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Amylin deposition in the brain: a second amyloid in Alzheimer's disease ?

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Abstract

Objectives—Hyperamylinemia, a common pancreatic disorder in obese and insulin resistant patients, is known to cause amylin oligomerization and cytotoxicity in pancreatic islets leading to β -cell mass depletion and development of type-2 diabetes. Recent data revealed that hyperamylinemia also affects the vascular system, heart and kidneys. We, therefore, hypothesized that oligomerized amylin might accumulate in cerebrovascular system and brain parenchyma of diabetic patients.

Methods—Amylin accumulation in the brain of diabetic patients with vascular dementia or Alzheimer's disease (AD), non-diabetic patients with AD, and age-matched healthy controls was assessed by quantitative real-time PCR, immunohistochemistry, western blot and ELISA.

Results—Amylin oligomers and plaques were identified in the temporal lobe gray matter from diabetic patients, but not controls. In addition, extensive amylin deposition was found in blood vessels and perivascular spaces. Intriguingly, amylin deposition was also detected in blood vessels and brain parenchyma of patients with late-onset AD without clinically apparent diabetes. Mixed amylin and A β deposits were occasionally observed. However, amylin accumulation leads to amyloid formation independent of A β deposition. Tissues infiltrated by amylin show increased interstitial space, vacuolation, spongiform change, and capillaries bended at amylin accumulation sites. Unlike the pancreas, there was no evidence of amylin synthesis in the brain.

Interpretations—Metabolic disorders and aging promote accumulation of amylin amyloid in cerebrovascular system and gray matter altering microvasculature and tissue structure. Amylin amyloid formation in the wall of cerebral blood vessels may also induce failure of elimination of A β from the brain, thus contributing to the etiology of AD.

Introduction

Type-2 diabetes (T2D) is a chronic metabolic disorder that increases the risk for cerebrovascular disease and dementia.^{1–6} Increased risk for these diseases develops years before^{7–9} the onset of clinically apparent diabetes and is higher in people with obesity and insulin resistance.^{2,4–11} A cluster of interconnected factors including hypertension,^{4,9,12–16} hyperglycemia,^{4,8,9,13,15–17} dyslipidemia,^{4,8,9,13,15,16} and hyperinsulinemia^{18–20} are considered major contributing mechanisms. Obese and insulin resistant patients also have increased blood concentrations of amylin (also known as islet amyloid polypeptide),^{21–24} a

hormone co-expressed and co-secreted with insulin by pancreatic β -cells. Hyperamylinemia coincides with hyperinsulinemia,²¹ is diabetogenic,²¹ and affects the cardiovascular system.^{22,23}

Deleterious effects of hyperamylinemia are associated with the high propensity of human amylin to form amyloids.²¹ Similar to prions²⁴ and other amyloid-assembling proteins,^{25–27} the toxic species of human amylin are the soluble oligomers. They form membrane-permeant oligomers^{28,29} that alter Ca^{2+} homeostasis and viability of various cells,^{22,30–33} including astrocytes³¹ and neurons.^{31,32} In addition, oligomeric amylin affects the cardiovascular system by stimulating lipolysis,^{21,34} elevating plasma free fatty acid levels,²¹ activating the renin-angiotensin-aldosterone system,³⁵ engaging the advanced glycation end-products receptors (RAGE),³⁵ and promoting inflammatory^{21,35} and oxidative^{21,34,36} stress.

Amylin oligomerization and amylin deposition in the pancreas are hallmark features of T2D.²¹ Recent experimental evidence^{22,23} shows that hyperamylinemia also induces toxicity in peripheral organs. Amylin deposition has been found in heart²² and kidneys²³ of obese and diabetic patients. Examination of human myocardium demonstrated accumulation of large amylin oligomers and amylin plaques in failing hearts from obese and diabetic patients, without such deposits in non-failing hearts or in failing hearts from lean, non-diabetic individuals.²² Buildup of oligomeric amylin in the heart accelerates diabetic heart failure.²²

Recognizing that the peripheral accumulation of amylin is associated with vascular and tissue damage to heart and kidneys, we hypothesized that a similar process may occur in the cerebrovasculature and brain structure of diabetic patients. To test this hypothesis, we investigated brain samples from diabetic humans with dementia, including cerebrovascular disease and Alzheimer's disease (AD), and compared the results to non-diabetic patients with AD and age-matched healthy individuals.

Methods

Detailed procedures are included in the Supplemental Material.

