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The amylin dyshomeostasis hypothesis: small vessel-type ischemic stroke in the setting of type-2 diabetes mellitus

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Abstract

Recent histological analyses of human brains show that small-vessel type injuries in the setting of type-2 diabetes are colocalized with deposits of amylin, an amyloid-forming hormone secreted by the pancreas. Amylin inclusions are also identified in circulating red blood cells in persons with type-2 diabetes and stroke or cardiovascular disease. In laboratory models of type-2 diabetes, accumulation of aggregated amylin in blood and the cerebral microvasculature induces brain microhemorrhages and reduces cerebral blood flow leading to white matter ischemia and neurological deficits. At the cellular level, aggregated amylin causes cell membrane lipid peroxidation injury, downregulation of tight junction proteins and activation of pro-inflammatory signaling pathways which, in turn, induces macrophage activation and macrophage infiltration in vascular areas positive for amylin deposition. We review each step of this cascade based on experimental and clinical evidence, and propose the hypothesis that systemic amylin dyshomeostasis may underlie the disparity between glycemic control and stroke risk and may be a therapeutic target to reduce the risk of small-vessel ischemic stroke in patients with type-2 diabetes.

There is a disparity between the level of glycemic control and the risk of macrovascular events such as myocardial infarction and stroke in persons with type-2 diabetes mellitus.[1,2] Amylin, an amyloidogenic peptide synthesized and co-secreted with insulin by pancreatic β-cells,[3] is overexpressed in individuals with prediabetic insulin resistance,[4–6] and forms pancreatic amyloid in those with type-2 diabetes.[7–9] Low molecular weight amyloidogenic proteins such as amylin and the Alzheimer's disease biomarker β-amyloid peptide generate a variety of cytotoxic aggregates.[10–13] Studies from multiple research teams find that diabetic states (prediabetic insulin resistance and type-2 diabetes) are associated with

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Disclosures

Dr. Goldstein and Dr. Despa report a patent on "Compositions and methods for enhancing neuro-repair" (Amylin skin test, USPTO 16/395,742)

Dr. Despa reports a patent on "Diagnosis of diabetes by detecting aggregated amylin in erythrocytes" (WO 2020/102566 A1)

increased circulating levels of aggregated amylin[14,15] and amylin deposition in extra-pancreatic tissues,[14–26.] including the brain microvasculature.[14,16–20.] By using rats with genetically manipulated amylin secretion, our team showed that accumulation of aggregated amylin in the blood and microvasculature causes brain microhemorrhages and reduced cerebral blood flow leading to white matter ischemic changes and neurological deficits.[16.] At the cellular level, amylin inclusions in vascular walls cause lipid peroxidation-related cell membrane injury, loss of endothelial cell coverage, and downregulation of tight junction proteins (Figure 1, red code pathway). Lipid peroxidation vascular injury contributes to a pro-inflammatory state affecting endothelial cells which, in turn, induces macrophage activation and macrophage infiltration in vascular areas positive for amylin deposition and microhemorrhages (Figure 1, magenta code pathway). Furthermore, amylin inclusions are identified in red blood cells (RBCs) of individuals with type-2 diabetes and stroke or cardiovascular disease.[26] Amylin-coated RBCs directly activate hypoxia signaling (Figure 1, green code pathway), have increased adhesion to vascular endothelial cells, and a tendency to aggregate.[26] Thus, amylin-mediated microvascular injury, macrophage infiltration, and reduced RBC flux due to amylin deposition on RBCs and endothelial cells may increase the risk of small vessel-type ischemic stroke (Figure 1, navy code pathway). Each step of this cascade is discussed in detail based on experimental and clinical evidence.

Circulating aggregated amylin induces brain microhemorrhages.

