Endothelin and Diabetic Complications: a Brain-Centric View

W. LI1,2, Y. ABDUL1,2, R. WARD3, A. ERGUL1,2

1Charlie Norwood Veterans Affairs Medical Center, Augusta, Georgia, USA, 2Department of Physiology, Augusta University, Augusta, Georgia, USA, 3Department of Neuroscience and Regenerative Medicine, Augusta University, Augusta, Georgia, USA

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Summary

The global epidemic of diabetes is of significant concern. Diabetes associated vascular disease signifies the principal cause of morbidity and mortality in diabetic patients. It is also the most rapidly increasing risk factor for cognitive impairment, a silent disease that causes loss of creativity, productivity, and quality of life. Small vessel disease in the cerebral vasculature plays a major role in the pathogenesis of cognitive impairment in diabetes. Endothelin system, including endothelin-1 (ET-1) and the receptors (ETA and ETA), is a likely candidate that may be involved in many aspects of the diabetes cerebrovascular disease. In this review, we took a brain-centric approach and discussed the role of the ET system in cerebrovascular and cognitive dysfunction in diabetes.

Key words

Endothelin • Cognitive impairment • Cerebrovascular dysfunction • Neurovascular unit • Diabetes

Corresponding author

A. Ergul, Department of Physiology, Augusta University, 1120 15th Street CA-2094, Augusta, Georgia 30912, USA. Fax: 706.721.7299. E-mail: aergul@augusta.edu

Introduction

According to International Diabetes Federation, 425 million people had diabetes worldwide in 2017 and this is expected to rise up to 629 million by 2045 (IDF 2017). Given that cardiovascular complications of diabetes are the leading cause of morbidity and mortality associated with the disease, this epidemic has a huge burden on global health. As such, microvascular (nephropathy, retinopathy, and neuropathy) and macrovascular (heart disease, stroke, and peripheral arterial disease) complications of diabetes represent intense areas of research. It is increasingly recognized that cognitive dysfunction is an emerging complication that should be added to the diabetic complications list (Simo et al. 2017). Small vessel disease, involving both structural and functional changes in the cerebral microvasculature, plays a major role in the pathogenesis and progression of cognitive dysfunction in diabetes (Blair et al. 2017, Gorelick et al. 2017). While the mechanisms are multifactorial, endothelial dysfunction, reduced cerebral blood flow (CBF) and hypoperfusion is believed to precede the negative changes in the cognitive function observed in both patients and experimental models.

Endothelium is an early target in diabetes and cerebrovascular dysfunction resulting from an imbalance of endothelium-derived vasoconstrictor and vasodilator substances can contribute to the early decrease in CBF which could trigger the neuroinflammation and oxidative/nitrative stress. Endothelin-1 (ET-1), a potent vasoconstrictor with proliferative, profibrotic, prooxidative and proinflammatory properties, is a likely candidate that may be involved in many facets of diabetic cerebrovascular disease (Daulatzai 2017, Faraco et al. 2013). It has been suggested that high glucose level as seen in diabetes up-regulates the ET-1 system in the endothelial cells through many different pathways (Chiu et al. 2008, Li et al. 2013, Manea et al. 2013, Wu et al. 2010). For example, early studies reported that high glucose-mediated disruption of endothelial barrier...
Cerebral perfusion and brain health

Adequate cerebral perfusion is essential for brain function, an organ that has high metabolic demands but minimal or almost no energy stores (Attwell et al. 2010, Iadecola et al. 2010). Blood delivers nutrients such as glucose and oxygen while removing metabolites. Hence, tight regulatory mechanisms operate to ensure optimum cerebral perfusion, which involve cerebrovascular autoregulation, neurovascular coupling and ligand-mediated vasoreactivity. These properties of the cerebral circulation require orchestrated action of the components of the nervous vascular unit comprised of neurons, glial cells, pericytes, vascular endothelial and SMCs, as well as perivascular cells and cerebrovascular matrix. In this complex system, endothelial cells regulate microvascular blood flow and integrity by releasing vasoactive agents such as ET-1 and nitric oxide (NO).