Human tissue specimens

Human tissue specimens were provided by the Alzheimer's Disease Center at University of California Davis. They were obtained through consented autopsies with the Institutional Review Board approval. We analyzed brain specimens from late-onset AD patients (>70 yo) and age-matched diabetic humans. Brain samples from age-matched non-diabetic individuals, without AD, served as controls.

Brain tissues were divided in pathologically distinct groups as follows. T2D-D represents the group of brain tissues from patients with overt T2D and dementia (N=15). Patients in this group were with either vascular dementia (N=8) or AD (N=7). The presence of diabetes in these patients was determined by their medical history or the use of diabetic medications. Vascular dementia was diagnosed when there was sufficient evidence of vascular pathology to explain the dementia in the absence of significant AD or other pathologies. Because of their overt diabetes, patients in this group were expected to show significant cerebral accumulation of amylin. The AD group (AD, N=14) includes brain samples from AD patients without history of T2D. Brain specimens from age-matched healthy individuals served as controls (Ctl, N=13). The absence of clinically evident diabetes in patients from AD and control groups was concluded from the review of the medical history including detailed medication information. We expected no significant amylin deposition in brain specimens from the control group. Etiology of cerebral and vascular disorders, age, gender,

and diabetes status of all cases included in the present study are summarized in Tables 1–3 (Supplemental Material).

Immunohistochemistry

Western blot, immunohistochemistry, and ELISA were used to test amylin accumulation in temporal cortex specimens. Western blot and ELISA assays were done on all brain specimens. A subset of four samples from each group was examined by immunohistochemistry. An anti-human amylin antibody (polyclonal, raised in rabbit, Bachem-Peninsula, T4149) was the primary antibody in immunohistochemistry and western blot tests. The anti-amylin antibody does not recognize A β (Supplemental Material; Fig S1). Human pancreas tissue from a T2D patient was the positive control for amylin deposition. To test whether amylin forms amyloid-like structures in the brain, we performed serial staining with anti-amylin antibody and Congo red (Fig 2A to D). In the immunohistochemistry tests, biotinylated goat anti-rabbit IgG (Vector) was the secondary antibody. The specificity of anti-amylin antibody in immunohistochemistry studies was tested by incubating sections only with the secondary antibody (Fig 2H).

For western blot, brain tissue was homogenized in homogenization buffer containing 10 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 0.1% sodium dodecyl sulfate, 1% TritonX-100, 1% sodium deoxycholate, 5 mmol/L EDTA, 1 mmol/L NaF, 1 mmol/L sodium orthovanadate and protease and phosphatase inhibitor cocktail (Calbiochem). Brain lysate was generated by centrifugation (12,000 g) of brain protein homogenate. After standard western blot assay, the signal intensity of each band was analyzed in Image J. To verify specific staining of protein bands, samples were loaded onto a gel in duplicate and after blotting and blocking, the membrane was cut and one half was incubated with the anti-amylin antibody while the other half was incubated in the absence of a primary antibody. Both halves were then incubated with the secondary antibody and developed and imaged together (Supplemental Material; Fig S2). Immunochemical results were verified with a second anti-human amylin antibody (polyclonal, raised in rabbit, Lifespan Biosciences, LS-C20521, see Supplemental Material, Fig S3). Possible presence of proamylin in human brains was assessed with an anti-proamylin antibody (polyclonal, raised in rabbit, Santa Cruz Biotechnology, H50). Equal loading in western blot experiments was verified by re-probing with a monoclonal anti- α -actin antibody (raised in mouse, clone BA3R, Thermo Scientific; Supplemental Material, Fig S4). An anti-A β antibody (monoclonal, raised in mouse, Covance, clone 6E10) was employed in assessing the A β -amylin co-localization. Total amylin content in supernatant of brain homogenates (250 μ g protein) was measured by ELISA using a total human amylin kit (EZHAT-51K, Millipore).

To immunoprecipitate amylin, 250 μ g of brain homogenate was incubated with anti-amylin antibody (20 μ g) overnight, at 4°C, with end-over-end rotation. The antigen-antibody complex was then incubated with Immobilized Protein A/G resin (Pierce) for 2 hours at room temperature with gentle mixing. The resin-bound complex was washed several times and immunoprecipitates were collected by elution with 50 μ L of elution buffer (0.08 N HCl, 200 mM glycine, pH=2–3), followed by brief centrifugation at 1,000 xg. The latter step was repeated and the supernatant fractions were combined, adjusted to physiological pH by adding 10 μ L of 1 M Tris solution (pH=7.5–9) and saved to load on the gel.

