Diabetic Complications Consortium

Application Title: Amylin amyloid, a link between pancreas and brain pathologies in diabetes

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1. Project Accomplishments:

We identified amylin deposits in post-mortem brain tissue from older people who had been diagnosed with Alzheimer's disease (AD) or cerebrovascular dementia (CVD) and type-2 diabetes (T2D). Our findings also indicated that amylin may play a similar role in the AD process as amyloid-β (Aβ) protein, a hallmark of the disorder.

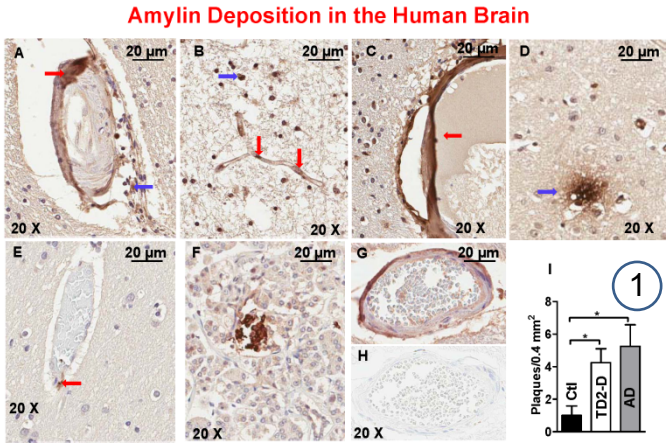
Amylin (also known as islet amyloid polypeptide) is a hormone synthesized and co-secreted with insulin and plays a role in glucose homeostasis. When amylin is oversecreted, the risk of developing type-2 diabetes increases. Like Aβ, amylin circulates in the blood and, during the disease process, is overproduced and not cleared normally, building up in the brain. Over time, both proteins lead to the loss of brain cells and brain damage. Our findings may explain why diabetics are at increased risk for cerebrovascular injury. Amylin buildup in the brain's blood vessels may also play a role in amyloid buildup and contribute to risk for AD.

Using a rat model that overexpresses human amylin in the pancreas, we found that brain amylin deposition is associated with reduced exploratory drive, recognition memory and vestibulomotor function[1]. The results suggest that hyperamylinemia may be an early contributor to the multifactorial mechanism by which IR predisposes to brain injury and cognitive decline.

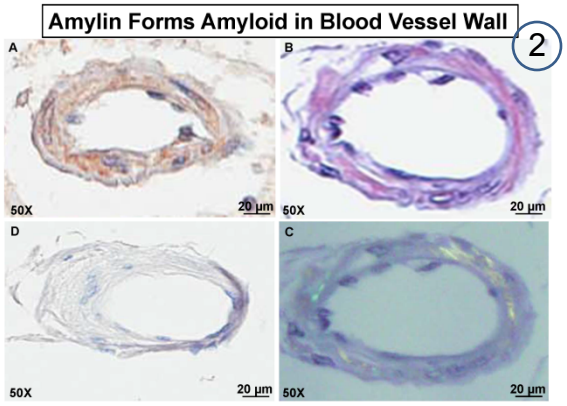
2. Specific Aims:

Specific Aim 1. **Test the specificity of cerebral deposition of amylin in type-2 diabetes.**

Results: We examined post-mortem temporal lobe (Brodmann areas 21/22) gray matter from 42 individuals. One control group consisted of 13 non-diabetic cognitively normal humans with low Braak and Braak scores. The second control group consisted of temporal lobe tissue from 14 non-diabetic AD patients. Diabetic patient group included 15 individuals with AD or CVD. Our study[2]



