebrovascular pathology in Alzheimer's disease. *Ann N Y Acad Sci* **893**, 113-125.
- [49] Kalaria RN (1992) The blood-brain barrier and cerebral microcirculation in Alzheimer disease. *Cerebrovasc Brain Metab Rev* **4**, 226-260.
- [50] Munoz DG, Erkinjuntti T, Gaytan-Garcia S, Hachinski V (1997) Serum protein leakage in Alzheimer's disease revisited. *Ann N Y Acad Sci* **826**, 173-189.
- [51] Skoog I, Wallin A, Fredman P, Hesse C, Aevrsson O, Karlsson I, Gottfries CG, Blennow K (1998) A population study on blood-brain barrier function in 85-year-olds: relation to Alzheimer's disease and vascular dementia. *Neurology* **50**, 966-971.
- [52] Blennow K, Wallin A, Fredman P, Karlsson I, Gottfries CG, Svennerholm L (1990) Blood-brain barrier disturbance in patients with Alzheimer's disease is related to vascular factors. *Acta Neurol Scand* **81**, 323-326.
- [53] Algotsson A, Winblad B (2007) The integrity of the blood-brain barrier in Alzheimer's disease. *Acta Neurol Scand* **115**, 403-408.
- [54] Bowman GL, Kaye JA, Moore M, Waichunas D, Carlson NE, Quinn JF (2007) Blood-brain barrier impairment in Alzheimer disease: stability and functional significance. *Neurology* **68**, 1809-1814.
- [55] Farkas E, Luiten PG (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol* **64**, 575-611.
- [56] Ujjiie M, Dickstein DL, Carlow DA, Jefferies WA (2003) Blood-brain barrier permeability precedes senile plaque formation in an Alzheimer disease model. *Microcirculation* **10**, 463-470.
- [57] Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, Welch D, Manness L, Lin C, Yu J, Zhu H, Ghiso J, Frangione B, Stern A, Schmidt AM, Armstrong DL, Arnold B, Liliensiek B, Nawroth P, Hofman F, Kindy M, Stern D, Zlokovic B (2003) RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. *Nat Med* **9**, 907-913.
- [58] Deane R, Wu Z, Sagare A, Davis J, Du Yan S, Hamm K, Xu F, Parisi M, LaRue B, Hu HW, Spijkers P, Guo H, Song X, Lenting PJ, Van Nostrand WE, Zlokovic BV (2004) LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. *Neuron* **43**, 333-344.
- [59] Deane R, Zlokovic BV (2007) Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease. *Curr Alzheimer Res* **4**, 191-197.
- [60] Zlokovic BV (1996) Cerebrovascular transport of Alzheimer's amyloid beta and apolipoproteins J and E: possible anti-amyloidogenic role of the blood-brain barrier. *Life Sci* **59**, 1483-1497.
- [61] Deane R, Sagare A, Hamm K, Parisi M, Lane S, Finn MB, Holtzman DM, Zlokovic BV (2008) apoE isoform-specific disruption of amyloid beta peptide clearance from mouse brain. *J Clin Invest* **118**, 4002-4013.
- [62] Selkoe DJ (2001) Clearing the brain's amyloid cobwebs. *Neuron* **32**, 177-180.
- [63] Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV (2000) Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest* **106**, 1489-1499.
- [64] Zlokovic BV, Martel CL, Matsubara E, McComb JG, Zheng G, McCluskey RT, Frangione B, Ghiso J (1996) Glycoprotein 330/megalin: probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer disease amyloid beta at the blood-brain and blood-cerebrospinal fluid barriers. *Proc Natl Acad Sci U S A* **93**, 4229-4234.
- [65] Tang K, Hynan LS, Baskin F, Rosenberg RN (2006) Platelet amyloid precursor protein processing: a bio-marker for Alzheimer's disease. *J Neurol Sci* **240**, 53-58.
- [66] Kuo YM, Emmerling MR, Lampert HC, Hempelman SR, Kokjohn TA, Woods AS, Cotter RJ, Roher AE (1999) High levels of circulating Abeta42 are sequestered by plasma proteins in Alzheimer's disease. *Biochem Biophys Res Commun* **257**, 787-791.
- [67] Kuo YM, Kokjohn TA, Watson MD, Woods AS, Cotter RJ, Sue LI, Kalback WM, Emmerling MR, Beach TG, Roher AE (2000) Elevated abeta42 in skeletal muscle of Alzheimer disease patients suggests peripheral alterations of AbetaPP metabolism. *Am J Pathol* **156**, 797-805.
- [68] Kalaria RN, Premkumar DR, Pax AB, Cohen DL, Lieberburg I (1996) Production and increased detection of amyloid beta protein and amyloidogenic fragments in brain microvessels, meningeal vessels and choroid plexus in Alzheimer's disease. *Brain Res Mol Brain Res* **35**, 58-68.
