**Supplementary Material**

Supplementary Material A

Shear-induced preferential migration to lower shear regions

Because of shear processes within the arteriolar PVP liquid environment, there is also an unexpected thermodynamic driving force on a shear-distorted Aβ\* molecule to migrate preferentially toward any lower shear region, e.g., an uneven surface of an arteriolar astrocyte foot, open OEPs between these feet, or adventitia PVP wall pocket or outlet (Supplementary Fig. 1). This is analogous to a process described by Metzner and coworkers, where there is a lower shear “environmental pocket” to which shear-stressed polymer molecules retreat [1]. Neither the adventitia nor the artery astrocyte foot PVP wall segments will be entirely smooth, with any indentations such as OEPs providing lower shear regions for Aβ\* molecules that have been shear-stressed by other PVP wall segments protruding into the CSF flow.

Increasing the Aβ concentration within the astrocyte OEP regions leave these molecules confined to very small spaces and vulnerable to shear-induced aggregation and OEP wall deposition if there is enough CSF pressure to push this fluid into the parenchyma or vice versa. Since the OEP opening is so relatively small, this wall coating could lead to clogging the opening should the flow rate through this opening increase, thus increasing the shear rate. There could also be dimers and oligomers formed near the OEP walls.

Supplementary Material B

Catalysis chemical mechanism where HSPG is the catalyst

HSPG molecules could help catalyze CAA deposits because of polar attractions between sheared Aβ\* molecules and HSPG, possibly by initially forming a metastable HSPG-Aβ\* complex. This immobilized HSPG complex could then interact with another passing Aβ\* molecule in the parenchymal ECS to form an (Aβ\*)**2** dimer and a free HSPG molecule, which then attracts another passing Aβ\* molecule, thus making the HSPG a dimer formation catalyst. If the HSPG molecule does not release the dimer, then it could be a focal point for the formation of an Aβ protofibril. The latter mechanism could well be dominant, since HSPG molecules have been found to be associated with AD plaque in the brain parenchyma. The question would be whether the Aβ molecule initiating such processes is an Aβ\* or an unexcited Aβ molecule. The author favors the Aβ\* for kinetic reasons. The proposed catalysis reaction can be summarized in equation form by the following set of reactions:

(1) 2Aβ + shear energy 🡪 2Aβ\*

(2) Aβ\* + HSPG 🡪 Aβ\*–HSPG

(3) Aβ\*–HSPG + Aβ\* 🡪 (Aβ\*)2 + HSPG

(4) (Aβ\*)2 🡪 (Aβ)2 + energy

Adding equations (1), (2), (3), and (4) yields equation (5) below

(5) 2Aβ+ shear energy + HSPG 🡪 (Aβ\*)2 + HSPG + thermal energy

which can be reduced to equation (6):

(6) 2Aβ+ shear energy (HSPG catalyst) 🡪 (Aβ\*)2 + thermal energy

Finally, equations (7) and (8) are anticipated:

(7) (Aβ)2 + Aβ\*or Aβ 🡪 🡪 oligomer formation 🡪 🡪 CAA formation

If the Aβ\*–HSPG bond in equation (2) is strong, equation (8) would be added:

(8) Aβ\*–HSPG + Aβ\*or Aβ 🡪 🡪 oligomer-HSPG formation 🡪 🡪 CAA formation

where the oligomer and/or CAA would contain HSPG.