To detect the presence of oligomerized amylin in the circulation, blood samples from AD patients were centrifuged at 3500 rpm to remove cellular components and analyzed by western blot.

Quantitative real-time PCR

Expression level of amylin in human pancreas and brain was assessed by quantitative reverse transcription real-time PCR (qRT-PCR) using a modification of the protocol described in Ref. 37 (Supplemental Material).

Statistical Analysis

Data are expressed as mean $\pm$ SEM. Statistical discriminations were performed using two-tailed unpaired Student's *t* test, with $P < 0.05$ considered significant. One way analysis of variance (ANOVA) with the Dunnett post hoc test was used when comparing multiple groups.

Results

Immunohistochemical examination of temporal lobe gray matter from diabetic patients (T2D-D group) with an anti-amylin antibody uncovered large distributions of amylin plaques (Fig 1A and B). Amylin deposition is present in blood vessel wall (Fig 1A; red arrow) and pericapillary spaces (Fig 1A; blue arrow), consistent with amylin influx from the circulation. Patches of amylin are also observed in areas remote from blood vessel walls (Fig 1B; blue arrow). 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 was similar to that in brain samples from the T2D-D group, including buildup on blood vessel walls (Fig 1C; red arrow) and extravascular patches (Fig 1D; blue arrow). In contrast, brain specimens from age-matched healthy humans (the control group) show only sporadic amylin patches in blood vessels and brain parenchyma (Fig 1E; arrow). Fig 1F displays a positive control for amylin deposition formed in pancreatic islets of 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 demonstrating the specificity of the anti-amylin antibody. Density distribution of large amylin plaques, i.e. $\sim 20\mu\text{m}$ in diameter or larger (Fig 1D), is significantly higher in the brain parenchyma from patients in T2D-D and AD groups (Fig 1) suggesting a potential role in the development of the disease. The extent of amylin accumulation (Fig 1I) is actually much larger taking into account the large density of smaller plaques (Fig 1A to D). Additional data on cerebral amylin deposits are shown in the Supplemental Material (Fig S5 to S7). Amylin deposits coincide with areas of increased interstitial space, vacuolation, and spongiform change (Fig 1B). In contrast, the brain parenchyma of control subjects is intact (Fig 1E).

The incorporated amylin 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 A immunoreactivity (Fig 2D and Supplemental Fig S8). The results demonstrate that amylin incorporation in blood vessel leads to amyloid formation independent of A β deposition. Total amylin level in protein homogenate supernatants measured by ELISA is also significantly larger in brain specimens from T2D-D (Fig 3A). A substantially smaller, but statistically significant, increase of amylin aggregates is also observed in AD group (Fig 3A). In line with the immunohistochemistry (Figs 1A to D) and ELISA (Fig 3A) results, western blot analysis of tissue protein homogenates revealed increased levels of oligomeric amylin in brain specimens from T2D-D and AD groups (Fig 3B and C). Molecular weight bands corresponding to trimers (12 kDa), tetramers (16 kDa), and pentamers (20 kDa) have significantly higher intensity signals in AD and T2D-D groups compared to controls (Fig 3D). An example of whole electrophoretic pattern is presented in the Supplemental Material (Fig S2). Amylin deposition in AD brains suggests undiagnosed insulin resistance in these patients, which is common to aging.³⁸

Proamylin, a precursor of amylin, which is also prone to aggregation within the secretory pathways,²¹ may also participate to amylin buildup in the brain. Western blot analysis indicates the presence of proamylin oligomers in the brain (Fig3E). Additional data are presented as Supplemental Material (Fig S4).