- [69] Natte R, de Boer WI, Maat-Schieman ML, Baelde HJ, Vinters HV, Roos RA, van Duinen SG (1999) Amyloid beta precursor protein-mRNA is expressed throughout cerebral vessel walls. *Brain Res* **828**, 179-183.
- [70] Miklossy J (2008) Chronic inflammation and amyloidogenesis in Alzheimer's disease – role of Spirochetes. *J Alzheimers Dis* **13**, 381-391.
- [71] Farkas IG, Czigner A, Farkas E, Dobo E, Soos K, Penke B, Endresz V, Mihaly A (2003) Beta-amyloid peptide-induced blood-brain barrier disruption facilitates T-cell entry into the rat brain. *Acta Histochem* **105**, 115-125.
- [72] Racke MM, Boone LI, Hepburn DL, Parsadainian M, Bryan MT, Ness DK, Piroozzi KS, Jordan WH, Brown DD, Hoffmann WP, Holtzman DM, Bales KR, Gitter BD, May PC, Paul SM, DeMattos RB (2005) Exacerbation of cerebral amyloid angiopathy-associated microhemorrhage in amyloid precursor protein transgenic mice by immunotherapy is dependent on antibody recognition of deposited forms of amyloid beta. *J Neurosci* **25**, 629-636.
- [73] Dickstein DL, Biron KE, Ujjiie M, Pfeifer CG, Jeffries AR, Jefferies WA (2006) Abeta peptide immunization restores blood-brain barrier integrity in Alzheimer disease. *FASEB J* **20**, 426-433.
- [74] Jancso G, Domoki F, Santha P, Varga J, Fischer J, Orosz K, Penke B, Becskei A, Dux M, Toth L (1998) Beta-amyloid (1-42) peptide impairs blood-brain barrier function after intracarotid infusion in rats. *Neurosci Lett* **253**, 139-141.
- [75] Eisenhauer PB, Johnson RJ, Wells JM, Davies TA, Fine RE (2000) Toxicity of various amyloid beta peptide species in cultured human blood-brain barrier endothelial cells: increased toxicity of dutch-type mutant. *J Neurosci Res* **60**, 804-810.

- [76] Sparks DL, Martin TA, Gross DR, Hunsaker JC, 3rd (2000) Link between heart disease, cholesterol, and Alzheimer's disease: a review. *Microsc Res Tech* **50**, 287-290.
- [77] Kivipelto M, Helkala EL, Hanninen T, Laakso MP, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J, Nissinen A (2001) Midlife vascular risk factors and late-life mild cognitive impairment: A population-based study. *Neurology* **56**, 1683-1689.
- [78] Sjogren M, Mielke M, Gustafson D, Zandi P, Skoog I (2006) Cholesterol and Alzheimer's disease – is there a relation? *Mech Ageing Dev* **127**, 138-147.
- [79] Jurevics H, Morell P (1995) Cholesterol for synthesis of myelin is made locally, not imported into brain. *J Neurochem* **64**, 895-901.
- [80] Maxfield FR, Tabas I (2005) Role of cholesterol and lipid organization in disease. *Nature* **438**, 612-621.
- [81] Marzolo MP, Bu G (2009) Lipoprotein receptors and cholesterol in APP trafficking and proteolytic processing, implications for Alzheimer's disease. *Semin Cell Dev Biol* **20**, 191-200.
- [82] Sing CF, Davignon J (1985) Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. *Am J Hum Genet* **37**, 268-285.
- [83] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921-923.
- [84] Hofman A, Ott A, Breteler MM, Bots ML, Slooter AJ, van Harskamp F, van Duijn CN, Van Broeckhoven C, Grobbee DE (1997) Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* **349**, 151-154.
- [85] Notkola IL, Sulkava R, Pekkanen J, Erkinjuntti T, Ehnholm C, Kivinen P, Tuomilehto J, Nissinen A (1998) Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. *Neuroepidemiology* **17**, 14-20.
- [86] Sparks DL, Scheff SW, Hunsaker JC, 3rd, Liu H, Landers T, Gross DR (1994) Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp Neurol* **126**, 88-94.
- [87] Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K (1998) Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci U S A* **95**, 6460-6464.
- [88] Frears ER, Stephens DJ, Walters CE, Davies H, Austen BM (1999) The role of cholesterol in the biosynthesis of beta-amyloid. *Neuroreport* **10**, 1699-1705.
- [89] Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, Runz H, Kuhl S, Bertsch T, von Bergmann K, Hennerici M, Beyreuther K, Hartmann T (2001) Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Abeta 42 and Abeta 40 *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A* **98**, 5856-5861.
- [90] Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA (2000) Statins and the risk of dementia. *Lancet* **356**, 1627-1631.