Supplementary Material C

Shear-based mechanism for amyloid cascade and toxic Aβ reactions

While parenchymal ISF flow is undoubtedly relatively slow, the narrow parenchymal flow pathways still apparently provide enough extensional and laminar shear to promote parenchymal ISF fluid Aβ42, but not Aβ40, to a higher conformational energy state (Aβ42\*). Then, depending on the lifetime of this state and, depending on the local concentration of Aβ42\* parenchymal molecules, there may be time enough for a collision between two high energy Aβ42\* molecules to form a (Aβ42\*)2 or de-energized (Aβ42)2 dimer that is capable of seeding an amyloid chemical cascade reaction within the ISF when the dimer collides with successive Aβ42\* molecules. However, even though there may be an overall small steady ISF flow, a transiting Aβ molecule would sense another type of oscillatory extensional shear pattern as it passed through a series of oscillating narrower- and wider-spaced flow obstacles. In addition, there are literature suggestions of oscillating back-and-forth ISF flows within these narrow spaces. Thus, the Aβ molecule may also be oscillating through a series of shear energy-induced distorted conformation states and relaxation of these altered conformation states during its parenchymal transit, depending on the dimensions of the flow rates, flow path constrictions, and the oscillation frequencies of the back-and-forth flows. Thus, whether or not dimerization of these Aβ42\* molecules takes place will thus be critically dependent on the lifetimes of these states.

Dunstan and coworkers [2] claim that shear rates that are low enough to break only one hydrogen bond per Aβ molecule are able to produce amyloid fibrils in sheared Aβ solutions. Ashton et al. [3], in a spectroscopic study of a number of different types of proteins, have proposed that there are reversible, laminar shear-rate dependent protein conformational changes that take place with shear energy inputs that are much lower than those necessary to unfold these molecules [4]. Thus, it is suggested that it is not necessary to “unfold” or even “partially unfold” an Aβ42 molecule by breaking large numbers of weak chemical bonds to ultimately form the low energy Aβ42 oligomers. Would exposure of these molecules to extensional shear probably cause even more excited, possibly different conformers? Could relatively weak shear forces cause concerted conformation changes in addition to the many different conformations of the intrinsically disordered Aβ molecule available in non-sheared Aβ solutions?

The calculations of Yang and Teplow [5] suggest that, with sufficient thermal energy, there may be rapid transitions among the many different conformers they postulate in the absence of shear. Their calculations indicate that conformational conversions among these molecules in quiescent conditions can be frequent and rapid and could require only random thermal energy spikes for many conformational transitions. Those conformations induced by shear are higher in energy because shear energy must be expended to maintain a stressed molecule, increasing the number of its hydrophobic parts exposed to an energetically unfavorable aqueous hydrophilic environment. This excess shear energy can be quickly lowered if two such exposed high-conformational-energy molecules (Aβ\*) are attracted to each other in such a way that a maximum number of their hydrophobic, hydrophilic and perhaps ionic regions join together to form a dimer molecule and shed the released energy as heat to the more stable dimer’s surroundings.

A very important finding by Cohen et al. [6] indicates that there is a significant quantitative and qualitative difference for Aβ42 in the kinetics of its fibril formation in the presence and absence of shear. In these experiments, swirling the solution in a 96-well plate probably generated only mild laminar shear. Couette shear experiments with higher laminar shear rates of similar solutions have also shown significant shortening of the flat time-lag phase in amyloid cascade reactions. It would appear from the Cohen et al. study that the mechanisms of formation and the resulting structures of Aβ fibrils [7] are different in the presence and absence of shear. These data could be an indication that toxic oligomer structures in the shear-initiated amyloid cascade are different, suggesting that oligomer and other amyloid cascade product studies in the presence of shear and comparisons with quiescent products should be a high priority. However, there is one complicating factor that needs to be considered, namely the role of surfaces in the formation and functions of toxic oligomers.

Shear-induced oligomers in membranes

Kotler et al. [8] have reviewed the interactions between Aβ and membranes. Pertinent points made for discussion here are: (a) Aβ oligomer toxicity depends on its ability to disrupt membrane function; (b) the presence of membranes has been shown to strongly influence the Aβ aggregation pathway; (c) gangliosides such as GM1 (Fig. 6) play a significant role in altering this aggregation pathway; (d) Aβ membrane binding is enhanced by the presence of negatively charged lipid head-groups such as those contained on the GM1 molecule; (e) Aβ has the ability to form flow path-like pores in membranes that may allow unregulated passage of Ca2+ ions through the membrane, thus disrupting neuron function; (f) the membrane promotes formation of Aβ fibrils that can disrupt the membrane.

