Blood-brain barrier disturbances in diabetes-associated dementia: Therapeutic potential for cannabinoids

Running title

Therapeutic potential of cannabinoids in diabetes associated dementia

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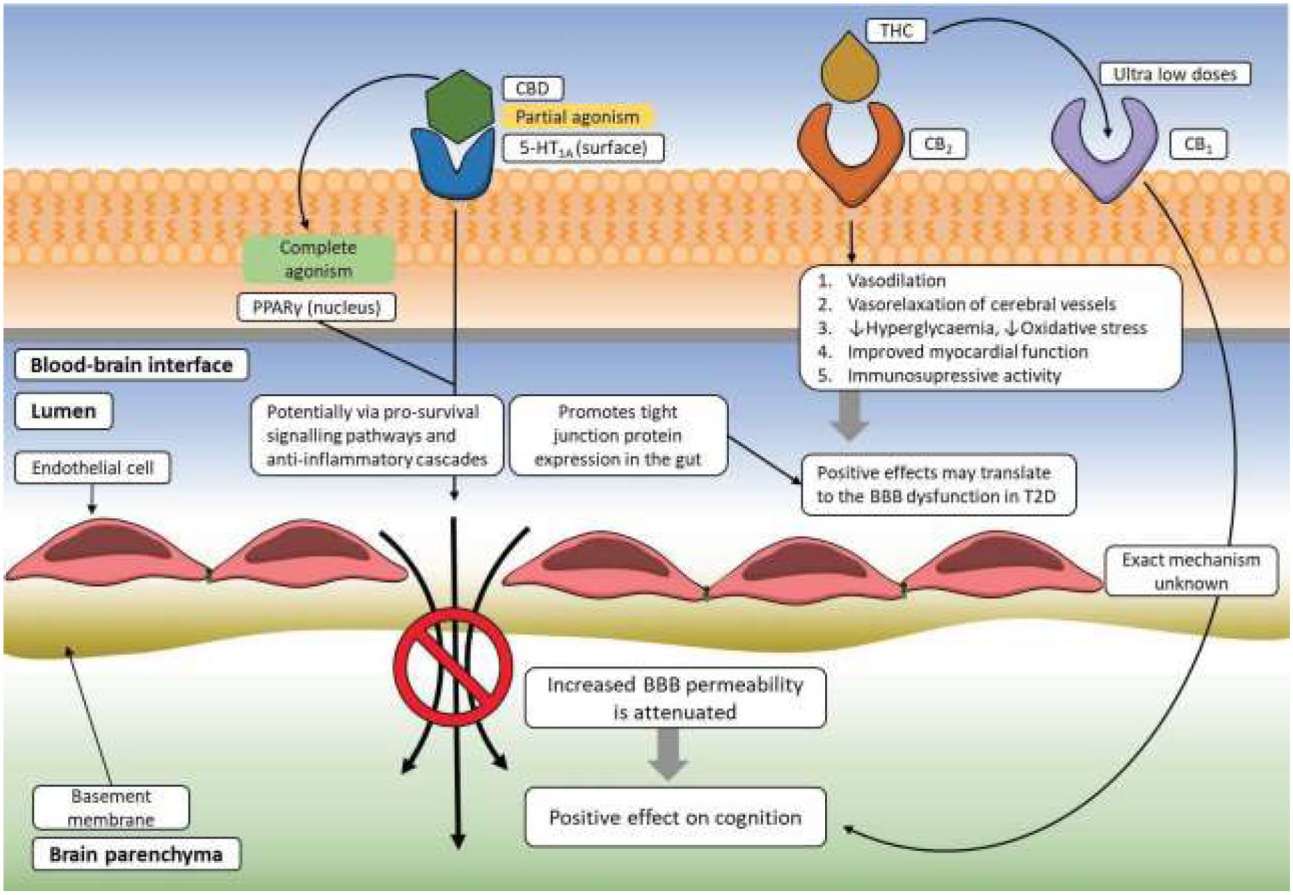
Graphical Abstract



Abstract

Type-2 diabetes (T2D) increases the risk of dementia by ~5-fold, however the mechanisms by which T2D increases dementia risk remain unclear. Evidence suggests that the heightened inflammation and oxidative stress in T2D may lead to disruption of the blood-brain barrier (BBB), which precedes premature cognitive decline. Studies show that vascular-targeted anti-inflammatory treatments protect the BBB by attenuating neuroinflammation, and in some studies attenuate cognitive decline. Yet, this potential pathway is understudied in T2D-associated cognitive impairment. In recent years, therapeutic potential of cannabinoids has gained much interest. The two major cannabinoids, cannabidiol and tetrahydrocannabinol, exert anti-inflammatory and vascular protective effects, however few studies report their potential for reversing BBB dysfunction, particularly in T2D. Therefore, in this review, we summarize the current findings on the role of BBB dysfunction in T2D-

associated dementia and consider the potential therapeutic use of cannabinoids as a protectant of cerebrovascular BBB protection.