To assess how deposition of amylin is distributed relative to A β plaques, brain specimens from AD patients were co-stained with anti-amylin and anti-A β antibodies (Fig 4 and 5). Profuse amylin (brown color) and A β (red in Fig 4A, B and 5 and dark blue in Fig 4C) patches are distinctly visible within blood vessels (Fig 4A,5 and A to D) and brain parenchyma (Fig 4B and C). Clusters of small amylin deposits are found next to much larger A β plaques (Fig 4C) or embedded in A β amyloids, as shown in Fig 4B. The fraction of mixed amylin-A β plaques is significantly lower in brain samples from the AD group compared to those in the T2D-D (Fig 4D). Brain samples from controls show no mixed amylin-A β plaques. Amylin is observed in the whole (Fig 5A) or part (Fig 5B) of the circumference of vessel wall in brain specimens from patients in T2D-D (Fig 5A) and AD (Fig 5B) groups, but not in controls (Figs 5E). Patchy distribution of amylin is similar (and complementary) to that of A β (Fig 5C and D) suggesting that both peptides are involved in the development of cerebral amyloid angiopathy (CAA). Fig 5F presents the estimated average fraction of the vessel wall circumference covered by amylin in brain samples from T2D-D, AD, and control groups. Although the anti-amylin and anti-A β antibodies clearly show mixtures of the two types of proteinaceous depositions within blood vessel walls (Fig 5) and brain parenchyma (Fig 4B and C), delineating accurately each contribution is difficult with current immunochemical methods.

Areas infiltrated by amylin and/or amylin-A β mixed plaques coincide with morphological changes of tissue, including bending of capillaries at the amyloid accumulation sites (Fig 1B, arrows), cell multinucleation, variation in nuclear size, and infiltrative cells (Fig 1A). These results suggest that amylin deposition alters the microvasculature and brain structure in a manner similar to A β pathology.²⁷ Assessing the true incidence and severity of mixed amylin-A β deposition in CAA and AD pathologies needs additional tests and will be addressed in future studies.

We also tested the presence of amylin mRNA in human brains using qRT-PCR. The results (Fig 6A) show that the level of amylin mRNA in the brain is $\sim 10^4$ lower than in the pancreas (the known source of insulin and amylin in vertebrates²¹). The level of amylin mRNA measured in the brain is within the error limit of detection. For instance, the same amount of human amylin mRNA was detected in rat tissues (Fig 6A), the negative controls in this experiment. Therefore, we interpret these results as lack of amylin expression in brain tissue and conclude that accumulation is from amylin secreted in the blood by pancreatic β -cells. Based on these data, we propose two possible mechanisms of amylin accumulation in the brain. One of these mechanisms may involve amylin oligomers that circulate in the blood and build up "homogenous" amylin plaques in various organs,^{22,23} including the brain (Fig 1A to D). To test this hypothesis, we assessed the presence of oligomeric amylin in plasma samples from AD patients (AD group) by western blot. Western blot analysis in Fig 6B indicates that amylin tetramers exist in both plasma and supernatant brain homogenates from patients in the AD group. Moreover, the immunoreactive signal of amylin is much higher in brain tissues suggesting ongoing accumulation. Another mechanism of amylin plaque formation in the brain may involve a direct interaction with A β , as suggested by our data (Fig 4A to C). To test this hypothesis, mixtures of amylin aggregates immunoprecipitated from brain protein extracts were analyzed by western blot using an anti-A β antibody (Fig 6C). A β immunoreactivity was detected in all these amylin-enriched samples indicating that the two peptides interact and aggregate forming soluble mixed oligomers (Fig 6C).

Discussion

Human amylin is a very aggressive amyloid-forming peptide.^{21–23,28–36} Over 95% of insulin resistant patients that develop overt T2D display amylin amyloids in the pancreas.²¹ The initial oligomerization process, which may also involve proamylin,^{39–41} occurs within the secretory pathway of pancreatic β -cells³⁹ and is associated with a chronic hyperglycemic stress.^{40,41} Amylin oligomerization and amyloid deposition contribute to pancreatic β -cell dysfunction and apoptosis leading to depletion of β -cell mass and development of T2D.²¹ Oligomerized amylin also accumulates in heart²² and kidneys²³ accelerating diabetic heart failure.²² Our study identified amylin deposits in the temporal lobe gray matter from diabetic patients (Fig 1B), but not in controls (Fig 1E). In addition, amylin deposition was found in both blood vessels and perivascular spaces (Fig 1A, B, 4A, and 5), consistent with amylin influx from the circulation. The absence of amylin transcript in the human brain (Fig 6A) confirms that accumulation is promoted by amylin oligomers circulating in the blood (Fig 6B) and derived from the pancreas. Accumulation of oligomerized amylin in the brain coincides with tissue morphological alteration, bending of capillaries at the site of amylin accumulation (Fig 1B; red arrows), areas of increased interstitial space, vacuolation, and spongiform change (Fig 1B). In contrast, the brain parenchyma of control subjects is intact (Fig 1E). Thus, these results implicate hyperamylinemia and consequent accumulation of oligomerized amylin in the cerebrovascular system as important contributors to diabetic brain damage and neurodegeneration. In addition, amylin amyloid formation in the wall of cerebral blood vessels (Fig 2, 4A and 5) may induce failure of elimination of A β from the brain, thus contributing to the etiology of AD.