Radiographically-defined brain microhemorrhages (vascular microlesions)[27,28] correspond histologically to hemosiderin-laden phagocytic microglia.[29–31] They are associated with cerebral amyloid angiopathy, hypertension, and cerebral hypoperfusion associated with arteriolosclerosis, in addition to other conditions.[21–34]

We found that amylin aggregation in pancreatic islets leads to a feed-forward pathologic process by which aggregated amylin is secreted into the blood, deposits in brain blood vessels, and provokes brain microvascular injury by degrading endothelial cell coverage and tight junctions in rats that express amyloid-forming human amylin within pancreatic β-cells (HIP, human amylin insulin promoter transgenic rats).[16] This amylin deposition in cerebral small blood vessels is associated with vessel wall disruption and abnormal surrounding neuropil in patients with type-2 diabetes and vascular dementia,[16] in HIP rats,[16,35] and in amylin knockout rats infused with aggregated amylin.[16] In HIP rats, amylin-mediated injury in the cerebral microvasculature leads to accelerated aging,[16] neuroinflammation,[35] brain parenchymal loss,[16] and impaired neurological functioning.[16,35.] These data identify amylin deposition in cerebral small vessels as a trigger of brain microhemorrhages and neurologic deficits that are modulated by the circulating level of aggregated amylin.[16,35].

Amylin-induced vascular microlesions activate Interleukin-1β (IL-1β) pro-inflammatory pathways.

In pancreatic islets, aggregated amylin induces oxidative stress[11,12] leading to NLRP3 inflammasome activation and release of IL-1β,[36,37] a cytokine involved in a plethora of inflammatory responses.[38] In vascular cells, IL-1β is involved in the signaling pathway that mediates leukocyte interactions.[38.] Histological analyses in human tissues (brain[39] and

heart[15]) in addition to *in vivo* experiments in transgenic animals and cell model systems showed that the interaction between aggregated amylin and cellular membranes destabilizes cellular membranes and generates reactive aldehydes.[15,39] The functional effects of these cellular processes include the increased synthesis of IL-1β[15,39] in extra-pancreatic tissues, consistent with amylin-mediated injury in pancreatic β-cells and elevated IL-1β in type-2 diabetes.[36,37] Thus, exacerbated synthesis of IL-1β may be a critical stress-activated signaling pathway in response to the interaction of aggregated amylin with cellular membranes. Reports from our team also show that generation of reactive aldehydes and increased synthesis of IL-1β are caused by amylin aggregation independent of hyperglycemia.[15,39.] As proof of concept for the proposed mechanism, we evaluated the effect of combined treatments with N-acetyl cysteine, an antioxidant, and a surfactant membrane stabilizer[15,39] and found they synergistically inhibit the lipid peroxidation chain reaction and renormalize IL-1β synthesis[15,39] (Figure 2). Thus, IL-1β might function as a sensor of cellular amylin uptake and potential mediator of pro-inflammatory responses to amylin-induced brain microhemorrhages.

Amylin-mediated vascular microlesions activate hypoxia signaling.

Using biochemical analyses of human blood and blood transfusions in transgenic rats, our team found that amylin accumulation in blood cells and microvasculature activate hypoxia-inducible transcription factors (HIF-1 and HIF-2) in endothelial cells.[26] Erythropoietin upregulation, a consequence of hypoxia signaling activation, correlate with lower hematocrit in HIP rats,[26] common in pathologic erythropoiesis.[40] These effects are present in diabetic HIP rats that express amyloid-forming human amylin in the pancreas, but not in age- and blood glucose-matched rats that express non-amyloid forming rat amylin demonstrating that amylin-induced hypoxia signaling is independent of glucotoxicity.[26] Vascular amylin deposition in HIP rats induces arginase dysregulation,[26] suggesting subsequent effects on nitric oxide production and endothelium-mediated regulation of vascular smooth muscle cell tone. Nitric oxide production is modulated via regulation of two opposing L-arginine metabolic pathways.[41.] HIF-1 induces expression of endothelial nitric oxide synthase whereas HIF-2 regulates arginase expression, with both enzymes dependent upon L-arginine as a substrate.[41] Increased arginase expression/activity can therefore result in nitric oxide deficiency and deleterious effects on endothelium-mediated regulation of vascular smooth muscle tone, which can exacerbate blood flow impairment in cerebral ischemia. Pharmacological downregulation of adhesion proteins in the vascular endothelium ameliorates the effects of circulating aggregated amylin while also reducing HIF-1,2 and arginase protein expression levels[26] (Figure 3). These results suggest that endothelial adhesion proteins are potential therapeutic targets to reduce vascular amylin deposition and pathology.

Amylin-coated RBCs and vascular amylin deposits promote microthrombi.