Diabetes, cerebrovascular dysfunction and ET-1

In this section, we will review the role of ET-1 on four mechanisms involved in regulation of cerebrovascular physiology, namely cerebral autoregulation, myogenic tone, neurovascular coupling, and ligand-mediated vasoreactivity in this order.

Cerebral autoregulation and myogenic tone

Cerebral autoregulation is one of the well-developed mechanisms in the brain to protect it from fluctuations in perfusion pressure (Cipolla 2016). While several mechanisms contribute to the process of cerebral autoregulation, the most prominent one is the myogenic response, which describes the ability of SMCs to react to changes in blood pressure in order to keep blood flow constant. SMCs usually contract as result of stretching, which is due to an increase in blood pressure, and dilate upon blood pressure reduction. As previously reviewed, diabetes affects myogenic tone emphasizing the direct effects of ET-1 on SMC contractility (Ergul et al. 2012). Although it is not in a diabetic model, ET-1-mediated peroxynitrite formation has been shown to augment baseline tone of penetrating arterioles of the brain. While in most models (Cipolla et al. 2013), diabetes increases the myogenic tone, this appears to be age-dependent (Kelly-Cobbs et al. 2011a).

ET-1 contributes to dysregulation of myogenic response and tone in diabetes. Dumont et al. (2003) demonstrated that diabetic rats develop a greater tone and treatment with bosentan, a dual antagonist that blocks both ET receptor subtypes, restores myogenic tone to control levels. We have shown that middle cerebral arteries (MCA) from spontaneously diabetic Goto-Kakizaki (GK) rats exhibit increased myogenic tone in the early disease course (10 weeks of age) but later (18 weeks of age) show decreased tone development (Kelly-Cobbs et al. 2011a, Kelly-Cobbs et al. 2012). Glycemic control with metformin started at the onset of diabetes prevented the decreased ability to develop tone and also restored the increased ET-1 levels in this model (Kelly-Cobbs et al. 2011a). Another study reported that bosentan treatment started at 18 weeks after the vascular disease was established could reverse the myogenic dysfunction in aged diabetic rats (Abdelsaid et al. 2014b).

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Neurovascular coupling

While cerebral autoregulation contributes to maintaining constant blood flow to the brain despite changes in cerebral perfusion pressure, fine tuning of the delivery of blood where it is needed is achieved by bidirectional communication between neurons and vasculature. This interaction is known as neurovascular coupling and results in an increase in CBF, a response referred as functional hyperemia. Vessels around the firing neurons dilate in response to the signals they receive and match blood flow to the increased metabolic demand of neurons. Our knowledge of neurovascular (un)coupling in diabetes, let alone the role of ET-1 in this process, is very limited. One study found that retinal arteriole dilation in response to light stimulation is reduced in type 1 diabetic rats compared to control (Mishra et al. 2010). Another study reported impaired neurovascular coupling in the cerebral vasculature of type 1 diabetic rats (Vetri et al. 2017, Vetri et al. 2012).

In type 2 diabetes, impaired functional hyperemia has been reported in the GK rats, which also display increased myogenic tone and activation of the ET system (Harris et al. 2005, Kelly-Cobbs et al. 2011b, Kelly-Cobbs et al. 2012). Studies in other disease models such as hypertension showed that ET-1 is involved in neurovascular uncoupling, but a direct role in diabetes remains to be established (Capone et al. 2012a, Capone et al. 2012b).

Agonist-induced vasoreactivity

Endothelium-dependent relaxation of basilar arteries but not mesenteric arteries is impaired in GK rats, a lean model of type 2 diabetes that presents with moderate hyperglycemia and insulin resistance without obesity (Sachidanandam et al. 2006). As discussed below, this model also displays hyperreactivity to ET-1 as evidenced by greater contraction. Blockade of ETA receptor improves relaxation but ETB selective blockade with A-192621 causes paradoxical constriction of basilar arteries (Harris et al. 2008). Intriguingly, dual blockade of both ETA and ETB receptors with bosentan improves the maximum relaxation response (Li et al. 2011). A recent study demonstrated that oral hypoglycemic linagliptin improves the ET-1-mediated cerebrovascular dysfunction observed in the GK model through a reduction in ET-1 plasma levels and reduced cerebrovascular hyperreactivity (Hardigan et al. 2016a, Hardigan et al. 2016c). This effect is potentially a result of linagliptin causing a decrease in endothelial toll like receptor 2 (TLR2) expression and a subsequent increase in NO bioavailability (Hardigan et al. 2016a, Hardigan et al. 2016c). Interestingly, linagliptin did not correct hyperglycemia suggesting that these effects are blood glucose-independent.