A surprising result of our study is the finding of amylin deposition in the brain of AD patients in the absence of clinically apparent diabetes (Fig 1C, D and 3). This amylin deposition may be a sign of (undiagnosed) insulin resistance, which is common in aging.³⁸ Additional epidemiological studies are needed to clarify this hypothesis. However, a relationship between insulin resistance and AD has previously been proposed multiple times,^{2,4–11,18–20} although the underlying molecular mechanism was not identified. Our results suggest at least one potential mechanism. In AD brains, amylin was detected as oligomers (Fig 3) and distinct plaques (Fig 1C and D). Amylin was also co-localized with A β (Fig 4 and 5). In some cases, patches of amylin were embedded in A β amyloid (Fig 4B). Because amylin deposits stain positive for Congo red (Fig 2) and, hence, have amyloid-like structures, the two accumulating peptides are indistinguishable by amyloid-specific staining. The propensity of both peptides to form amyloid is a possible reason why they have not been previously identified. Our data showing complex amylin-A β patches (Fig 4 and 5) are in agreement with *in vitro* studies⁴² that demonstrated that mature amylin fibrils promote a robust growth of mixed amylin-A β amyloids. The presence of a distinct amylin core in some A β plaques suggests that A β interacts with amylin and/or amylin plaques *in vivo*. We can only speculate on the impact of this interaction, but one possibility is that amylin-mediated precipitation of A β amyloid may have a protective role by reducing the amount of toxic A β molecular species in the brain. Alternatively, these two amyloid species could act together or independently to accentuate toxicity. Amylin oligomerization is cytotoxic^{21,22,28,29} and may induce deleterious effects to brain. Amylin oligomerization can injure the neurons by forming membrane-permeant oligomers,^{28,29} similar to the A β oligomer-induced pathology.^{25,27,31,43} Indeed, previous results demonstrated that amylin oligomers alter Ca²⁺ homeostasis and viability of cultured astrocytes³¹ and neurons.^{31,32} Recent data⁴⁴ suggest that both human amylin and A β alter Ca²⁺ homeostasis in neurons through a mechanism involving the activation of AMY3 isoform of the calcitonin gene-related peptide receptors. Potential mechanism(s) of hyperamylinemia-mediated brain injury will be examined in future studies using animal models.

Factors contributing to the metabolic derangement in aging,³⁸ i.e. insulin resistance, changes in body composition, and altered secretion of hormones, are known to accelerate the neurodegenerative process, but the underlying molecular mechanisms are poorly understood. Our data suggest that hyperamylinemia and accumulation of oligomerized amylin in the brain are intrinsic components of the pathological mechanisms linking age-related metabolic disorders with both diabetic brain injury and AD. Greater understanding of the complex pathology contributing to (pre)diabetic brain injury may improve diagnosis and potentially lead to new treatments. For example, if hyperamylinemia and amylin oligomerization damage the brain, as suggested by our study, then it would require avoiding treatments that aim to increase insulin and amylin secretion in favor of treatments that prevent overstimulation of pancreatic β -cells, or insulin itself. Furthermore, if amylin accumulation is indeed important in the pathology of diabetic cerebrovascular and neurodegenerative disorders, the amylin oligomer may potentially be a new therapeutic target in the treatment of these diseases. For instance, drugs that prevent amylin oligomerization and/or increase clearance of amylin oligomers from the circulation may reduce the amylin deposition toxic effect on the brain.

In conclusion, our study shows, for the first time, that amylin derived from the pancreas accumulates in the brain precipitating as independent plaques or co-precipitating with A β to form complex amylin/A β plaques. The mechanism by which amylin accumulates in the brain seems multimodal and are likely involving insulin resistance/hyperamylinemia and circulating amylin oligomers. Deficient clearance of proteinaceous residues from the brain^{27,45} may also play a role in cerebral accumulation of oligomeric amylin. Based on these data, we propose that accumulation of oligomerized amylin in the brain is a direct effect of the pre-diabetic insulin resistance and may be a second amyloid that contributes to the complex pathology^{1–20} of age-related cerebrovascular and neurodegenerative disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.

