Journal Club

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Pursuing the Molecular Nexus Connecting Diabetes and the Associated Cognitive Impairments

Daniel J. Torres,¹ Naghum Alfulaij,¹ Alexandru R. Sasuclark,¹ Lígia M. Watanabe,^{1,2} and Herena Y. Ha¹

¹Department of Cell & Molecular Biology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii 96813, and ²Department of Internal Medicine, Division of Nutrition and Metabolism, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, 14048-900 Brazil

Review of Yamamoto et al.

Emerging evidence from clinical and animal studies has demonstrated that Type 2 diabetes mellitus (T2DM) and obesity are associated with an increased risk for cognitive impairments (Biessels and Reagan, 2015). The hippocampus appears to be particularly susceptible to damage, such as morphological changes and reduced synaptic function, related to obesogenic and prodiabetic diets (Granhölm et al., 2008; Stranahan et al., 2008). A clear understanding of the mechanistic underpinnings of these relationships has been elusive, but growing evidence implicates degradation and dysfunction of the cerebrovascular blood–brain barrier (BBB) as a key factor that leads to cognitive decline (Valcarcel-Ares et al., 2019).

BBB integrity is vital for neuronal health, and many neurological disorders are associated with BBB breakdown (Van Dyken and Lacoste, 2018). Multiple cell types constitute the BBB, including endothelial cells, pericytes, astrocytes, and microglia. This cellular conglomeration, known as the “neurovascular unit,” pro-

TECTS the brain from toxic substances and regulates the transport of nutrients and hormones into the brain (Rhea et al., 2017). Endothelial cells, which reside on the luminal side of the brain microvasculature and are connected by tight junction proteins, are especially important for regulating BBB permeability. Pericytes provide additional structural stability by lining the abluminal surface of the endothelial monocellular layer. Multiple studies in rodent models of T2DM and obesity have reported that tight junctions degrade, reducing BBB integrity, particularly in the hippocampus. Therefore, BBB breakdown might be a causative factor leading to cognitive dysfunction in these diseases (Rhea et al., 2017; Takechi et al., 2017; Salameh et al., 2019).

T2DM and obesity have many interrelated effects, including chronic hyperglycemia, insulin resistance, altered hormone levels, and redox imbalances (Reagan, 2012). The extent to which each of these plays a role in neurological impairments is poorly understood. Moreover, multiple repercussions of chronic metabolic stress, such as astrogliosis (Calvo-Ochoa et al., 2014) and pathological angiogenesis (Yi et al., 2012), may adversely affect BBB function. Thus, the precise order of pathological events leading to cognitive decline is difficult to discern.

Yamamoto et al. (2019) sought to address these uncertainties by placing mice on low- or high-fat diets (LFD and HFD, respectively) and monitoring metabolic parameters and BBB integrity over time. To assess BBB permeability, mice were intraperitoneally injected with two fluorescent tracers; then hippocampal tissue was evaluated for the presence of each tracer to determine whether there were moderate (NaFl) or more severe (Evans Blue) increases in BBB permeability. After 8 weeks, HFD-fed mice were heavier than LFD-fed controls, but fasting glycemia, insulinemia, and BBB permeability were similar in the two groups (Yamamoto et al., 2019, their Fig. 1A–C). By 12 weeks, BBB permeability to NaFl had increased in HFD-fed mice; and at 16 weeks, BBB permeability to Evans Blue had increased, along with fasting glucose and insulin levels. Increased BBB permeability was accompanied by a multitude of structural alterations to the neurovascular unit following 16 weeks of HFD administration. Tight junction proteins were significantly reduced (Yamamoto et al., 2019, their Fig. 1E), and electron microscopy revealed tight junction involution and atrophy. Additionally, the authors observed swollen astrocytic endfeet (Yamamoto et al., 2019, their Fig. 2) and pericyte regression, indicated by reduced pericyte coverage of the endothelial surface area. The potential

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Correspondence should be addressed to Daniel J. Torres at djtorr@hawaii.edu.