RBC amylin content can be a useful maker of abnormally increased secretion of amyloid-forming amylin species from pancreatic islets.[26.] Because amylin deposition on RBCs directly affects rheological properties of the blood and increases the adhesion of RBCs to

endothelium,[26] these processes likely contribute to the complex mechanisms underlying diabetic microvascular disease.[42,43]

RBCs passively incorporate into the growing fibrin network of thrombi via binding to leukocytes and platelets.[44] Because the inter-cell interaction appears more important in RBC aggregation than adhesion of RBCs to vascular endothelium,[45,46] we hypothesize that the amyloidogenic nature of amylin in amylin-coated RBCs promotes aggregation. Aggregated RBCs could further interact with clotting factors to form microthrombi. Indeed, increased RBC aggregation is common in chronic cerebral ischemia,[47,48] acute stroke[49,50] and microvascular angina.[51] Slowed capillary RBC flow owing to amylin deposition on RBCs and endothelial cells likely leads to impaired neuronal oxygen delivery. Furthermore, because RBCs act as both oxygen carriers and mediators of oxygen sensing and signaling pathways within blood capillary walls,[40] amylin-coated RBCs may directly contribute to cerebral hypoxic-ischemic injury.

Clinical perspectives.

Amylin-mediated microvascular injury, macrophage infiltration and reduced RBC flow due to amylin deposition on RBCs and endothelial cells likely exacerbate circulatory disturbances leading to ischemic tissue injury in the setting of type-2 diabetes mellitus. Noteworthy, amylin-positive occluded small blood vessels can be identified both in brain[16] and peripheral tissues,[23,25] and were also induced in healthy rats by RBC transfusions from diabetic rats expressing amyloid-forming human amylin.[26] Because human amylin is amyloidogenic, whereas rodent amylin is not,[52] this provides the opportunity to advance mechanistic studies on the potential role of systemic amylin dyshomeostasis in ischemic stroke. Future clinical and experimental studies are needed to: 1, determine the prevalence of amylin-mediated formation of microthrombi in early stages of diabetes-associated ischemic stroke; 2, improve the understanding of whether and how amylin-mediated microthrombi lead to ischemic tissue injury and stroke in patients with type-2 diabetes mellitus; and 3, determine whether lowering amylin accumulation in blood cells and microvasculature could provide a novel strategy for reducing the risk of ischemic stroke and small-vessel tissue injury in patients with diabetes.

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Non-standard Abbreviations and Acronyms

HIP rats	Human amylin insulin promoter transgenic rats
IL-1β	Interleukin-1β

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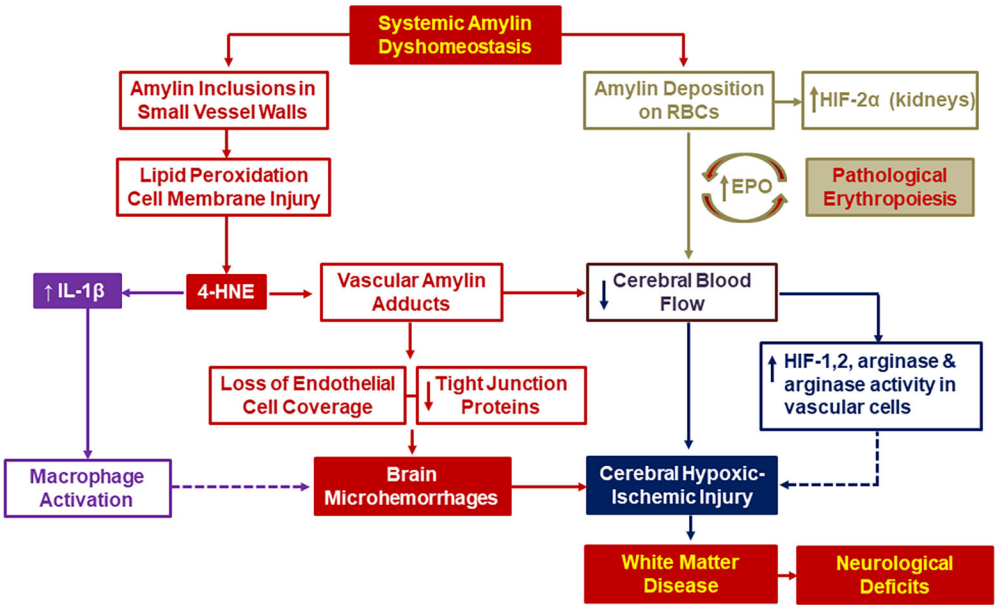

Figure 1. The amylin dyshomeostasis hypothesis of small vessel-type ischemic stroke in the setting of type-2 diabetes mellitus.