Keywords

Blood-brain barrier; diabetes; dementia; cannabidiol; cognitive impairment; inflammation; tetrahydrocannabidiol

Abbreviations

5-HT_{1A}, 5-Hydroxytryptamine$_{1A}$ receptor; BBB, blood-brain barrier; CBD, cannabidiol; EC, endothelial cell; PPARγ, peroxisome proliferator receptor-gamma; T2D, type-2 diabetes; THC, tetrahydrocannabinol;

Introduction

Type-2 diabetes (T2D) is a chronic metabolic disorder that has been linked to numerous other age-related diseases such as cardiovascular disease and dementia. Indeed, reported by both cross-sectional and longitudinal studies, T2D significantly increases the risk of developing dementia prematurely and thus, is an established risk factor for dementia. Recent lines of evidence suggest that disturbances to the function and structure of cerebral capillary blood-brain barrier (BBB) are a pivotal and early event that precedes cognitive decline and by extension, dementia. Consequently, compromised brain capillaries may be central to the onset and progression of dementia. Interestingly, emerging evidence also suggests that the BBB is compromised in T2D. As such, protecting BBB integrity may be a treatment target for T2D-associated dementia.

The BBB is a key interface separating circulating blood compounds and brain. However, the BBB is susceptible to chronic systemic inflammation. Chronic systemic inflammation leads to cerebral and neuronal inflammation, thereby compromising BBB integrity. Thus, it is important to explore potential anti-inflammatory therapies, which can protect and restore the integrity and function of brain capillaries.

In recent years, research surrounding the therapeutic potential of cannabinoids as an anti-inflammatory agent has gained significant interest. Most notably, cannabidiol (CBD), which is the second most abundant compound derived from Cannabis. It exhibits not only potent anti-inflammatory activity but is also able to modulate endothelial and epithelial barriers, and ameliorate cognitive deficits. Other lines of research have focused on the therapeutic potential of delta-9-tetrahydrocannabinol (THC). Although, THC is responsible for the psychoactive effects associated with the use of Cannabis, emerging research suggests that THC may also possess therapeutic potential for T2D-associated dementia.

Literature regarding the therapeutic potential of cannabinoids in diabetes-associated BBB dysfunction are scarce. However, these links can be postulated from 1) *in vitro* studies, 2) studies investigating the effect of cannabinoids on the BBB dysfunction in other disease paradigms and 3) the effect of the cannabinoids on peripheral vasculopathies as this may translate to the cerebral vascular system.

The Blood-brain barrier: A brief overview of its structure, function and breakdown

Structure and Function

The BBB serves as mechanical and functional interface between the central nervous system (CNS) and peripheral circulation (1). The primary role of the BBB is to protect the brain by excluding circulating potentially neurotoxic and inflammatory agents, such as cytokines, erythrocytes, leukocytes and pathogens (2). The BBB is comprised of blood vessels which are lined by endothelial cells (ECs) tightly linked together by tight junction complexes. Surrounding the ECs is the basement membrane and astrocyte endfeet supporting the structure of BBB. Pericytes also support the BBB and are embedded within the basement membrane. Extending beyond the BBB, the microglia serve as the primary line of defence in the CNS.

BBB Breakdown

Inflammation is a key mediator of BBB dysfunction. Chronic systemic inflammation promotes cerebrovascular inflammation, which in turn potentiates the dysregulation of regulatory proteinases leading to the breakdown of BBB tight junction complex. For example, matrix metalloproteinase (MMP) degrades tight junction proteins and the basement membrane surrounding the BBB. As such, heightened MMP activity is considered a likely trigger for BBB breakdown (3). Additionally, cytokines produced during heightened systemic inflammation, such as IL-2 and IL-1β are reported to activate NF-kB in BBB ECs, which in turn promotes the secretion of additional pro-inflammatory cytokines including IL-1β, TNF-α and IL-6 (4). These cytokines are also documented to degrade BBB

tight junction proteins and dysregulate its translocation. The dysregulation of BBB EC tight junction complex results in increased permeability of BBB, leading to paracellular extravasation of blood-borne molecules and the infiltration of circulating immune cells into the brain parenchyma. The invasion of blood derived products into the brain perpetuates cerebral inflammation, and thus the cycle of inflammation and BBB breakdown continues (5, 6).