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role of inflammation was also revealed by an increase in VEGF and VEGF receptor expression in micro-vessel preparations from HFD-fed mice (Yamamoto et al., 2019, their Fig. 6E). Importantly, mice fed HFD for 16 weeks displayed deficits in hippocampal LTP (Yamamoto et al., 2019, their Fig. 3D) and spatial memory retention (Yamamoto et al., 2019, their Fig. 7A–C), reflecting the cognitive effects associated with diabetes and T2DM.

Past studies demonstrated that the Adenosine A2A receptor (Adora2a) promotes BBB permeability through a mechanism proposed to involve intracellular signaling in endothelial cells and tight junction protein alteration (Carman et al., 2011). Therefore, Yamamoto et al. (2019) next administered the Adora2a antagonist SCH58261 to HFD-fed mice via intraperitoneal injection. Adora2a antagonism reversed BBB permeability to NaFl and Evans Blue (Yamamoto et al., 2019, their Fig. 3E) and rescued hippocampal LTP. Similarly, Tie2 promoter-driven KO of Adora2a in endothelial cells and pericytes protected HFD-fed mice from tight junction degradation, BBB hyperpermeability (Yamamoto et al., 2019, their Fig. 4E,F), and deficits in LTP (Yamamoto et al., 2019, their Fig. 7D,E). Finally, Adora2a antagonism and ablation prevented HFD-induced pericyte regression and upregulation of both VEGF and VEGF receptor.

From the results obtained, the authors concluded that obesity (present at 8 weeks) increases BBB permeability (which is present at 12 weeks), whereas insulin resistance (which did not appear until 16 weeks) leads to a more severe breakdown of the BBB. This hypothesis is consistent with previous work suggesting that proper insulin signaling at the BBB helps maintain tight junction structure and, thus, permeability (Rhea and Banks, 2019). Mice with endothelial-cell-specific KO of the insulin receptor display increased BBB permeability and reduced tight junction protein expression (Konishi et al., 2017). Furthermore, insulin-deficient diabetes-model mice experience BBB deterioration via astrocytic retraction, resulting in increased permeability (Salameh et al., 2016). Finally, insulin administration restores BBB permeability in streptozotocin-injected diabetes-model mice (Sun et al., 2015). Further investigation is needed, however, to determine whether the correlation between insulin resistance and severe BBB deterioration truly represents a causal relationship.

Since the interventions used to target Adora2a may have also affected pericyte-

resident Adora2a, the potential role of pericytes must also be considered. For example, a human pericyte and microvascular endothelial cell coculture study suggested that Adora2a activation in pericytes stimulates the release of large amounts of VEGF, which may, in turn, cause pericyte detachment (Caporarello et al., 2018). Thus, it is possible that the HFD-induced increase in VEGF and VEGF receptor expression observed by Yamamoto et al. (2019) resulted from Adora2a activation in pericytes. Pericytes could, in this way, provide a driving force in generating inflammation and causing HFD-induced BBB breakdown. Further studies are needed to fully characterize this cascade of pathological events leading to BBB dysfunction and the resulting cognitive deficits.

The identification of Adora2a as a key mediator of obesity-induced cognitive dysfunction by Yamamoto et al. (2019) is complemented by another recent article in which Zhou et al. (2019) demonstrated that endothelial Adora2a mediates inflammation and BBB permeability caused by thromboembolic stroke. While the precise molecular insults caused by thromboembolic stroke are likely not identical to those incurred by chronic HFD intake, the study reinforces the role of endothelial Adora2a as a major mediator of BBB dysfunction. Additionally, Zhou et al. (2019) determined that Adora2a-induced endothelial inflammation may involve activation of the Nlrp3 inflammasome, a multiprotein complex that triggers inflammatory processes in response to various stimuli. The Nlrp3 inflammasome is associated with metabolic disorders and is preferentially expressed in neurovascular endothelial cells (Yang et al., 2014), presenting another potential molecular target for further investigation of Adora2a-mediated BBB dysfunction.