Aβ molecules that are generated in the synapse are either flushed out or diffuse out of the synaptic gap. It is suggested here that they then encounter very slow, possibly even oscillating, ISF flow through the very narrow ECS flow paths and some are exposed to liquid shear, especially those next to or very near the highly confining ECS flow path walls. If the Aβ molecule is initially flowing next to a neuron or astrocyte membrane surface, it is exposed to the maximum possible values of liquid shear within the brain parenchyma.

Because of their experimentally demonstrated extreme sensitivity to shear, it is proposed that Aβ42 molecules undergo shear-induced conformation change to form higher energy, conformation-altered Aβ42\* molecules. However, it is also proposed that Aβ40 molecules next to the cell walls are not affected because of the comparatively low absolute shear rates in the parenchyma and the smaller sensitivity of Aβ40 to shear. It is suggested that shear-distorted Aβ42\* molecules next to or very close to the wall then quickly and temporarily adsorb directly on the neuron or astrocyte membrane surface because of the various types of molecular attractions between the cell membrane and the shear distorted Aβ42\* molecules. Following a certain relaxation time, the metastable Aβ42\*-membrane complex releases either the Aβ42\* or, more likely, an unexcited Aβ42 molecule back into the ISF mobile phase. It should be remembered that the mobile ISF Aβ molecule was born by being released from a synaptic membrane.

Thus, at low Aβ concentrations, there is postulated to be a continuous cycle of shear-induced Aβ42 excitation/adsorption/desorption represented as:

Aβ42 /Aβ42\*/Aβ42\*–membrane /Aβ42–membrane/Aβ42.

When the local Aβ concentration increases, there is an increased probability that two Aβ\* membrane-adsorbed molecules will be within a critical reaction distance on the fluid membrane surface and the two adsorbed molecules form a dimer on that surface that subsequently sheds its excess thermal energy into and remains bound on the membrane. As more Aβ\* molecules adsorb to the membrane they can react with the (Aβ42)**2** dimer to form higher oligomers. Hong et al. [9] report that *in* *vivo* Aβ42 oligomers formed in the mouse brain ISF are sequestered much more effectively than single Aβ molecules by brain membranes and are recovered in part as bound to GM1 gangliosides. But this deposition of oligomers formed in the ISF may not be the only mechanism. The Aβ42\* may adsorb to the membrane one stressed molecule at a time, ultimately forming dimers and neurotoxic higher Aβ42 oligomers on the membrane surface, with these then migrating in the fluid membrane to the surface-embedded GM. Alternatively the Aβ42\* could initially bind to an embedded GM1 molecule, attaching to it because of its strong polar attractions and then attracting successive Aβ42\* molecules to form a GM1-bound oligomer.

The above is suggested to be a potential mechanism whereby Aβ40 is the overwhelming isoform found in vascular CAA and very little free Aβ42 is found in ISF in AD patients. If the mechanism outlined above and illustrated symbolically in Supplementary Figure 2 below of oligomer formation within the neuron membrane is valid, the shear-induced Aβ42\* molecular precursor of the Aβ42\*-membrane complex and this complex itself may be the most critical intermediates in amyloid oligomer neurotoxicity. This mechanism is represented in equation form below. Equations (1) through (4) represent the proposed mechanism at low Aβconcentrations.

(1) Aβ42 + shear energy 🡪 Aβ42\*

(2) Aβ42\* + M(membrane) 🡪 Aβ42\*–M (complex)

(3) Aβ42\*–M 🡪 Aβ42–M +energy

(4) Aβ42–M 🡪 Aβ42 + M

Equations (1) through (4) can be represented as the cycle: Aβ42/ Aβ42\*/ Aβ42\*-M/ Aβ42–M/ Aβ42.