BBB disturbances in diabetes: the possible link between dementia and diabetes

There is an accumulating body of evidence implicating BBB dysfunction and neuroinflammation in the pathogenesis of diabetes-associated dementia (7). Diabetes-induced alterations in BBB were first addressed in the 1980s (8)., however, emerging evidence now indicates that cerebrovascular integrity is markedly compromised in diabetes. Studies utilising streptozotocin (STZ)-induced rodent models of T2D demonstrate that the expression of tight junction proteins, occludin-1 and claudin-5, are significantly down-regulated, which progressively increase BBB permeability, consequently, allowing the penetration of plasma neuroinflammatory molecules into the brain (9, 10). Further studies in our laboratory employing a diet-induced model of insulin resistance showed a significant association between obesity, diabetes and increased cerebral capillary permeability (11-14). Studies employing genetic models of T2D also report similar findings. For example, Zucker diabetic fatty (ZDF) rats exhibited an increased production of inflammatory cytokines, activated microglia, reduced expression of tight junction proteins and elevated BBB permeability in the hippocampus (15-17). Further studies utilising db/db mice have also shown an association between obesity, inflammation, T2D and BBB breakdown (18, 19). Moreover, in clinical T2D, magnetic resonance imaging (MRI) revealed that people with well-controlled T2D had significantly greater BBB permeability compared to control non-diabetic individuals (20). Although the evidence indicates BBB dysfunction may be an early and pivotal event leading to diabetes associated dementia, only a few studies to date have explored BBB dysfunction as a potential mechanism in the development of dementia and restoring function as a therapeutic target.

Proposed mechanisms driving diabetes associated BBB dysfunction in T2D

Numerous direct and indirect mechanisms support the hypothesis of a causal association of BBB breakdown in T2D and cognitive decline. Chronically elevated oxidative stress is a common pathological characteristic of T2D, which directly degrades BBB tight junction proteins, or induce BBB EC apoptosis by activating caspase-3 (21). Chronic low-grade systemic inflammation indicated by elevated plasma pro-inflammatory cytokines is also another pathological feature of T2D. The chronic exposure of BBB ECs to such inflammatory state directly increases the BBB EC inflammation, resulting in the BBB tight junction dysregulation. Additionally, hyperglycaemia inT2D may also indirectly increase BBB permeability by up-regulating BBB EC inflammation and oxidative stress through several pathways. Acute hyperglycaemia promotes the production of superoxide, which in turn led to diminished BBB integrity (22). In a more recent study, two different genetic T2D mouse models (Goto-Kakizaki rats and db/db mice) exhibited dysfunctional neovascularisation. The authors conclude that hyperglycaemia is a potential key factor contributing to the generation of disturbed brain capillaries and that early intervention of glycaemic control is able prevent such neovascularisation (23). Furthermore, hyperglycaemia leads to excessive production of reactive oxygen species (ROS), impaired glycolysis, and importantly the accumulation of advanced glycation end products (AGE). AGE are the result of chronic and prolonged hyperglycaemia, which in-part promote cellular dysfunction instigated by ROS and inflammation (24). BBB breakdown in T2D is summarised in Figure 1.

Prevention of BBB dysfunction

With hyperglycaemia as a hallmark of T2D and potential amplified of BBB dysfunction, it is hypothesised that optimising glycaemic control may prevent disturbances to the cerebral capillaries that will in turn ameliorate accelerated cognitive impairment. However, there is limited evidence demonstrating the efficacy of intensive glycaemic control in attenuating premature cognitive decline.

Furthermore, intensive glycaemic control may elevate the risks of severe hypoglycaemia in T2D (25). Even in well-controlled T2D subjects, a clinical study has shown that their BBB remained compromised (20).