A major question that remains to be addressed is how Adora2a becomes activated by HFD administration. One possibility is that chronic stress and inflammation within the endothelial monolayer of the BBB cause the release of abnormally high amounts of ATP into the extracellular space, leading to adenosine generation (Bynoe et al., 2015). The ectonucleotidases CD39 and CD73, which reside on the cell surface and contain extracellular-oriented catalytic sites, rapidly convert ATP into AMP (CD39), and then adenosine (CD73), which can then activate Adora2a (Burnstock and Boeynaems, 2014). Although CD73 expression is low in BBB endothelial cells under

normal physiological conditions, it is increased by cell stress and inflammation (Bynoe et al., 2015).

As previously mentioned, the multiple interacting factors in cases of T2DM make it difficult to pinpoint causative factors of the associated neurological impairments. Indeed, a review of the current literature on T2DM and BBB permeability presents contrasting hypotheses on the effects of hyperglycemia on BBB integrity. In brief, while analyzing BBB composition and function in the *Lepr^{db/db}* mouse strain that develops obesity-induced insulin resistance, Corem et al. (2019) observed transient BBB leakage during prehyperglycemic stage. The authors concluded that hyperglycemia-induced BBB permeability is likely not the underlying cause of T2DM-associated cognitive impairment, instead concluding that mitochondrial degeneration in the endothelium may be a driving force. Mäe et al. (2018) came to a similar conclusion using a mouse model in which insulin-producing β -cell-specific death is genetically induced, resulting in chronic hyperglycemia, but no changes in BBB function. The conclusions drawn from these studies may be specific to the models and methodology used, however, and caution must be used when extrapolating these findings to the complicated context of T2DM and obesity in humans.

This study by Yamamoto et al. (2019) implicates Adora2a as a potential molecular mediator of HFD-induced BBB degradation and cognitive impairment. Further investigation of the cause of Adora2a activation and characterization of Adora2a actions in the neurovascular unit may help develop therapeutic treatments for T2DM and obesity-associated cognitive decline.

References

- Biessels GJ, Reagan LP (2015) Hippocampal insulin resistance and cognitive dysfunction. *Nat Rev Neurosci* 16:660–671.
- Burnstock G, Boeynaems JM (2014) Purinergic signalling and immune cells. *Purinergic Signal* 10:529–564.
- Bynoe MS, Viret C, Yan A, Kim DG (2015) Adenosine receptor signaling: a key to opening the blood–brain door. *Fluids Barriers CNS* 12:20.
- Calvo-Ochoa E, Hernández-Ortega K, Ferrera P, Morimoto S, Arias C (2014) Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. *J Cereb Blood Flow Metab* 34:1001–1008.
- Caporarello N, Olivieri M, Cristaldi M, Scalia M, Toscano MA, Genovese C, Addamo A, Salmieri M, Lupo G, Anfuso CD (2018) Blood–