The chemical equations for the case of increased Aβ concentrations in the B section of Figure 8, where the membrane is represented by M, are:

(5) 2 Aβ42 + shear energy 🡪 2 Aβ42\*

(6) Aβ42\* + M 🡪 Aβ42\*–M

(7) Aβ42\*– M + Aβ42\* 🡪 (Aβ42\*)2-M

(8) (Aβ42\*)2-M 🡪 (Aβ42)2-M + thermal energy

Adding equations (5), (6), (7), and (8) yields equation (9)

(9) 2 Aβ42 + shear energy + M 🡪 (Aβ42\*)2-M + thermal energy

Finally, equations (10) and (11) are anticipated to follow reaction (9):

(10) (Aβ42\*)2-M🡪 (Aβ42)2-M+ thermal energy

(11) (Aβ42)2-M🡪 (Aβ42)2 + M

(12) (Aβ42)2-M+ Aβ42\*or Aβ42 🡪 🡪 oligomer formation in or on the membrane.

However, not all of the Aβ42\* molecules within the ISF are created directly at the neuron cell membrane and not all will necessarily collide with a membrane surface, but instead may undergo shear-induced dimerization and further aggregation within the ISF phase and ultimately produce oligomers that adsorb to the neuron membrane. Are the final oligomer structures different because the origins of these oligomers are different, one formed in a neuron membrane surface and the other within the ISF? *In vitro* experiments may be able to answer this question. Attention is now turned to the character of the ECS flow paths.

These flow paths narrow with increasing age in mice, probably also increasing the ISF parenchymal shear rates, even though flow rates may slow even further. The shapes of these flow pathways are quite irregular, so the dimensional dependence of the shear rates in these flow paths on their dimensions is difficult to model. However, the limits should be somewhere between that in a capillary with a circular cross section, where the shear rate depends on the inverse cube of the radius, and two parallel plates, where shear rate depends on the inverse distance between the plates. Thus, although flow rates through these extracellular space flow paths may be quite slow in comparison with those in the perivascular segments of the brain [10], the flow-limiting dimensions in these two regions are estimated to differ by a factor of approximately 5,000 [11]. Thus, a flow rate of ISF in the brain ECS parenchyma flow paths approximately 5,000 times smaller than that of perivascular CSF in the PVC could conceivably generate roughly equal, or probably even greater, shear forces on Aβ molecules than in the perivascular PVC region. However, this “unobstructed flow path” argument does not take into account the other molecular inhabitants of these parenchymal ECS flow paths that could contribute to increased shear over and above that provided by a “clear flow path.”

Role of ECS flow path-blocking molecules

As in the case of the vascular basement membranes, ECS flow path membranes are associated with both heparin sulfate proteoglycans (HSPG) and a complex mixture of extracellular matrix components (ECM) [11]. One of these HSPG molecules, agrin, has been shown to be involved in the formation of Aβ fibrils [12]. This complex network of HSPG and ECM, forming a molecular mesh called perineuronal nets through which the ISF is forced to flow, however slowly, may cause Aβ to undergo extensional shear. In addition, such flow may produce the same kind of shear pattern induced by flow a dissolved polymer through the grid that caused the formation of a stringy solid polymer in the low shear region immediately behind a flow-blocking wire mesh observed by Metzner et al. (Figure 4 in reference [13]), especially when Aβ concentrations are above a critical threshold. Such ISF flow blockages could also initiate the formation of solution Aβ oligomers that are different from those postulated above to form within the membrane. For those who claim that convectional flow is completely prevented by narrow ECS flow paths and molecular flow path blockers, it should be noted that if there is that much resistance and yet there is even a very small amount of flow, much of the absorbed energy that is expended to allow this small flow is used to force Aβ molecules through and around tightly spaced flow obstacles and therefore probably exposes the Aβ and its oligomers to strong extensional shear forces, thereby inducing changes in Aβ conformation, causing the formation of Aβ\*. These relatively flexible IDP Aβ molecules probably absorb a significant fraction of this energy to change conformation to enable them to transit through tortuous flow pathways, around molecular obstacles, and flow near membrane surfaces. The narrower the flow paths are, the higher the flow path shear, even though the resistance may have slowed the flow rate quite significantly. As long as there is flow, there will be significant shear forces, especially in this crowded ECS environment. Supplementary Figure 3 suggests symbolically the many different types of Aβ aggregation reactions that can take place in and around the PNN region.