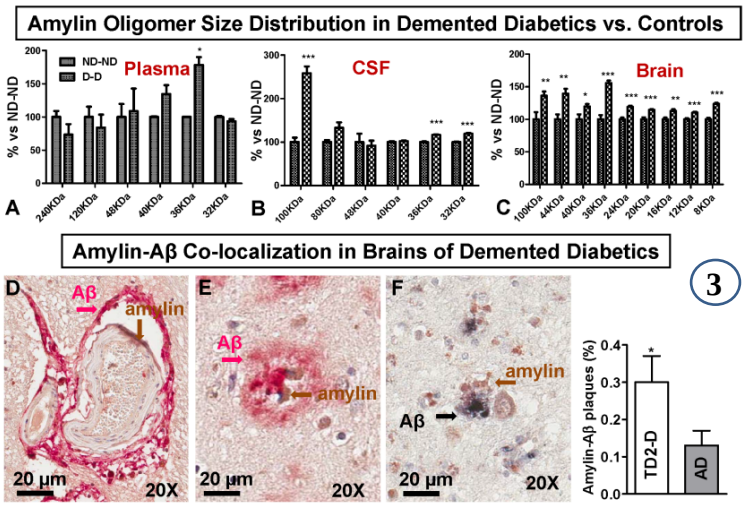
demonstrated that brain amylin deposition is associated with AD and CVD (**Fig 1**). Large amylin deposits were identified in the temporal lobe (Brodmann areas 21/22) gray matter from diabetic patients with dementia (T2D-D group) (**Fig 1A** and **B**). Amylin deposits are present in blood vessel wall (**Fig 1A**; red arrow) and pericapillary spaces (**Fig 1A**; blue arrow), consistent with amylin influx from the circulation. Amylin patches (plaques) are also observed in parenchyma, away from blood vessel walls (**Fig 1B;** blue arrow). Deposits coincide with areas of increased interstitial space, vacuolation, and spongiform change (**Fig 1B**). Bending of capillaries at the amyloid accumulation sites (**Fig 1B,** arrows), cell multinucleation, variation in nuclear size, and infiltrative cells (**Fig 1A**) are also observed in areas infiltrated by amylin. Intriguingly, amylin deposition was also detected in brain specimens from patients with AD without clinically apparent diabetes (AD group; **Fig 1C** and **D**). Moreover, the amylin distribution in AD brain was similar to that in brain samples from the T2D-D group, including buildup on blood vessel walls (**Fig 1C**; red arrow) and parenchyma (**Fig 1D**; blue arrow). In contrast, brain specimens from age-matched healthy humans show only sporadic amylin deposits in blood vessels and brain parenchyma (**Fig 1E**; arrow). Density distribution of large amylin plaques, i.e. ~ 20µm in diameter or larger (**Fig 1D**), is significantly higher in the brain parenchyma from patients in T2D-D and AD groups (**Fig 1I**) suggesting a potential role of amylin in plaque formation. The extent of amylin accumulation (**Fig 1I**) is actually much larger taking into account the large density of smaller plaques (**Fig 1A** to **D**). **Fig 1F** displays a positive control for amylin deposition (pancreas from T2D patients). **Fig 1G** shows amylin deposition in a blood vessel similar to **Fig 1A** and **C**, while **Fig 1H** displays the same blood vessel in a brain section incubated only with the secondary antibody.



We also showed[2] that the amylin deposited in the blood vessel wall (**Fig 2A**) exhibits apple-green birefringence in the Congo red stain (**Fig 2B** and **2C**). The same blood vessel shows no Aβ immunoreactivity (**Fig 2D**). The results demonstrate that amylin amyloid incorporation in blood vessel can comprise non-Aβ amyloid deposition. Deposition of amylin amyloid in the vascular media and adventitia (as observed in **Fig 2A**) may lead to loss of integrity of the vessel wall (cerebral amyloid angiopathy; CAA) and dementia.

Specific Aim 2. **Test the co-localization of amyloid β and amylin in the brain of demented diabetics.**

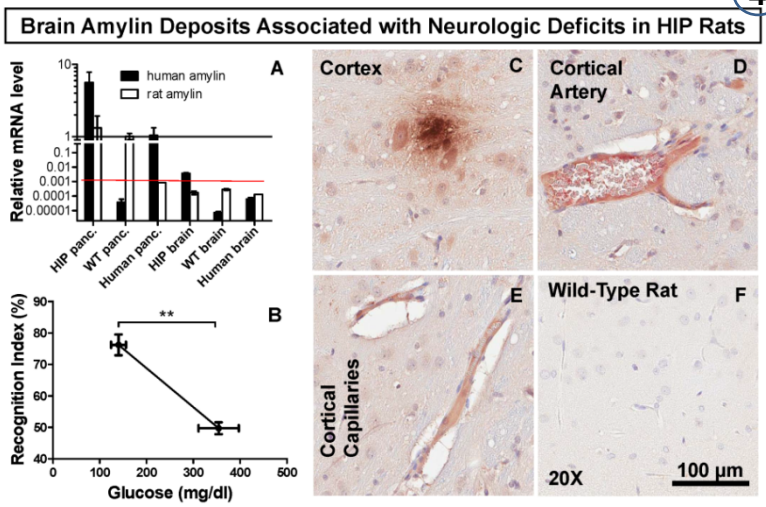
Results: Our preliminary data (unpublished) show that the oligomerized amylin levels in plasma (**Fig 3A**), CSF (**Fig 3B**) and brain tissue (**Fig 3C**) are higher in individuals with T2D and AD/ CVD (Di-De group, n=8) compared to healthy controls (non-diabetic, noon-demented; NDi-NDe group, n=24). This ongoing study suggests that the oligomerized amylin may be a bio-marker of increased risk for dementia in diabetic population.