Contractile response to ET-1 is augmented in the rat and rabbit basilar arteries of type 1 diabetic rats, respectively (Alabadi et al. 2004, Matsumoto et al. 2004). Arrick and coworkers showed that type 1 diabetes impairs endothelium-dependent vasorelaxation by dysregulation of both eNOS and nNOS in an oxidative stress manner (Alomar et al. 2016, Arrick et al. 2010, Arrick et al. 2007b, Arrick et al. 2007a, Arrick et al. 2011, Mayhan et al. 2017, Mayhan et al. 2011). Furthermore, the same group, using a cranial window approach to study pial vascular responses, reported that local ETA blockade by BQ-123 restored the impaired eNOS- and nNOS-dependent vasodilation (Arrick et al. 2010). This finding appeared to be specific for NOS-dependent reactivity since response to nitroglycerin were not altered by BQ-123. Additionally, the production of superoxide anion was increased from parietal cortex tissue in diabetic rats and BQ-123 inhibited this basal superoxide production. A recent study showed that overexpression of ET-1 in the endothelium exacerbates endothelial dysfunction in resistance arteries in diabetes via excessive generation of free radicals. This detrimental effect involved multiple mechanisms including decreased eNOS expression, increased vascular superoxide production and attenuation of the antioxidant capacity as indicated by an inability to upregulate superoxide dismutase expression (Idris-Khodja et al. 2016). Similar findings have been reported in the ophthalmic circulation (Granstaw et al. 2011). While no study explored the mechanistic link between ET-1-mediated reactive species generation and cognitive dysfunction in metabolic diseases, a recent review summarizes the role of oxidative stress in cognitive dysfunction in general (Grochowski et al. 2018). Collectively, these studies strongly suggest that ET-1 is a significant contributor to cerebrovascular dysfunction in diabetes via generation of reactive oxygen and nitrogen species.

In summary, activation of the ET system in the cerebrovasculature promotes vascular dysfunction at multiple levels and contributes to dysregulation of CBF in diabetes.
Diabetes, cerebrovascular remodeling/neo-vascularization and ET-1

Changes in cerebrovascular structure are equally important for proper brain function in diabetes. While cerebrovascular restructuring is beneficial to maintain physiological integrity in health, pathological remodeling can occur in disease settings such as diabetes and affect both cerebrovascular function and integrity of the blood brain barrier (BBB) leading to increased permeability.

Cerebrovascular remodeling

Type 2 diabetic GK rats develop significant cerebrovascular remodeling of middle cerebral arteries characterized by reduced lumen diameter, increased wall-to-lumen ratio and wall thickness in a relatively short time after the onset of diabetes (Harris et al. 2005). This remodeling causes an increase in the MCA wall stiffness that is associated with an increased expression of extracellular matrix proteins and an increase in the activity of matrix metalloproteases (MMPs) (Harris et al. 2005, Li et al. 2010). A significant decrease in the cerebral perfusion came in parallel with the increase in media-to-lumen ratio (Kelly-Cobbs et al. 2012). The role of ET-1 in this cerebrovascular remodeling response appears to be quite complex. While selective ET_A (atrasentan) or ET_B receptor (A192621) blockade partially blocks remodeling, dual antagonism with bosentan completely attenuates this response (Harris et al. 2005, Kelly-Cobbs et al. 2011b). The results with selective ET_B receptor blockade were surprising. Given that genetic or pharmacological inhibition of ET_B receptors worsen vascular remodeling in a wire injury model (Murakoshi et al. 2002), it was originally hypothesized that diabetes decreases protective endothelial ET_B receptors contributing to vascular remodeling and antagonism of this receptor exacerbates the changes in the vascular structure. Diabetes did not influence endothelial ET_B receptors but ET_A and ET_B receptors on smooth muscles were increased. This was prevented by chronic bosentan treatment. On the other hand, A192621 treatment augmented remodeling in control animals indicating a physiological protective role for the ET_B receptor subtype (Kelly-Cobbs et al. 2011b). The finding that bosentan treatment prevents the changes in ET receptor profile suggests that ET-1 has a positive feedback on the expression of its receptors in the cerebrovasculature, underscoring the fact that the ET receptor antagonism may yield different results in healthy and diseased states.