A number of studies report that certain anti-inflammatory therapies are effective in protecting the integrity of BBB. In the KKAy mouse model of T2D, anti-hypertensive drugs (i.e. telmisartan and losartan) prevented the BBB hyperpermeability by significantly attenuating inflammation (Min et al). However, results from our laboratory using another angiotensin II blocker, candesartan, found the drug did not suppress inflammation and BBB dysfunction persisted in a dietary-induced mouse model of T2D. The paradoxical findings with hypotensive agents suggest other mechanisms such as inflammatory triggers significantly modulate BBB function (Mamo et al 2017). Consistent with the latter, our previous studies further demonstrated that other pharmacological and nutraceutical agents that have substantial anti-inflammatory properties prevent BBB breakdown. In dietary induced models of inflammation and insulin resistance, a historic lipid-lowering drug, probucol, significantly attenuated the breakdown of BBB by substantially suppressing the neurovascular inflammation of BBB ECs, independent of its lipid-lowering effect (12, 14). In the same studies, the protection of BBB by probucol resulted in significant reduction of neuroinflammation, neurodegeneration, and cognitive decline. In a study using another lipid lowering with pleiotropic anti-inflammatory propoerties, atorvastatin was found to restore BBB integrity in a high fat induced model (26). Furthermore, we showed that selected anti-inflammatory nutraceutical agents including α-lipoic acid and aged garlic extract effectively attenuate inflammation and oxidative stress, and prevent BBB hyperpermeability and neuroinflammation in a dietary induced model of inflammation (13). These data collectively suggest that vascular-focussed anti-inflammatory may be a key strategy to prevent BBB dysfunction and cognitive decline in T2D. Further studies are needed to identify more effective agents that can protect BBB in T2D.

Potential use of cannabinoids to attenuate BBB dysfunction and dementia in T2D

In recent years the medical use of cannabinoids has risen significantly. However, potential therapeutic effects of cannabinoids in diabetes-associated dementia have not been fully investigated. Therefore, we hereby summarize the potential effects of cannabinoid on counteracting neuroinflammation, cognitive decline, and dementia in T2D through the aspect of cerebrocapillary BBB integrity.

Delta-9-Tetrahydrocannabinol and associated receptors

There are a number of active cannabinoid compounds that are found in Cannabis. THC is the most abundant cannabinoid, however its percentage and cannabinoid ratios can vary across different Cannabis plant strains. THC is known to exert psychoactive and appetite modulating effects, which are predominantly mediated through cannabinoid type-1 and type-2 receptors (CB_1, CB_2).

CB_1 is major presynaptic cannabinoid receptor located on subsets of inhibitory neurons. CB_1 is expressed at varying densities throughout the brain and thus, the activation of CB_1 is linked to the psychoactive properties associated with cannabis use. Greatest binding activity between CB_1 and agonists such as THC occurs within the basal ganglia, cerebellum, olfactory bulb, hippocampal formation and the neocortex. Whilst moderate CB_1-agonist binding occurs within the forebrain and scarce interaction within the brain stem and spinal cord (27-29). However, CB_1 is not exclusively expressed within the brain but is also expressed peripherally such as thyroid gland and adipose tissue (30, 31).

CB_2 is primarily expressed by circulating immune cells and therefore have a principal role in mediating anti-inflammatory activity (32, 33). The activation of CB_2 downregulates pro-inflammatory transcription factors such as NFKβ and Activator protein 1, resulting in diminished levels of peripheral pro-inflammatory cytokines (34). Similar to CB_1, CB_2 expression is not restricted

to its primary expression site (immune cells), but is also expressed in various areas of the brain and throughout the gastrointestinal system (35, 36).

The potential of THC in BBB protection

As summarized previously, vascular-focussed anti-inflammatory treatment may effectively ameliorate BBB dysfunction in T2D. In the STZ-induced T1D mouse model, treatment with THC (150 mg/kg) significantly attenuated pancreatic islet inflammation by reducing TNF-α, IL-12 and IFN-γ (37). Furthermore, THC was reported to reduce lipopolysaccharide (LPS)-stimulated mRNA expression of various pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 in rat cerebral microglia in vitro, which was independent of CB_2 receptor pathways (38). Another *in vitro* study demonstrated that THC inhibits the secretion of TNF-α and IFN-γ by human T, B, $CD8^+$ cells and NK cells (39). Additionally, a study by Fischer-Stenger demonstrated that THC suppressed tumoricidal activity of soluble macrophage by attenuating the intracellular conversion of presecretory TNF-α to its secretary form (40). However, there has been no studies specifically testing vascular protective effects of THC through its anti-inflammatory properties, and no studies to date examined whether THC protects BBB in T2D through its anti-inflammatory properties.