The circulating oligomeric amylin may be an intrinsic mechanism of accumulation in the brain. In addition, we found that oligomeric amylin interacts with Aβ forming mixed amylin-Aβ plaques in perivascular area (**Fig 3D**) and brain tissue (**Fig 3E,F**). The results suggest that T2D and pre-diabetes may accelerate accumulation of amyloid-like deposits in the brains of individuals with impaired Aβ homeostasis (i.e., increased expression of Aβ or low clearance of Aβ from the brain).

Using a rat model of brain amylin deposition which overexpresses human amylin in the pancreas (the HIP rat; **Fig 4A**), we demonstrated that hyperamylinemia is associated with neurologic deficits[1]. Brain function in HIP rats was assessed by home cage activity monitoring, rotarod and novel object recognition (NOR) protocols. Based on the home cage activity monitoring, there were no significant differences in horizontal (x, y) activity. Total travel distance and velocity were similar in HIP rats and wild-type (WT) rats. The count of spontaneous rear up was lower in HIP rats compared to WT rats suggesting decreased exploratory activity in HIP rats. The vestibulomotor performance of HIP rats and WT rats was analyzed by using the rotarod test. In the first day of training, HIP rats and WT rats had similar vestibulomotor performance. However, HIP rats demonstrated no ability to improve performance on the rotarod. In contrast, WT rats increased the rotarod time with each day of training. Because HIP rats have preserved locomotion ability, their poor rotarod test performance suggests impaired coordination or/and learning deficits. The novel object recognition (NOR) protocol was used to assess possible changes in recognition memory in HIP rats versus age-matched WT rats. Both pre-diabetic HIP rats and diabetic HIP rats were included in the study[1]. The time spent exploring a familiar object and a novel object was recorded during a fixed duration trial. Diabetic HIP rats showed significant memory impairment indicated by reduced exploration of

the novel object, as compared to WT rats. Recognition memory function of pre-diabetic HIP rats was intermediate, but not significantly different from either WT rats or diabetic HIP rats. To assess possible regression in the recognition memory with the development of disease, a subset of animals with low blood glucose levels at the time of the initial NOR test were re-tested at a later time (~ 8 weeks), after they developed full-blown T2D (i.e., blood glucose >200 mg/dl). (4) Progressing to full diabetes led to a significant drop in the recognition memory in HIP rats (P=0.0022; **Fig 4B**). Brain tissue from pre-diabetic HIP rats was analyzed by immune-histochemistry with an anti-amylin antibody (**Fig 4C-F**). Large amylin deposits, i.e. > 50µm diameter, similar to those detected in brains from AD patients, were occasionally seen in HIP rat brains (**Fig 4C**). Brain blood vessels in HIP rats are positive for amylin deposition (**Fig 4D,E**). As expected, WT rats lack brain amylin deposition (**Fig 4F**). Hence, the feasibility study[1] suggests that the HIP rat is a clinically relevant animal model to study dynamics of cerebral amylin deposition and its impact on brain function.



3. Publications:

1. Srodulski S, Sharma S, Bachstetter AB, Brelsfoard JM, Pascual C, Xie S, Saatman KE, Van Eldik LJ, Despa F. Neuroinflammation and neurologic deficits in diabetes linked to brain accumulation of amylin. Mol Neurodegener. 2014; 9 30
2. Jackson K, Barisone G, Jin L-W, DeCarli C, Despa F: Amylin deposition in the brain: A second amyloid in Alzheimer disease? *Ann Neurol* 2013, 74(4):517-526.