- [91] Wolozin B, Kellman W, Ruosseau P, Celesia GG, Siegel G (2000) Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arch Neurol* **57**, 1439-1443.
- [92] Ghribi O, Golovko MY, Larsen B, Schrag M, Murphy EJ (2006) Deposition of iron and beta-amyloid plaques is associated with cortical cellular damage in rabbits fed with long-term cholesterol-enriched diets. *J Neurochem* **99**, 438-449.
- [93] Ghribi O, Larsen B, Schrag M, Herman MM (2006) High cholesterol content in neurons increases BACE, beta-amyloid, and phosphorylated tau levels in rabbit hippocampus. *Exp Neurol* **200**, 460-467.
- [94] Woodruff-Pak DS, Agelan A, Del Valle L (2007) A rabbit model of Alzheimer's disease: valid at neuropathological, cognitive, and therapeutic levels. *J Alzheimers Dis* **11**, 371-383.
- [95] Sparks DL, Schreurs BG (2003) Trace amounts of copper in water induce beta-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proc Natl Acad Sci U S A* **100**, 11065-11069.
- [96] Chen X, Gawryluk JW, Wagener JF, Ghribi O, Geiger JD (2008) Caffeine blocks disruption of blood brain barrier in a rabbit model of Alzheimer's disease. *J Neuroinflammation* **5**, 12.
- [97] Ferreira A, Caceres A, Kosik KS (1993) Intraneuronal compartments of the amyloid precursor protein. *J Neurosci* **13**, 3112-3123.
- [98] Perez RG, Soriano S, Hayes JD, Ostaszewski B, Xia W, Selkoe DJ, Chen X, Stokin GB, Koo EH (1999) Mutagenesis identifies new signals for beta-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including Abeta42. *J Biol Chem* **274**, 18851-18856.
- [99] Soriano S, Chyung AS, Chen X, Stokin GB, Lee VM, Koo EH (1999) Expression of beta-amyloid precursor protein-CD3gamma chimeras to demonstrate the selective generation of amyloid beta(1-40) and amyloid beta(1-42) peptides within secretory and endocytic compartments. *J Biol Chem* **274**, 32295-32300.
- [100] Chyung JH, Selkoe DJ (2003) Inhibition of receptor-mediated endocytosis demonstrates generation of amyloid beta-protein at the cell surface. *J Biol Chem* **278**, 51035-51043.
- [101] Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286**, 735-741.
- [102] Shimizu H, Tosaki A, Kaneko K, Hisano T, Sakurai T, Nukina N (2008) Crystal structure of an active form of BACE1, an enzyme responsible for amyloid beta protein production. *Mol Cell Biol* **28**, 3663-3671.
- [103] Rajendran L, Schneider A, Schlechtingen G, Weidlich S, Ries J, Braxmeier T, Schwiller P, Schulz JB, Schroeder C, Simons M, Jennings G, Knölker HJ, Simons K (2008) Efficient inhibition of the Alzheimer's disease beta-secretase by membrane targeting. *Science* **320**, 520-523.
- [104] Cataldo AM, Petanceska S, Terio NB, Peterhoff CM, Durham R, Mercken M, Mehta PD, Buxbaum J, Haroutunian V, Nixon RA (2004) Abeta localization in abnormal endosomes: association with earliest Abeta elevations in AD and Down syndrome. *Neurobiol Aging* **25**, 1263-1272.
- [105] Cataldo AM, Peterhoff CM, Troncoso JC, Gomez-Isla T, Hyman BT, Nixon RA (2000) Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. *Am J Pathol* **157**, 277-286.

- [106] Ohyagi Y (2008) Intracellular amyloid beta-protein as a therapeutic target for treating Alzheimer's disease. *Curr Alzheimer Res* **5**, 555-561.
- [107] Vance JE, Karten B, Hayashi H (2006) Lipid dynamics in neurons. *Biochem Soc Trans* **34**, 399-403.
- [108] Meresse S, Delbart C, Fruchart JC, Cecchelli R (1989) Low-density lipoprotein receptor on endothelium of brain capillaries. *J Neurochem* **53**, 340-345.
- [109] Dehouck B, Fenart L, Dehouck MP, Pierce A, Torpier G, Cecchelli R (1997) A new function for the LDL receptor: transcytosis of LDL across the blood-brain barrier. *J Cell Biol* **138**, 877-889.
- [110] Ignatius MJ, Gebicke-Haerter PJ, Pitas RE, Shooter EM (1987) Apolipoprotein E in nerve injury and repair. *Prog Brain Res* **71**, 177-184.
- [111] Swanson LW, Simmons DM, Hofmann SL, Goldstein JL, Brown MS (1988) Localization of mRNA for low density lipoprotein receptor and a cholesterol synthetic enzyme in rabbit nervous system by in situ hybridization. *Proc Natl Acad Sci U S A* **85**, 9821-9825.