In addition to the anti-inflammatory effects, studies report beneficial effects of THC on certain vascular conditions that are associated with T2D. Studies from the 1970s implicate a therapeutic role for THC in hypertension. Oral treatment with THC at 25 mg/kg dose for 10 days substantially ameliorated chronic hypertension in mutant spontaneous hypertensive rats, whilst THC did not alter the blood pressure in healthy control rats (41). O'Sullivan *et al* (2004) investigated the potential vasorelaxent effects of THC *in vitro* by using aortae isolated from adult Wistar rats in comparison to other cannabinoids, N-arachidonoyl-dopamine (NANDA) and CP55,940. THC induced an approximately 20-25% relaxation of the constricted aortae, and its vasorelaxent properties were interestingly, mediated by CB_2, not CB_1 (42). NADA and CP55,940 also showed equipotent

vasorelaxation effects, however their action pathways differed from THC. THC is also reported to exert vasodilation effects on mesenteric arteries in rabbits and rats (43, 44). Furthermore, in an *in vitro* study using cerebral arteriole isolated from New Zealand White rabbits, it was reported that THC induced relaxation of cerebral vessels by stimulating release and metabolism of endogenous arachidonic acid (45). Recently, a low dose (0.15 mg/kg) chronic administration of THC to STZ-diabetic Wistar-Kyoto rats for 8 weeks was shown to prevent cardiovascular dysfunction, attenuating hyperglycaemia and oxidative stress, concomitant with improved myocardial and vascular function (46). Collectively, these findings consistently suggest that THC has potential vascular-focussed anti-inflammatory/-oxidative effects and thus, may effectively protect the structure and function of BBB in T2D. The potential positive effects of THC on BBB integrity are summarised in Figure 2.

Cannabidiol and associated receptors

CBD is the second most abundant cannabinoid sourced from Cannabis. CBD possesses anti-inflammatory properties, which are primarily mediated by the activation of 5-Hydroxytryptamine receptor $_{1A}$ (5-HT$_{1A}$). 5-HT$_{1A}$ can be categorised into two distinct classes. 5-HT$_{1A}$ autoreceptors are expressed on the cell body and dendrites of serotonergic neurons in the raphe nucleus (a cluster of nuclei located in the brain stem). Once activated, 5-HT$_{1A}$ autoreceptors supress firing of serotonergic neurons. Whereas, 5-HT$_{1A}$ heteroreceptors are located on non-serotonergic neurons but distributed in high densities throughout the limbic areas of the brain, such as the cerebral cortex and hippocampus. Similar to 5-HT$_{1A}$ autoreceptors, the activation of 5-HT$_{1A}$ heteroreceptors also reduces neuronal excitability and firing (47-50). The neuroprotective effects of 5-HT$_{1A}$ activation are dependent on growth factor associated signalling, such as MAPK and Akt transduction, in addition to the inhibition of NMDA mediated excitotoxicity (49).

CBD also acts as a complete agonist for PPARγ. PPARs are a family of nuclear hormone receptors with three isoforms; α, δ and γ. PPARγ is predominantly expressed in adipose tissue, macrophages and the colon, with a principal role in promoting adipocyte differentiation, fatty acid storage, insulin

sensitivity, and glucose metabolism. (51-54). PPARγ is also found throughout the brain, highly abundant in microglia mediating inflammatory cascade (55), thus may mediate similar neuroprotective effects as 5-HT_{1A} activation by CBD (56). Furthermore, studies report that PPARγ is expressed in endothelial cells, and the activation of PPARγ markedly attenuated monocyte adhesion and infiltration in primary human brain endothelial cells, indicating potential for BBB protection (57). Collectively, CBD may exert potent BBB protective effects through the activation of 5-HT_{1A} and PPARγ attenuating endothelial inflammation.