ECS flow path shear-stressed solution monomers or oligomers can undergo chemical reactions and attraction with molecules attracted and attached to the stationary ECM mesh within the ECS. This mesh has been postulated to act as anchor points for the formation of AD plaque, within which HSPG molecules are found. For example, one of the blocking molecules could, because of hydrophobic and/or hydrophilic attractive forces, attract a distorted Aβ42\* molecule, momentarily hold it in its Aβ\* conformation through attractive forces until it is released or another Aβ42\* molecule approaches and is attracted to the membrane-complexed Aβ42\* molecule, thereby forming a dimer (Fig. 9d). Thus these blocking molecules could act to catalyze dimer and oligomer formation in somewhat the same manner as the previously proposed membrane-catalyzed vascular dimer formation. Both of these catalytic processes are highly dependent on and very sensitive to local Aβ42\* concentrations because of limited lifetimes of the Aβ42\* complexes, especially if there is an oscillating flow. Other structural molecules involved in perineuronal nets and the ECM add additional ECS flow restrictions that have not been addressed, but surely add further shear opportunities to flowing Aβ molecules.

Cecchi and Stefani [14] have summarized the complex nature of the interactions among Aβ, oligomers, and membranes. These depend heavily on both the manner of preparation of the oligomers as well as the chemical makeup of the membrane. Therefore, it is a reasonable assumption that the amount and type of shear force applied and whether formed in solution or on the membrane surface may determine the shear-induced conformation of Aβ\* molecules as well as the toxicity of the resulting oligomers.

Thus, it is suggested that the Aβ42 dimerization process that takes place in ISF flow paths a short distance away from the membrane walls, but within the PNN, is an initiation event that leads to fibril and plaque formation, whereas the membrane-based dimerization may be a critical part of an amyloid cascade process that ends with the formation of toxic membrane bound oligomers. Dimers formed on Aβ fibrils as suggested by Cohen et al. [6] could contribute to a third group of oligomers that may also be neurotoxins.

Possible roles for astrocytes and microglia in shear-induced Aβ\* chemistry

Although the prime focus in the above discussion and most other amyloid literature is on the effects of toxic Aβ oligomers on neurons, it should be pointed out that astrocyte and glia surfaces are also are an integral part of the critical shear-inducing CSF and ISF flow paths discussed above. A recent hypothesis [15] on the importance of astrocytes and glia in neuron control raises the possibility that the membranes of astrocytes and glia, which apparently are involved in the control of Aβ clearance [15], may also be affected by shear processes and thereby impede Aβ clearance.

Variable shear energy level events and medical consequences

The results from the study of the effects of shear on protein spectra of Ashton and coworkers [3] are quite significant in terms of the above hypotheses. In their study, shear rates were far below those needed to denature or even partially unfold the protein. Yet reversible spectral changes were observed as shear rates were gradually increased and decreased, implying reversible conformational changes. The general Aβ\* symbol has been used above for any non-thermal, shear-induced conformational excited state. In these discussions, there is a distinction made regarding the amount of shear conformational energy needed in order to shear activate Aβ40 and Aβ42 molecules sufficiently to form aggregates in the PVP and parenchyma, respectively. Given the concept of Stone et al. [16] of sharpening the systolic pulse in the aged because of atherosclerosis, it would seem that there would be increased amounts of shear energy available to promote even higher conformational Aβ\* energy levels, so much so, that entirely new oligomer and fibril products might possibly be formed.

Taking this idea one energy level step further, one can speculate about the kind of shear forces resulting from the very sharp, very high energy events present in brain fluids in the event of concussions and traumatic brain injury. There is certainly a possibility that even higher Aβ\* conformational energy states are formed during these events with quite different amyloid-based medical consequences. Perhaps it is such high energy events that lead to true unfolding and actual subsequent “misfolding” of brain proteins. Such speculation is subject to experimental testing using the apparatus that will be described in the next paper in this series.

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