- [112] Beffert U, Danik M, Krzykowski P, Ramassamy C, Berrada F, Poirier J (1998) The neurobiology of apolipoproteins and their receptors in the CNS and Alzheimer's disease. *Brain Res Brain Res Rev* **27**, 119-142.
- [113] Waldron E, Jaeger S, Pietrzik CU (2006) Functional role of the low-density lipoprotein receptor-related protein in Alzheimer's disease. *Neurodegener Dis* **3**, 233-238.
- [114] Pietrzik CU, Busse T, Merriam DE, Weggen S, Koo EH (2002) The cytoplasmic domain of the LDL receptor-related protein regulates multiple steps in APP processing. *Embo J* **21**, 5691-5700.
- [115] Pietrzik CU, Yoon IS, Jaeger S, Busse T, Weggen S, Koo EH (2004) FE65 constitutes the functional link between the low-density lipoprotein receptor-related protein and the amyloid precursor protein. *J Neurosci* **24**, 4259-4265.
- [116] Bu G, Cam J, Zerbinatti C (2006) LRP in amyloid-beta production and metabolism. *Ann N Y Acad Sci* **1086**, 35-53.
- [117] Kounnas MZ, Moir RD, Rebeck GW, Bush AI, Argraves WS, Tanzi RE, Hyman BT, Strickland DK (1995) LDL receptor-related protein, a multifunctional ApoE receptor, binds secreted beta-amyloid precursor protein and mediates its degradation. *Cell* **82**, 331-340.
- [118] Ulery PG, Beers J, Mikhailenko I, Tanzi RE, Rebeck GW, Hyman BT, Strickland DK (2000) Modulation of beta-amyloid precursor protein processing by the low density lipoprotein receptor-related protein (LRP). Evidence that LRP contributes to the pathogenesis of Alzheimer's disease. *J Biol Chem* **275**, 7410-7415.
- [119] Holland JA, Pritchard KA, Rogers NJ, Stemeran MB (1992) Atherogenic levels of low-density lipoprotein increase endocytotic activity in cultured human endothelial cells. *Am J Pathol* **140**, 551-558.
- [120] Guretzki HJ, Gerbitz KD, Olgemoller B, Schleicher E (1994) Atherogenic levels of low density lipoprotein alter the permeability and composition of the endothelial barrier. *Atherosclerosis* **107**, 15-24.
- [121] Calne DB, Langston JW (1983) Aetiology of Parkinson's disease. *Lancet* **2**, 1457-1459.
- [122] Hunot S, Hirsch EC (2003) Neuroinflammatory processes in Parkinson's disease. *Ann Neurol* **53 Suppl 3**, S49-58; discussion S58-60.
- [123] Rogers J, Kovelowski CJ (2003) Inflammatory Mechanisms in Parkinson's Disease In *Neuroinflammation, 2nd Edition: Mechanisms and Management*, Wood PL, ed. Humana Press Inc., Totowa, NJ, pp. 391-403.
- [124] Hawkins BT, Davis TP (2005) The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* **57**, 173-185.
- [125] Abbott NJ, Ronnback L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* **7**, 41-53.
- [126] Stolp HB, Dziegielewska KM (2009) Review: Role of developmental inflammation and blood-brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases. *Neuropathol Appl Neurobiol* **35**, 132-146.
- [127] Weiss N, Miller F, Cazaubon S, Couraud PO (2009) The blood-brain barrier in brain homeostasis and neurological diseases. *Biochim Biophys Acta* **1788**, 842-857.
- [128] Gupta A, Agarwal R, Shukla GS (1999) Functional impairment of blood-brain barrier following pesticide exposure during early development in rats. *Hum Exp Toxicol* **18**, 174-179.
- [129] Behzadian MA, Wang XL, Windsor LJ, Ghaly N, Caldwell RB (2001) TGF-beta increases retinal endothelial cell permeability by increasing MMP-9: possible role of glial cells in endothelial barrier function. *Invest Ophthalmol Vis Sci* **42**, 853-859.
- [130] Pflugfelder SC, Farley W, Luo L, Chen LZ, de Paiva CS, Olmos LC, Li DQ, Fini ME (2005) Matrix metalloproteinase-9 knockout confers resistance to corneal epithelial barrier disruption in experimental dry eye. *Am J Pathol* **166**, 61-71.
- [131] Asahi M, Wang X, Mori T, Sumii T, Jung JC, Moskowitz MA, Fini ME, Lo EH (2001) Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. *J Neurosci* **21**, 7724-7732.
- [132] Petty MA, Lo EH (2002) Junctional complexes of the blood-brain barrier: permeability changes in neuroinflammation. *Prog Neurobiol* **68**, 311-323.