While these early studies suggested that ET receptor antagonism can be an effective prevention strategy, follow up studies showed that adverse remodeling of middle cerebral arteries can be reversed by glycemic control with metformin or ET receptor antagonism with bosentan, suggesting that ET system can be therapeutically targeted as well (Abdelsaid et al. 2014a). Furthermore, treatment with a relatively new class of oral hypoglycemic linagliptin is effective in reversing established pathological cerebrovascular remodeling in the GK model (Yasir et al. 2016). As briefly discussed above, this treatment did not lower blood glucose in this model. In vitro studies showed that linagliptin prevented the ET-1-mediated increase in SMC ET_A receptors under high glucose conditions, suggesting that attenuation of the ET system could be a pleiotropic effect of linagliptin that provides vascular protection (Yasir et al. 2016).

Cerebral neovascularization

Our understanding of the impact of diabetes on neovascularization mostly came from peripheral and retinal circulation. While diabetes is known to impair angiogenesis and collateral formation in the heart and peripheral circulation, in the retinal circulation, pathological angiogenesis occurs leading to diabetic retinopathy. In the brain, diabetes causes microangiopathy similar to the retina. Diabetes causes increased, yet dysfunctional, neovascularization in the cortex and striatum of type 2 diabetic GK rats (Prakash et al. 2013b, Prakash et al. 2012). Vascular density, volume and surface area are increased but associated with poor vessel wall maturity as indicated by reduced pericytes and increased permeability. On the other hand, another group reported decreased vascularization and capillary branching in the dentate gyrus of the hippocampus, an area associated with memory and learning processes, in GK rats (Beauquis et al. 2010). It is also possible that there are differences in the angiogenic response in very specialized areas of the brain. Comparative studies with the db/db mouse model and high fat diet/low dose streptozotocin-induced rat model of type 2 diabetes demonstrated similar findings to the GK model suggesting that pathological vascularization is a common finding in diabetes (Prakash et al. 2013a, Qu et al. 2014).

ET-1 contributes to tumor angiogenesis, another form of pathological angiogenesis characterized by irregular vascularization, via the activation of hypoxia-
inducible factor and VEGF-A (Garrafa et al. 2012, Rosano et al. 2013, Spinella et al. 2010). A recent study showed that dual ET receptor blockade with bosentan reverses pathological cerebral neovascularization in GK rats with established cerebrovascular disease and improved BBB integrity (Abdelsaid et al. 2014a). A follow-up study reported that treatment with oral hypoglycemic linagliptin also reduces cerebral neovascularization as well as formation of string vessels, a marker of vascular degeneration. Moreover, linagliptin normalizes the augmented angiogenic properties of brain microvascular endothelial cells isolated from diabetic animals and bosentan blocks this response. While linagliptin significantly decreases ET-1 levels, it increases ETB receptors (Abdelsaid et al. 2016). ETA receptor antagonism has been shown to reduce string vessels and neuronal death in a diabetic retinopathy model providing additional evidence that ET receptor blockade is neurovascular protective in diabetes (Chou et al. 2014).

Collectively, these studies suggest that ET-1 negatively impacts cerebrovascular structure, density, and integrity in diabetes. All these changes can contribute to the development of neuroinflammation and neurovascular degeneration in cognitive impairment by reducing blood flow and increasing BBB permeability.