- brain barrier in a *Haemophilus influenzae* type A in vitro infection: role of adenosine receptors A2A and A2B. *Mol Neurobiol* 55:5321–5336.
- Carman AJ, Mills JH, Krenz A, Kim DG, Bynoe MS (2011) Adenosine receptor signaling modulates permeability of the blood–brain barrier. *J Neurosci* 31:13272–13280.
- Corem N, Anzi S, Gelb S, Ben-Zvi A (2019) Leptin receptor deficiency induces early, transient and hyperglycaemia-independent blood–brain barrier dysfunction. *Sci Rep* 9:2884.
- Granholm AC, Bimonte-Nelson HA, Moore AB, Nelson ME, Freeman LR, Sambamurti K (2008) Effects of a saturated fat and high cholesterol diet on memory and hippocampal morphology in the middle-aged rat. *J Alzheimers Dis* 14:133–145.
- Konishi M, Sakaguchi M, Lockhart SM, Cai W, Li ME, Homan EP, Rask-Madsen C, Kahn CR (2017) Endothelial insulin receptors differentially control insulin signaling kinetics in peripheral tissues and brain of mice. *Proc Natl Acad Sci U S A* 114:E8478–E8487.
- Mäe MA, Li T, Bertuzzi G, Raschperger E, Vanlandewijck M, He L, Nahar K, Dalheim A, Hofmann JJ, Laviña B, Keller A, Betsholtz C, Genové G (2018) Prolonged systemic hyperglycemia does not cause pericyte loss and permeability at the mouse blood–brain barrier. *Sci Rep* 8:17462.
- Reagan LP (2012) Diabetes as a chronic metabolic stressor: causes, consequences and clinical complications. *Exp Neurol* 233:68–78.
- Rhea EM, Banks WA (2019) Role of the blood–brain barrier in central nervous system insulin resistance. *Front Neurosci* 13:521.
- Rhea EM, Salameh TS, Logsdon AF, Hanson AJ, Erickson MA, Banks WA (2017) Blood–brain barriers in obesity. *AAPS J* 19:921–930.
- Salameh TS, Shah GN, Price TO, Hayden MR, Banks WA (2016) Blood–brain barrier disruption and neurovascular unit dysfunction in diabetic mice: protection with the mitochondrial carbonic anhydrase inhibitor topiramate. *J Pharmacol Exp Ther* 359:452–459.
- Salameh TS, Mortell WG, Logsdon AF, Butterfield DA, Banks WA (2019) Disruption of the hippocampal and hypothalamic blood–brain barrier in a diet-induced obese model of type II diabetes: prevention and treatment by the mitochondrial carbonic anhydrase inhibitor, topiramate. *Fluids Barriers CNS* 16:1.
- Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, Mattson MP (2008) Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus* 18:1085–1088.
- Sun YN, Liu LB, Xue YX, Wang P (2015) Effects of insulin combined with idebenone on blood–brain barrier permeability in diabetic rats. *J Neurosci Res* 93:666–677.
- Takechi R, Lam V, Brook E, Giles C, Fimognari N, Mooradian A, Al-Salami H, Coulson SH, Nesbit M, Mamo JCL (2017) Blood–brain barrier dysfunction precedes cognitive decline and neurodegeneration in diabetic insulin resistant mouse model: an implication for causal link. *Front Aging Neurosci* 9:399.
- Valcarcel-Ares MN, Tucsek Z, Kiss T, Giles CB, Tarantini S, Yabluchanskiy A, Balasubramanian P, Gautam T, Galvan V, Ballabh P, Richardson A, Freeman WM, Wren JD, Deak F, Ungvari Z, Csiszar A (2019) Obesity in aging exacerbates neuroinflammation, dysregulating synaptic function-related genes and altering eicosanoid synthesis in the mouse hippocampus: potential role in impaired synaptic plasticity and cognitive decline. *J Gerontol A Biol Sci Med Sci* 74:290–298.
- Van Dyken P, Lacoste B (2018) Impact of metabolic syndrome on neuroinflammation and the blood–brain barrier. *Front Neurosci* 12:930.
- Yamamoto M, Guo DH, Hernandez CM, Stranahan AM (2019) Endothelial Adora2a activation promotes blood–brain barrier breakdown and cognitive impairment in mice with diet-induced insulin resistance. *J Neurosci* 39:4179–4192.
- Yang F, Wang Z, Wei X, Han H, Meng X, Zhang Y, Shi W, Li F, Xin T, Pang Q, Yi F (2014) NLRP3 deficiency ameliorates neurovascular damage in experimental ischemic stroke. *J Cereb Blood Flow Metab* 34:660–667.
- Yi CX, Tschöp MH, Woods SC, Hofmann SM (2012) High-fat-diet exposure induces IgG accumulation in hypothalamic microglia. *Dis Model Mech* 5:686–690.
- Zhou Y, Zeng X, Li G, Yang Q, Xu J, Zhang M, Mao X, Cao Y, Wang L, Xu Y, Wang Y, Zhang Y, Xu Z, Wu C, Chen JF, Hoda MN, Liu Z, Hong M, Huo Y (2019) Inactivation of endothelial adenosine receptor 2A protects mice from cerebral ischemia-induced brain injury. *Br J Pharmacol* 176:2250–2263.