- [133] Zhao C, Ling Z, Newman MB, Bhatia A, Carvey PM (2007) TNF-alpha knockout and minocycline treatment attenuates blood-brain barrier leakage in MPTP-treated mice. *Neurobiol Dis* **26**, 36-46.
- [134] Faucheux BA, Bonnet AM, Agid Y, Hirsch EC (1999) Blood vessels change in the mesencephalon of patients with Parkinson's disease. *Lancet* **353**, 981-982.
- [135] Carvey PM, Zhao CH, Hendey B, Lum H, Trachtenberg J, Desai BS, Snyder J, Zhu YG, Ling ZD (2005) 6-Hydroxydopamine-induced alterations in blood-brain barrier permeability. *Eur J Neurosci* **22**, 1158-1168.
- [136] Rite I, Machado A, Cano J, Venero JL (2007) Blood-brain barrier disruption induces *in vivo* degeneration of nigral dopaminergic neurons. *J Neurochem* **101**, 1567-1582.
- [137] Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* **51**, 83-133.
- [138] Nehlig A, Daval JL, Debry G (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res Brain Res Rev* **17**, 139-170.
- [139] Olah ME, Stiles GL (1995) Adenosine receptor subtypes: characterization and therapeutic regulation. *Annu Rev Pharmacol Toxicol* **35**, 581-606.
- [140] Jacobson KA, von Lubitz DK, Daly JW, Fredholm BB (1996) Adenosine receptor ligands: differences with acute versus chronic treatment. *Trends Pharmacol Sci* **17**, 108-113.

- [141] Varani K, Portaluppi F, Merighi S, Ongini E, Belardinelli L, Borea PA (1999) Caffeine alters A2A adenosine receptors and their function in human platelets. *Circulation* **99**, 2499-2502.
- [142] Biaggioni I, Paul S, Puckett A, Arzubia C (1991) Caffeine and theophylline as adenosine receptor antagonists in humans. *J Pharmacol Exp Ther* **258**, 588-593.
- [143] Fredholm BB, Hedqvist P (1980) Modulation of neurotransmission by purine nucleotides and nucleosides. *Biochem Pharmacol* **29**, 1635-1643.
- [144] Dall'Igna OP, Porciuncula LO, Souza DO, Cunha RA, Lara DR (2003) Neuroprotection by caffeine and adenosine A2A receptor blockade of beta-amyloid neurotoxicity. *Br J Pharmacol* **138**, 1207-1209.
- [145] Rudolph KA, Keil M, Fastbom J, Fredholm BB (1989) Ischaemic damage in gerbil hippocampus is reduced following upregulation of adenosine (A1) receptors by caffeine treatment. *Neurosci Lett* **103**, 275-280.
- [146] Sutherland GR, Peeling J, Lesiuk HJ, Brownstone RM, Rydzy M, Saunders JK, Geiger JD (1991) The effects of caffeine on ischemic neuronal injury as determined by magnetic resonance imaging and histopathology. *Neuroscience* **42**, 171-182.
- [147] Bona E, Aden U, Fredholm BB, Hagberg H (1995) The effect of long term caffeine treatment on hypoxic-ischemic brain damage in the neonate. *Pediatr Res* **38**, 312-318.
- [148] Lindsay J, Laurin D, Verreault R, Hebert R, Helliwell B, Hill GB, McDowell I (2002) Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging. *Am J Epidemiol* **156**, 445-453.
- [149] Arendash GW, Schleif W, Rezai-Zadeh K, Jackson EK, Zacharia LC, Cracchiolo JR, Shippy D, Tan J (2006) Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain beta-amyloid production. *Neuroscience* **142**, 941-952.
- [150] Maia L, de Mendonca A (2002) Does caffeine intake protect from Alzheimer's disease? *Eur J Neurol* **9**, 377-382.
- [151] Ritchie K, Carriere I, de Mendonca A, Portet F, Dartigues JF, Rouaud O, Barberger-Gateau P, Ancelin ML (2007) The neuroprotective effects of caffeine: a prospective population study (the Three City Study). *Neurology* **69**, 536-545.
- [152] Benedetti MD, Bower JH, Maraganore DM, McDonnell SK, Peterson BJ, Ahlsgog JE, Schaid DJ, Rocca WA (2000) Smoking, alcohol, and coffee consumption preceding Parkinson's disease: a case-control study. *Neurology* **55**, 1350-1358.
- [153] Ross GW, Abbott RD, Petrovitch H, Morens DM, Grandinetti A, Tung KH, Tanner CM, Masaki KH, Blanchette PL, Curb JD, Popper JS, White LR (2000) Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA* **283**, 2674-2679.
- [154] Ross GW, Abbott RD, Petrovitch H, White LR, Tanner CM (2000) Relationship between caffeine intake and parkinson disease. *JAMA* **284**, 1378-1379.