Amylin deposition in brain specimens from T2D-D and AD patient groups demonstrated by immunohistochemistry with an anti-amylin antibody on thin tissue sections. A and B are representative images of amylin deposition in the brain of diabetic patients with vascular dementia. C and D show amylin plaques in brains from the AD group (non-diabetics with AD). Amylin is largely present on blood vessel walls (A, C; red arrows) and as small plaques within pericapillary areas (A; blue arrow) and even remote regions from capillaries (B, D; blue arrows). In contrast, brain samples of age-matched healthy humans show only occasional amylin deposition in blood vessels (E; arrow). F is a positive control for amylin deposition in pancreatic islets of T2D patients. G shows amylin deposition in a blood vessel similar to (A) and (C), while H displays the same blood vessel in a brain section incubated only with the secondary antibody demonstrating the specificity of the anti-amylin antibody. I shows the average surface density of amylin deposits in the brain parenchyma of patients in T2D-D, AD, and control groups (N=4 per group). Amylin deposition was assessed on a field of view 0.4 X 1mm. *P < 0.05.

Fig 2.

The same blood vessel stained for amylin (A), Congo red (B and C) and A (D). The incorporated amylin in the blood vessel wall exhibits apple-green birefringence under a polarized light microscope (Congo red stain; C) demonstrating that amylin deposition in the blood vessel forms amyloid-like structure. A larger scale view of the area selected in this figure is displayed in Supplemental Fig S8.

Fig 3.

Accumulation of oligomeric amylin in supernatant protein homogenates from brains in T2D-D, AD, and control groups demonstrated by ELISA (A) and western blot (B–D). B and C show the relative increase of amylin tetramers (B) and trimers (C) in T2D-D and AD brains versus controls. D displays representative electrophoretic patterns in these groups. * $P < 0.05$; ** $P < 0.01$. E shows that the amylin precursor, i.e. proamylin, may also be present in the brain. The test with an anti-proamylin antibody on western blot suggests that the band at ~60 kDa (right side of the panel) is specific to proamylin as it is not likely present on the western blot with the amylin antibody (left side of the panel). The specific band may represent a proamylin octamer (MW of proamylin is ~7.5kDa). Additional data are in Supplemental Material.

Fig 4.

Amylin and A β co-localization demonstrated by co-staining brain sections from AD patients with anti-amylin and anti-A β antibodies. Distinct patches of amylin (brown color) and A β (red color in **A**, and dark blue in **C**) can be observed in blood vessels (**A**, arrows) and brain parenchyma (**B**, **C**). Clusters of small amylin plaques (brown) adjacent to, or surrounded by, much larger A β deposits (red in **B** and dark blue in **C**) are also present in brain tissues. **A** and **C** are brain sections from a diabetic patient with AD (T2D-D group). **B** is a brain section from an AD patient without clinically demonstrated T2D. In **D**, we estimate the fraction of mixed amylin-A β plaques on a 0.4 X 1mm field of view in T2D-D and AD patient groups. * $P < 0.05$.

Fig 5.

Amylin is observed in the whole (A) or part (B) of the circumference of vessel wall. Co-staining with anti-amylin and anti-A β antibodies (C, D) shows a patchy distribution of amylin (brown) which is similar and complementary to that of A β (red) suggesting that both peptides are involved in CAA development. A and C are representative images of brain tissue from patients with vascular dementia. B and D are immunohistochemical images of brain tissue from AD patients. The inset in D shows a 40X magnification image of a capillary presenting amylin-A β mixed amyloid angiopathy. E is a representative image of tissue in control brain specimens showing lack of amylin deposition in brain parenchyma and blood vessels. F presents the estimated average fraction of the vessel wall circumference covered by amylin in a 0.4 X 1mm field of view of brain samples from T2D-D, AD, and control groups. * $P < 0.05$; ** $P < 0.01$.

Fig 6.

In A, qRT-PCR data show amylin mRNA levels in brain specimens from AD patients and healthy human controls relative to that in the human pancreas (note logarithmic scale and scale break). Rat brain and pancreas samples are negative controls for human amylin transcript. In B, western blot analysis indicates that amylin tetramers exist in both plasma and supernatant brain homogenates from patients in the AD group. In C, amylin was immunoprecipitated from brain specimens corresponding to control, T2D-D, and AD groups. Immunoprecipitates of amylin were then analyzed by western blot using an anti-A antibody. *P < 0.05.