**Diabetes, cognitive impairment and ET-1**

Individuals with diabetes have been shown to have changes in brain structure and cognitive function (Franc et al. 2011, Stiles et al. 2010). Yet, cognitive impairment remains to be one of the less understood and less studied complications of diabetes. As recently reviewed, there may be differences in the pathogenesis of the diseases between type 1 and type 2 diabetes (Hardigan et al. 2016b, Moheet et al. 2015, Riederer et al. 2017). Readers are referred to these reviews on diabetes and cognitive impairment in general. In this review, we will summarize what is known about the role of the ET system in cognitive impairment and especially in diabetes.

ET-1 has been implicated to contribute to cognitive deficits in various models of cognitive impairment and dementia (Daulatzai 2017). In a series of studies, Palmer and colleagues demonstrated that ET-1 and endothelin converting enzyme (ECE-1 and 2) activities to be upregulated in the brains of patients with Alzheimer's Disease (AD) (Palmer et al. 2011, Palmer et al. 2012, Palmer et al. 2013). ET-1-mediated vascular dysfunction and ensuing cognitive deficits have been reported in transforming growth factor-β1 transgenic mice that mimic the vascular pathology of AD (Papadopoulos et al. 2010, Tong et al. 2015) as well as under water deprivation conditions (Faraco et al. 2013, Faraco et al. 2014). On a sweet note, flavonol-rich dark cocoa has been shown to decrease plasma endothelin-1 and improve cognition in urban children (Calderon-Garciduenas et al. 2013).

A causal role of ET-1 in cognitive impairment in different disease models has been tested by using selective or dual ET receptor blockers. Interestingly, Freeman and colleagues showed that ET-1 mediates brain microvascular dysfunction leading to long-term cognitive impairment in a model of experimental cerebral malaria (Freeman et al. 2016). ETA receptor antagonists BQ123 or BMS182874 prevented cognitive dysfunction in a model of AD in which disease is induced by injection of amyloid B (Aβ1-40) in the lateral cerebral ventricles (Briyal et al. 2011). However, nonspecific ETα/ETβ receptor antagonist TAK-044 had no effect in this model. On the other hand, treatment with bosentan improved learning and memory as well as mitochondrial and carotid artery endothelial function in a similar yet slightly different model of AD. In this study, cognitive impairment was induced by combined administration of single intracerebroventricular infusion of Aβ and chronic oral administration of L-methionine resulting in hyperhomocysteinemia (Singh et al. 2017). The same group also reported that bosentan improves cognition in a two-kidney one-clip model of hypertension. It has to be noted that, in this study, ET receptor antagonism also attenuated the increase in mean arterial blood pressure (Singh et al. 2016). In contrast to these studies, Briyal et al. (2015) reported that stimulation of ETB receptors with ILR1620 decreases the progression of AD. This particular study used a model in which cognitive deficits were induced by Aβ1-40 injection into the brain. IRL1620 and ETB receptor antagonist BQ788 were also administered locally by stereotactic injection.

In the context of diabetes, ETB receptor activation has been reported to prevent cerebral oxidative stress and improve cognitive function in type 1 diabetic rats that received intracerebroventricular Aβ1-40 injections along with IRL1620 and BQ788 (Briyal et al. 2014). Behavioral and cognitive outcomes were determined two weeks after the administration of Aβ1-40 and drug. On the other hand, Singh et al. (2014) assessed...
cognitive function two months after the onset of type 1 diabetes and treated the animals with bosentan or donepezil during the second month. This study demonstrated that bosentan improved endothelial function and cognitive behavior significantly although donepezil was more effective (Singh et al. 2014).

**Integration**

This review on the role of the ET system in diabetic complications focuses on the central nervous system with an emphasis on cognitive impairment. While studies described above demonstrate conflicting conclusions with regard to the use of ET receptor antagonism or ET$_B$ activation in the prevention or treatment of cognitive impairment in preclinical models, they also offer an opportunity to evaluate the role of ET-1 as well as its isoforms and receptors on memory and cognitive function in the context of 1) neurovascular unit, and 2) models of diabetes and cognitive impairment.