- [155] Ascherio A, Zhang SM, Hernan MA, Kawachi I, Colditz GA, Speizer FE, Willett WC (2001) Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. *Ann Neurol* **50**, 56-63.
- [156] Higdon JV, Frei B (2006) Coffee and health: a review of recent human research. *Crit Rev Food Sci Nutr* **46**, 101-123.
- [157] Rosso A, Mossey J, Lippa CF (2008) Caffeine: neuroprotective functions in cognition and Alzheimer's disease. *Am J Alzheimers Dis Other Demen* **23**, 417-422.
- [158] Daly JW (2007) Caffeine analogs: biomedical impact. *Cell Mol Life Sci* **64**, 2153-2169.
- [159] Johnson-Kozlow M, Kritiz-Silverstein D, Barrett-Connor E, Morton D (2002) Coffee consumption and cognitive function among older adults. *Am J Epidemiol* **156**, 842-850.
- [160] van Gelder BM, Buijsse B, Tijhuis M, Kalmijn S, Giampaoli S, Nissinen A, Kromhout D (2007) Coffee consumption is inversely associated with cognitive decline in elderly European men: the FINE Study. *Eur J Clin Nutr* **61**, 226-232.
- [161] van Boxtel MP, Schmitt JA, Bosma H, Jolles J (2003) The effects of habitual caffeine use on cognitive change: a longitudinal perspective. *Pharmacol Biochem Behav* **75**, 921-927.
- [162] Eskelinen MH, Ngandu T, Tuomilehto J, Soininen H, Kivipelto M (2009) Midlife coffee and tea drinking and the risk of late-life dementia: a population-based CAIDE study. *J Alzheimers Dis* **16**, 85-91.
- [163] Arendash GW, Mori T, Cao C, Mamcarz M, Runfeldt M, Dickson A, Rezai-Zadeh K, Tan J, Citron BA, Lin X, Echeverria V, Potter H (2009) Caffeine reverses cognitive impairment and decreases brain amyloid-beta levels in aged Alzheimer's disease mice. *J Alzheimers Dis* **17**, 661-680.
- [164] Cao C, Cirrito JR, Lin X, Wang L, Verges DK, Dickson A, Mamcarz M, Zhang C, Mori T, Arendash GW, Holtzman DM, Potter H (2009) Caffeine suppresses amyloid-beta levels in plasma and brain of Alzheimer's disease transgenic mice. *J Alzheimers Dis* **17**, 681-697.
- [165] Fuxe K, Ungerstedt U (1974) Action of caffeine and theophyllamine on supersensitive dopamine receptors: considerable enhancement of receptor response to treatment with DOPA and dopamine receptor agonists. *Med Biol* **52**, 48-54.
- [166] Chen JF, Xu K, Petzer JP, Staal R, Xu YH, Beilstein M, Sonsalla PK, Castagnoli K, Castagnoli N, Jr., Schwarzschild MA (2001) Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson's disease. *J Neurosci* **21**, RC143, 141-146.
- [167] Geiger JD, Buscemi L, Fotheringham JA (2006) *Role of Adenosine in the Control of Inflammatory Events Associated With Acute and Chronic Neurodegenerative Disorders*, CRC Taylor and Francis.
- [168] Schwarzschild MA (2007) Adenosine A2A antagonists as neurotherapeutics: crossing the bridge. *Prog Neurobiol* **83**, 261-262.
- [169] de Mendonca A, Sebastiao AM, Ribeiro JA (2000) Adenosine: does it have a neuroprotective role after all? *Brain Res Brain Res Rev* **33**, 258-274.
- [170] Aoyama S, Kase H, Borrelli E (2000) Rescue of locomotor impairment in dopamine D2 receptor-deficient mice by an adenosine A2A receptor antagonist. *J Neurosci* **20**, 5848-5852.
- [171] Ikeda K, Kurokawa M, Aoyama S, Kuwana Y (2002) Neuroprotection by adenosine A2A receptor blockade in experimental models of Parkinson's disease. *J Neurochem* **80**, 262-270.
- [172] Morelli M (2003) Adenosine A2A antagonists: potential preventive and palliative treatment for Parkinson's disease. *Exp Neurol* **184**, 20-23.
- [173] Xu K, Bastia E, Schwarzschild M (2005) Therapeutic potential of adenosine A(2A) receptor antagonists in Parkinson's disease. *Pharmacol Ther* **105**, 267-310.
- [174] Pierri M, Vaudano E, Sager T, Englund U (2005) KW-6002 protects from MPTP induced dopaminergic toxicity in the mouse. *Neuropharmacology* **48**, 517-524.
- [175] LeWitt PA, Guttman M, Tetrad JW, Tuite PJ, Mori A, Chaikin P, Sussman NM (2008) Adenosine A2A receptor antagonist

- istradefylline (KW-6002) reduces "off" time in Parkinson's disease: a double-blind, randomized, multicenter clinical trial (6002-US-005). *Ann Neurol* **63**, 295-302.