Potential CBD protection of BBB function

Similar to THC, CBD is also known to exert anti-inflammatory effects. In human coronary artery endothelial cell lines, CBD suppressed the inflammation by significantly suppressing NF-kB activation induced by hyperglycaemic environment (58). In the same study, CBD was also shown to down-regulate mitochondrial production of reactive oxygen species and monocyte infiltration. Studies using models of Rheumatoid arthritis also reported that CBD treatment effective decreased TNF-α and IFN-γ (59). Consistently, in non-obese diabetes mouse model, CBD treatment significantly reduced Th1-associated cytokine production and attenuated the plasma concentrations of pro-inflammatory cytokines, TNF-α and IFN-γ (60). Moreoever, a study using STZ-induced rat model of diabetes, CBD treatment significantly suppressed the retinal oxidative stress and inflammation, and protected integrity of the blood-retinal barrier (61). Thus, it is highly plausible that CBD may also prompt the expression of tight junction proteins in the BBB and protect its integrity. Indeed, a limited number of studies investigated the role of CBD in BBB protection. BBB disruptions observed in a viral model of multiple sclerosis and in a mouse model of systemic inflammation induced by LPS, an i.p. administration of CBD attenuated BBB hyperpermeability by inhibiting VCAM-1 mediated monocyte infiltration and neurovascular inflammation (62, 63). Moreover, in an *in vitro* model of the BBB, CBD prevented BBB dysfunction following oxygen-glucose deprivation by activating 5-HT_{1A} and PPARγ receptor pathways (56), indicating that CBD mediated activation of

5-HT_{1A} and PPARγ receptors may play a pivotal role in ameliorating BBB dysfunction. Similarly, CBD is shown to be beneficial in other tight junction structures of the body. A recent *in vitro* study used *Clostridium difficile* toxin A to induce intestinal barrier dysfunction showed that the intestinal barrier dysfunction was marked by decreased expression of tight junction proteins, zonula occludin-1 and occludin. In a dose dependent response, CBD was able to inhibit the insult of *Clostridium difficile* toxin A on the gut tight junction proteins. Interestingly, CBD exerted its protective effects via $CB_{1,}$ despite the limited affinity between CBD and CB_1 (64). Similar findings were also reported in an earlier study using Caco-2 human intestinal cell line, demonstrating the tight junction protective effects of CBD (65). However, no studies to date tested the effects of CBD on BBB structure and function in T2D. Positive effects of CBD are summarised in Figure 2.

Evidence of cannabinoid as a therapeutic agent to ameliorate cognitive impairment

Although THC is the psychoactive constituent of cannabis, recent studies are demonstrating that low dose THC may have positive effects on cognition. A recent study demonstrated that chronic low THC dose of 3 mg/kg, mediated by CB_1 activation, was able to reverse cognitive decline via increased synaptic expressions and hippocampal spine density in aged wild-type C57Bl/6 mice (66). Similarly, another study using wild-type aged mice (24-month old) showed that after a single administration of an even lower THC dose of 0.002 mg/kg resulted in significant improvements in various aspects of memory and learning across six behavioural tests; effects of which lasted at least 7 weeks (67). In the same study, the authors found that the neuroprotective and cognitive benefits of THC was mediated through increased Situin1 in the frontal cortex. However, the positive benefits of THC as seen in preclinical studies failed to translate in humans. A clinical trial reported that memory and cognition were compromised amongst schizophrenic and healthy controls after THC administration (68). It should be noted that the doses used in this study were between 2.5 to 5 mg, which may be excessive.

A myriad of studies are emerging documenting the neuroprotective and memory enhancing effects of CBD in a diverse range of models of cognitive decline. CBD was shown to recover memory impairments induced by iron overload in rats (69). Furthermore, in a sepsis rat model, CBD treatment was able to reduce not only oxidative stress and mortality, but also cognitive impairments (70). CBD, via 5-HT_{1A} activation, was able to ameliorate cognitive and motor impairments in bile-duct ligated mice and such findings were abrogated by the 5-HT_{1A} antagonist, WAY-100635 (71). However, none of these studies considered the effects of cannabinoids on cognitive function through the aspect of cerebrovascular BBB structure and function. Furthermore, currently there is no available literature exploring the potential positive effects of CBD on cognitive decline in animal models of T2D or in humans, despite consistent evidence indicating that it may have substantial benefits through the protection of BBB integrity.

Concluding remarks

T2D is a chronic metabolic disease closely associated with prolonged systemic inflammation, and leads to other metabolic diseases, including dementia. In both T2D and dementia the cerebral capillary architecture is disturbed. Compromised integrity of the BBB in T2D is both an early and critical event preceding cognitive decline and potentially dementia. As such, targeting the BBB is a novel therapeutic approach for diabetes-associated dementia. Cannabinoids have emerged as a potential treatment option for prevention and is currently undergoing rigorous investigation. However, research exploring the potential of cannabinoids in T2D associated BBB breakdown and cognitive function are yet to reported.