![Diagram of the ET system in the neurovascular unit](https://example.com/diagram)

**Fig. 1.** The ET system is present in all components of the neurovascular unit and may contribute to the development of cognitive impairment in diabetes by increasing the cerebrovascular tone and the reduction of cerebral blood flow, disrupting blood brain barrier integrity, impairing cell survival, and causing Aβ deposition.

When we approach the issue from neurovascular unit perspective, we need to think about neurons, astrocytes, pericytes, vascular endothelial and SMCs as well as perivascular cells and cerebrovascular matrix (Fig. 1). For example, some studies showed that ET$_A$ or dual ET$_A$/ET$_B$ receptor antagonism is neuroprotective and improves outcomes after ischemia reperfusion injury of the brain (Dawson et al. 1999, Gupta et al. 2005, Matsuo et al. 2001, Zhang et al. 2008). Moreover, endothelial cell specific overexpression of ET-1 worsened cerebral damage (Leung et al. 2009) and ET-1 induced degeneration of cultured motor neurons (Ward et al. 2016). On the other hand, some studies suggested that the ET receptor blockade exacerbates brain infarction (Chuquet et al. 2002) and ET-1 improves neuronal survival in cell culture models (Leung et al. 2009, Ward et al. 2016).

In astrocytes, targeted overexpression of ET-1 exacerbates ischemia reperfusion injury of the brain (Yeung et al. 2009) and contributes to dementia associated with ischemic stroke by exaggerating astrocyte-derived amyloid secretion (Hung et al. 2015). An earlier study showed that glial-derived ET-1 contributes to delayed neuronal death in the hippocampus (Yamasita et al. 2000). Readers are referred to a recent review article for further reading, which highlighted astrocytes as the neglected co-star in cognitive impairment and emphasized ET-1 and Aβ interactions (Jo et al. 2014).

From a vascular perspective, there is no doubt that ET$_A$ receptors mediate vasoconstriction in many vascular beds. In the cerebral circulation, especially under hypoxic conditions, vascular smooth muscle cell ET$_B$ receptors are upregulated and mediate vasoconstriction as demonstrated by Kelly-Cobbs et al. (2011b) in a diabetes model as well as many studies by the Edvinsson laboratory (Henriksson et al. 2003, Stenman et al. 2002). A recent study by this group provided evidence for the first time that contractility of intraparenchymal arterioles, bottleneck of cerebral perfusion, is augmented after global cerebral ischemia in an ET-1 and ET$_B$ receptor-dependent manner contributing delayed cerebral hypoperfusion (Spray et al. 2017). Early studies showed that ET-1 by binding to ET$_A$ receptors regulates the arrangement of actin cytoskeleton in pericytes, contractile cells at the capillary level, and upregulation of both receptors contribute to disturbed microvascular autoregulation and sustained reduction of CBF following trauma to the brain (Dehouck et al. 1997, Kallakuri et al. 2007).

Collectively, studies summarized above show that the ET system is present in all components of the neurovascular unit. The interaction(s) between these cellular compartments and potential contribution(s) in regulation of cognition are quite complex. Moreover, diabetes impacts structure and function of the neurovascular unit significantly. As discussed above, there are only two studies that directly tested the role of ET receptors in cognitive impairment in diabetes. Both of
these studies used type 1 model of diabetes. While one of the studies looked at the direct effect of diabetes, the other study accelerated the development of cognitive deficits by Aβ injection, a model of AD. Thus, future studies are critically needed to investigate either the spontaneous development of cognitive deficits over time and/or in combination with cerebral hypoperfusion, the most common model of vascular cognitive impairment in type 2 diabetes models. We need pharmacological tools to manipulate the ET<sub>B</sub> receptors. The limited availability of ET<sub>B</sub> selective antagonists limited the progress in this area. At the cellular level, the specific role of the ET system in different members of the neurovascular unit can be teased out by cell specific knock-out or overexpression studies in the diabetic background. Equally important, we need to incorporate female animals into the experimental design. Most, if not all, of the studies highlighted in this review were conducted using male animals. It is encouraging that the cognitive outcomes will be assessed as a secondary endpoint in the ongoing Study Of Diabetic Nephropathy With Atrasentan (SONAR).

**Conflict of Interest**

There is no conflict of interest.

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