- [176] Jankovic J (2008) Are adenosine antagonists, such as istradefylline, caffeine, and chocolate, useful in the treatment of Parkinson's disease? *Ann Neurol* **63**, 267-269.
- [177] Chen X, Lan X, Roche I, Liu R, Geiger JD (2008) Caffeine protects against MPTP-induced blood-brain barrier dysfunction in mouse striatum. *J Neurochem* **107**, 1147-1157.
- [178] Phillis JW (1989) Adenosine in the control of the cerebral circulation. *Cerebrovasc Brain Metab Rev* **1**, 26-54.
- [179] Schaddelee MP, Voorwinden HL, van Tilburg EW, Pateman TJ, Ijzerman AP, Danhof M, de Boer AG (2003) Functional role of adenosine receptor subtypes in the regulation of blood-brain barrier permeability: possible implications for the design of synthetic adenosine derivatives. *Eur J Pharm Sci* **19**, 13-22.
- [180] Eckle T, Faigle M, Grenz A, Laucher S, Thompson LF, Eltzschig HK (2008) A2B adenosine receptor dampens hypoxia-induced vascular leak. *Blood* **111**, 2024-2035.
- [181] Laghi Pasini F, Guideri F, Picano E, Parenti G, Petersen C, Varga A, Di Perri T (2000) Increase in plasma adenosine during brain ischemia in man: a study during transient ischemic attacks, and stroke. *Brain Res Bull* **51**, 327-330.
- [182] Frenguelli BG, Wigmore G, Laudet E, Dale N (2007) Temporal and mechanistic dissociation of ATP and adenosine release during ischaemia in the mammalian hippocampus. *J Neurochem* **101**, 1400-1413.
- [183] Pearson T, Damian K, Lynas RE, Frenguelli BG (2006) Sustained elevation of extracellular adenosine and activation of A1 receptors underlie the post-ischaemic inhibition of neuronal function in rat hippocampus *in vitro*. *J Neurochem* **97**, 1357-1368.
- [184] Pignataro G, Maysami S, Studer FE, Wilz A, Simon RP, Boison D (2008) Downregulation of hippocampal adenosine kinase after focal ischemia as potential endogenous neuroprotective mechanism. *J Cereb Blood Flow Metab* **28**, 17-23.
- [185] Folcik VA, Smith T, O'Bryant S, Kawczak JA, Zhu B, Sakurai H, Kajiwarra A, Staddon JM, Glabinski A, Chernosky AL, Tani M, Johnson JM, Tuohy VK, Rubin LL, Ransohoff RM (1999) Treatment with BBB022A or rolipram stabilizes the blood-brain barrier in experimental autoimmune encephalomyelitis: an additional mechanism for the therapeutic effect of type IV phosphodiesterase inhibitors. *J Neuroimmunol* **97**, 119-128.
- [186] van Schaick EA, Zuideveld KP, Tukker HE, Langemeijer MW, Ijzerman AP, Danhof M (1998) Metabolic and cardiovascular effects of the adenosine A1 receptor agonist N6-(p-sulphophenyl)adenosine in diabetic Zucker rats: influence of the disease on the selectivity of action. *J Pharmacol Exp Ther* **287**, 21-30.
- [187] Schoelch C, Kuhlmann J, Gossel M, Mueller G, Neumann-Haefelin C, Belz U, Kalisch J, Biemer-Daub G, Kramer W, Juretschke HP, Herling AW (2004) Characterization of adenosine-A1 receptor-mediated antilipolysis in rats by tissue microdialysis, 1H-spectroscopy, and glucose clamp studies. *Diabetes* **53**, 1920-1926.
- [188] Reiss AB, Rahman MM, Chan ES, Montesinos MC, Awadallah NW, Cronstein BN (2004) Adenosine A2A receptor occupancy stimulates expression of proteins involved in reverse cholesterol transport and inhibits foam cell formation in macrophages. *J Leukoc Biol* **76**, 727-734.
- [189] Gonzalez C, Klein G, Satre M (1990) Caffeine, an inhibitor of endocytosis in Dictyostelium discoideum amoebae. *J Cell Physiol* **144**, 408-415.
- [190] Aubry L, Klein G, Satre M (1993) Endo-lysosomal acidification in Dictyostelium discoideum amoebae. Effects of two endocytosis inhibitors: caffeine and cycloheximide. *Eur J Cell Biol* **61**, 225-228.
- [191] Carini R, Castino R, De Cesaris MG, Splendore R, Demoz M, Albano E, Isidoro C (2004) Preconditioning-induced cytoprotection in hepatocytes requires Ca(2+)-dependent exocytosis of lysosomes. *J Cell Sci* **117**, 1065-1077.