Conflict of Interest

The authors declare no conflicts of interest

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Figure Legends

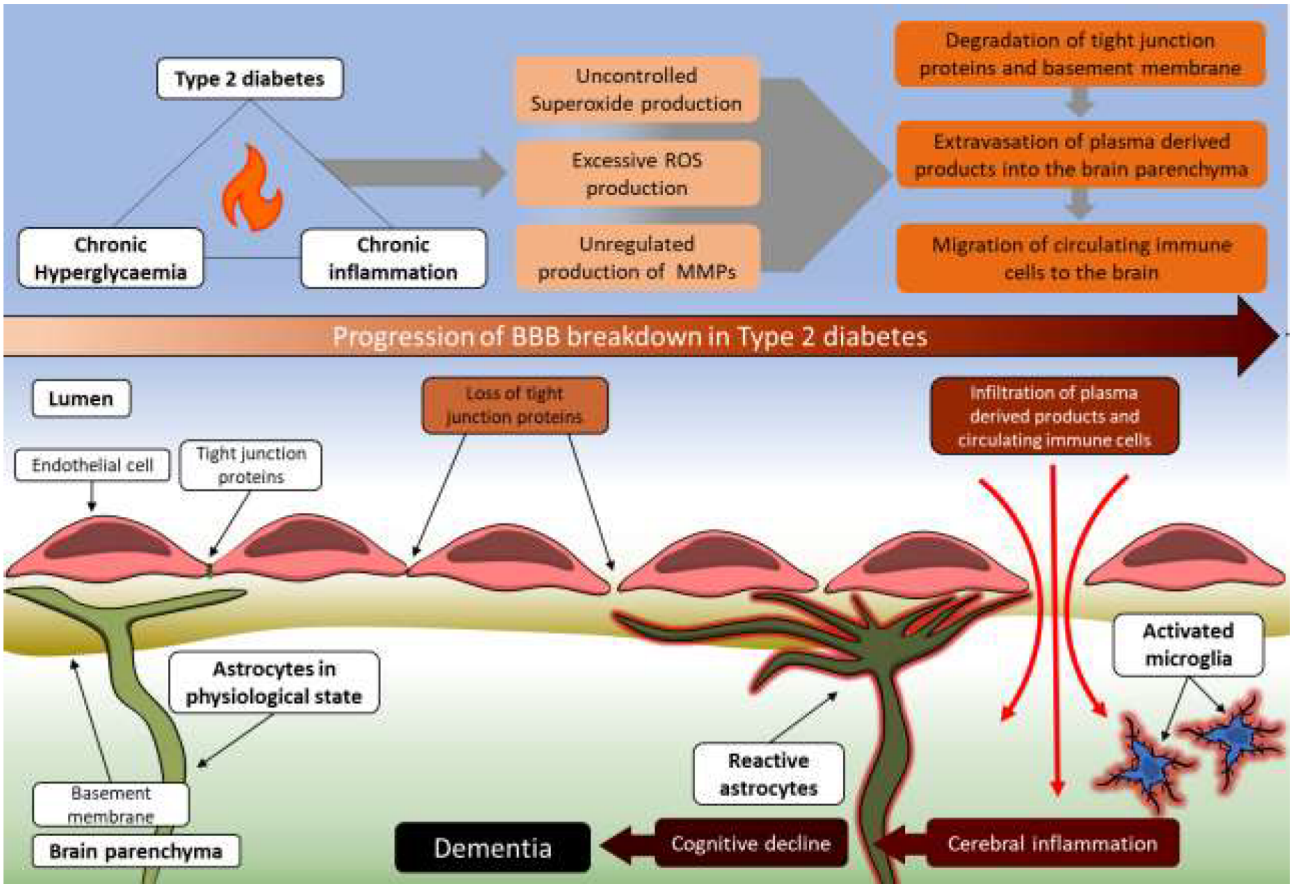

Figure 1

Type 2 diabetes, and the associated inflammation and hyperglycaemia promote uncontrolled ROS, oxidative stress and the dysregulation of regulatory MMPs. These events promote the loss of tight junction proteins and disturb the architecture of the BBB. The increased permeability of the BBB for the infiltration of circulating immune cells and neuroinflammatory blood borne products causing further inflammation. The perpetual systemic and cerebral inflammation potentially precedes cognitive decline and eventually dementia.