Systemic amylin dyshomeostasis is characterized by accumulation of aggregated amylin in blood and microvasculature, brain microhemorrhages and reduced cerebral blood flow leading to white matter injury and neurological deficits. At the cellular level, amylin inclusions in vascular walls generate reactive aldehydes such as 4-hydroxinonenal (4-HNE) and vascular amylin adducts causing loss of endothelial cell coverage and downregulation of tight junction proteins (red code pathway). Increased 4-HNE levels activates pro-inflammatory IL-1β signaling pathways leading to macrophage activation and macrophage infiltration in vascular areas positive for amylin deposition (magenta code pathway). In addition, amylin-coated RBCs directly activate hypoxia signaling (green code pathway), have increased adhesion to vascular endothelial cells and tendency to aggregate exacerbate the risk of small vessel-type ischemic stroke (navy code pathway).

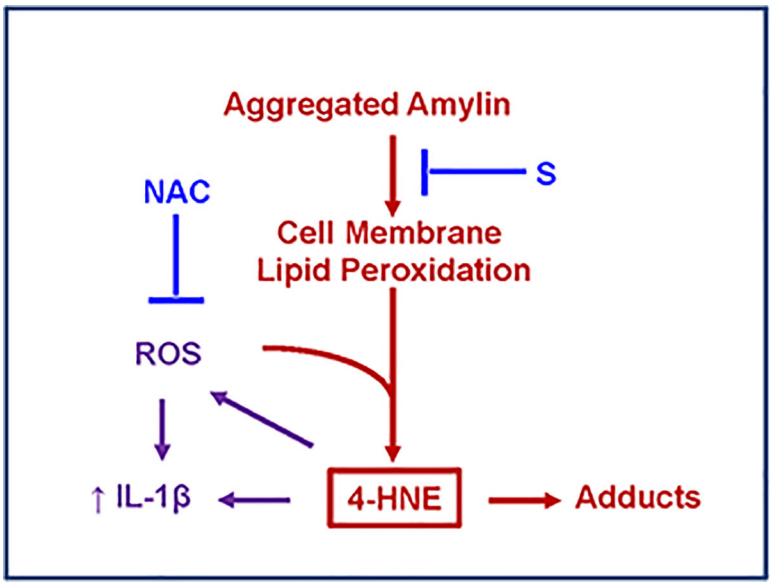

Figure 2. Proposed mechanism for amylin-mediated lipid peroxidation cell membrane injury and activation of IL-1β pro-inflammatory signaling pathway

(adapted from Ref. 15). Amylin inclusions in cellular membranes generate reactive aldehydes such as 4-hydroxnonenal (4-HNE) that perturb intracellular homeostasis, leading to increased synthesis of IL-1β. Blocking either cellular amylin uptake (by a surfactant cell membrane stabilizer; S), or the lipid peroxidation chain reaction (by N-acetyl cysteine; NAC), demonstrate that peroxidative membrane injury is upstream of IL-1β increased synthesis.

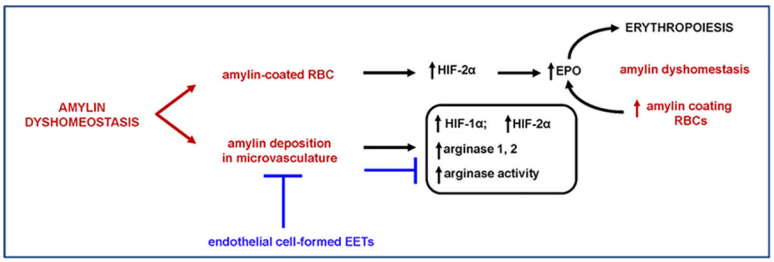

Figure 3. RBC-vascular endothelium interaction is altered by amylin deposition on RBCs and microvasculature, and is reversed by endothelial cell-secreted epoxyeicosatrienoic acids (EETs) (adapted from Ref. 26). Amylin deposition on RBCs activates HIF-mediated hypoxia signaling pathways in kidneys and downstream upregulation of erythropoietin (EPO). These cellular processes are associated with pathologic erythropoiesis and arginase dysregulation within vascular tissue. EETs reduce this effect by downregulation of adhesion proteins in the vascular endothelium.