- [192] Liu J, Lu W, Reigada D, Nguyen J, Laties AM, Mitchell CH (2008) Restoration of lysosomal pH in RPE cells from cultured human and ABCA4(-/-) mice: pharmacologic approaches and functional recovery. *Invest Ophthalmol Vis Sci* **49**, 772-780.
- [193] McGeer PL, McGeer EG (2008) Glial reactions in Parkinson's disease. *Mov Disord* **23**, 474-483.
- [194] Schwab C, McGeer PL (2008) Inflammatory aspects of Alzheimer disease and other neurodegenerative disorders. *J Alzheimers Dis* **13**, 359-369.
- [195] Mogi M, Togari A, Ogawa M, Ikeguchi K, Shizuma N, Fan D, Nakano I, Nagatsu T (1998) Effects of repeated systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to mice on interleukin-1beta and nerve growth factor in the striatum. *Neurosci Lett* **250**, 25-28.
- [196] Przedborski S, Jackson-Lewis V (1998) Experimental developments in movement disorders: update on proposed free radical mechanisms. *Curr Opin Neurol* **11**, 335-339.
- [197] Tichauer J, Saud K, von Bernhardt R (2007) Modulation by astrocytes of microglial cell-mediated neuroinflammation: effect on the activation of microglial signaling pathways. *Neuroimmunomodulation* **14**, 168-174.
- [198] Koutsilieri E, Scheller C, Grunblatt E, Nara K, Li J, Riederer P (2002) Free radicals in Parkinson's disease. *J Neurol* **249**(Suppl 2), II1-5.
- [199] Schwaninger M, Neher M, Viegas E, Schneider A, Spranger M (1997) Stimulation of interleukin-6 secretion and gene transcription in primary astrocytes by adenosine. *J Neurochem* **69**, 1145-1150.
- [200] Hasko G, Pacher P, Vizi ES, Illes P (2005) Adenosine receptor signaling in the brain immune system. *Trends Pharmacol Sci* **26**, 511-516.
- [201] Horrigan LA, Kelly JP, Connor TJ (2006) Immunomodulatory effects of caffeine: friend or foe? *Pharmacol Ther* **111**, 877-892.
- [202] Yu L, Huang Z, Mariani J, Wang Y, Moskowitz M, Chen JF (2004) Selective inactivation or reconstitution of adenosine A2A receptors in bone marrow cells reveals their significant contribution to the development of ischemic brain injury. *Nat Med* **10**, 1081-1087.
- [203] Shaftel SS, Griffin WS, O'Banion MK (2008) The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. *J Neuroinflammation* **5**, 7.
- [204] Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* **298**, 789-791.
- [205] Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol* **30**, 572-580.
- [206] Spires-Jones TL, Meyer-Luehmann M, Osetek JD, Jones PB, Stern EA, Bacskai BJ, Hyman BT (2007) Impaired spine

- stability underlies plaque-related spine loss in an Alzheimer's disease mouse model. *Am J Pathol* **171**, 1304-1311.
- [207] Dong H, Martin MV, Chambers S, Csernansky JG (2007) Spatial relationship between synapse loss and beta-amyloid deposition in Tg2576 mice. *J Comp Neurol* **500**, 311-321.
- [208] Rutten BP, Van der Kolk NM, Schafer S, van Zandvoort MA, Bayer TA, Steinbusch HW, Schmitz C (2005) Age-related loss of synaptophysin immunoreactive presynaptic boutons within the hippocampus of APP751SL, PS1M146L, and APP751SL/PS1M146L transgenic mice. *Am J Pathol* **167**, 161-173.
- [209] Prescott IA, Dostrovsky JO, Moro E, Hodaie M, Lozano AM, Hutchison WD (2009) Levodopa enhances synaptic plasticity in the substantia nigra pars reticulata of Parkinson's disease patients. *Brain* **132**, 309-318.
- [210] Calabresi P, Maj R, Pisani A, Mercuri NB, Bernardi G (1992) Long-term synaptic depression in the striatum: physiological and pharmacological characterization. *J Neurosci* **12**, 4224-4233.
- [211] Calabresi P, Galletti F, Saggese E, Ghiglieri V, Picconi B (2007) Neuronal networks and synaptic plasticity in Parkinson's disease: beyond motor deficits. *Parkinsonism Relat Disord* **13 Suppl 3**, S259-262.
- [212] Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopamine-mediated regulation of corticostriatal synaptic plasticity. *Trends Neurosci* **30**, 211-219.
- [213] Goldberg MS, Pisani A, Haburcak M, Vortherms TA, Kitada T, Costa C, Tong Y, Martella G, Tschertner A, Martins A, Bernardi G, Roth BL, Pothos EN, Calabresi P, Shen J (2005) Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. *Neuron* **45**, 489-496.