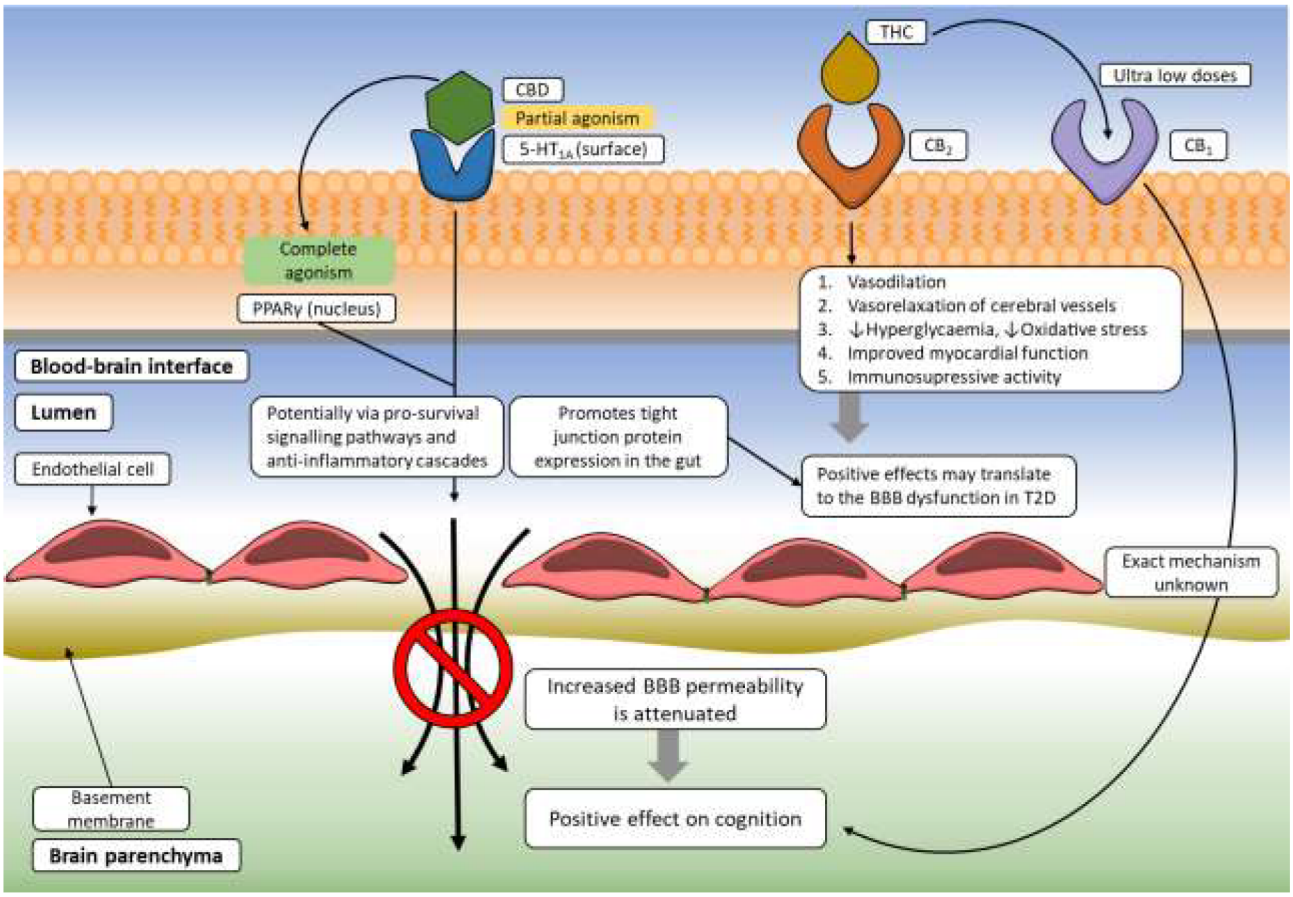

Figure 2

CBD activates 5-HT$_{1A}$ and PPARγ. Activation of these receptors promotes anti-inflammatory and pro-survival signalling pathways. Activation of such signalling cascades may attenuate or protect the loss of tight junction proteins, attenuating increased BBB permeability. By protecting the BBB, CBD my indirectly ameliorate cognitive deficits. THC primarily activates CB$_1$ and CB$_2$. Via CB$_2$ activation, THC is able to exert vasodilation properties, s. reduce oxidative stress, inflammation and hyperglycaemia. Although such effects have not yet been observed in models of BBB breakdown, they may be translated the cerebral capillaries. Ultra-low doses of THC via CB1 also have positive effects on cognition.