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Discussion

Alzheimer Research Forum Live Discussion: Now you see them, now you don't: The amyloid channel hypothesis[1]

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Tom Fagan: I am Tom Fagan and I will be moderating today when necessary. Bruce Kagan is going to start us off with a brief introduction.

Bruce Kagan: In 1993, Arispe et al. [1] reported that Aβ formed ion channels, and proposed that channels could cause cellular pathology and toxicity. Abundant evidence now supports this view, including the facts that virtually all amyloids form channels and that Aβ can inhibit long-term potentiation (LTP), depolarize neurons, allow cytochrome C efflux from mitochondria [2], and kill cells. Channel formation readily explains most amyloid pathophysiology, including memory disturbance, calcium dysfunction, membrane depolarizations, increased reactive oxygen species (ROS), sensitivity to toxins, and apoptosis.

Larry Nault: Bruce, do these channels have a hypothetical geometry? A pipe or a duct? Bounded by what?

Bruce Kagan: Larry, Arispe and Guy proposed several possible models for the pore. Recently, Arispe has published evidence [3] that specific regions of the peptide seem to line the pore.

Tom Fagan: Bruce, how many channels does it take to kill a cell?

Bruce Kagan: Tom, it depends on the size of the cell. The Aβ channels are large, electrically, and would create a significant leak. A single pore could reduce the sodium (Na+) concentration by about 10 micromolar per second.

Tom Fagan: Bruce, how does that compare to normal leakage?

Bruce Kagan: Tom, it again depends on the cell. For a neuron, which must maintain a tight membrane for signaling, a single pore would pose a significant but not lethal leak.

Wonmuk Hwang: How specific are these channels? I understand that they have a distribution of sizes, which likely cause them to be nonspecific.

Bruce Kagan: Won, the channels have been described as permeable to a variety of cations including sodium and calcium. There is little selectivity amongst cations.

[1] Note: The transcript has been edited for clarity and accuracy.

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Tom Fagan: This fits in with what Charlie reported recently, right Charlie?

Charlie Glabe: We see fluorescent dyes that are about 600 Daltons (Da) leaking; it is not ion-specific.

Tom Fagan: Charlie, 600 Da is pretty big; do you mean such molecules are getting through these pores?

Charlie Glabe: Yes, going both ways; I am not sure of the mechanism. Large proteins like lactate dehydrogenase (LDH) do not leak detectably.

Bruce Kagan: Charlie, we see non-electrolyte leakage of sugars with a diameter up to about 10–12 angstroms. Of course, we should temper this with the observation that we see many species of channels and they may all have different sizes and selectivities.

Tom Fagan: Bruce, so are these channels in a state of flux? Can their size change dynamically with time, and might there be large and small coexisting in the same cell?

Bruce Kagan: Tom, Charlie sees a very different result from ours, but Joseph Kourie has described at least three different Aβ channels with varying kinetics, selectivity lifetime, and so on [4].

Tom Fagan: Everyone, any ideas on how these channels can be stoppered? Would this be a therapeutic strategy?

Charlie Glabe: We have not seen any known channel blockers that inhibit the permeabilization, but that does not mean that there are none.

Bruce Kagan: Tom, the only blockers we have so far are very nonspecific, such as zinc (Zn^{2+}) or tromethamine ions (e.g., Tris buffer). The development of more specific blockers could prove useful as a therapeutic strategy. Zn^{2+} does block the toxicity of Aβ on fibroblasts [5].

Wonmuk Hwang: About Charlie's reply: How about the oligomer-specific antibodies that you published? Do they not block permeabilization?

Charlie Glabe: Wonmuk, it is not known, but that is a reasonable hypothesis. We find that you need to start with oligomers first. I am not sure that they insert into the hydrocarbon region in a traditional fashion, either.

Ratnesh Lal: Calcium uptake has been blocked specifically by anti-amyloid antibody and zinc in both liposomes and cells [6–9].

Tom Fagan: So how does the channel hypothesis fit with the slow progression of the disease? Given the degree of leakage, it would seem that channels would be pretty lethal and that the disease should progress more rapidly, or am I being too simplistic?

Charlie Glabe: Maybe a small leak just contributes to chronic stress as the cell has to pump more to keep up with the leak.

Bruce Kagan: Tom, Dennis Selkoe's group has shown that oligomers can impair memory in a reversible fashion. Since we know Aβ channels can inhibit LTP, this might be the first step in the pathology [10].

Tom Fagan: Bruce, but in the LTP experiments, basal transmission is okay, which seems odd, given the degree of leakage you could get. I wonder if there is something modulating the channels?

Bruce Kagan: Tom, the LTP experiments require very small doses (less than toxic) of Aβ. I suspect that they are altering membrane potential in a subtle way to inhibit LTP, but not enough to derail normal transmission.

Wonmuk Hwang: About channel formation: Is it known whether they are formed in solution, then incorporated into the cell membrane, or is it more likely that channels are formed through interaction with the membrane or the substrate (in case of *in vitro* experiments)?

Ratnesh Lal: Evidence is published suggesting that both preformed oligomers can insert in the membrane or monomers can insert and oligomerize to form ion channels.

Bruce Kagan: Wonmuk, there is clear evidence that the presence of lipids or membranes affects the folding of Aβ and other amyloids, tending to promote beta-sheet formation and oligomerization.

Wonmuk Hwang: Bruce, that is consistent with the finding that fibrils form in partially denaturing conditions–possibly the hydrophobic environment of the lipid tails enhances the condition for fibril formation.

Charlie Glabe: We find that monomers are pretty inert. It is not necessarily easy to make pure monomers.

Bruce Kagan: Charlie, we find that monomers are inert also, but that with time in aqueous solution, oligomers form and insert into the membrane.

Ratnesh Lal: As mentioned above, there is published work showing that monomers can induce cell toxicity as well as calcium uptake in both cells and liposomes.

Bruce Kagan: Ratnesh, how can you be certain that the peptide remains as a monomer throughout the experiment?

Ratnesh Lal: I do not think they remain monomers throughout the experiments. Instead, they do oligomerize. We have imaged those monomers in real time using atomic force microscopy (AFM). We have shown that, for an extended period of time, in physiological buffer and in physiologically relevant concentration, they do not form oligomers. Nevertheless, as I mentioned above, monomers will oligomerize once in association with lipids in the bilayer/liposomes.

Bruce Kagan: Ratnesh, so that would be consistent with all the recent work suggesting toxicity of oligomers?

Ratnesh Lal: Yes, that is all we have published in the last 7 years.

Wonmuk Hwang: Ratnesh, I think that Aβ peptides are produced throughout the lifespan.

Ratnesh Lal: Membrane-induced conversion from inert to an active form will be consistent with the expected conformational change in many amyloids associated with misfolding diseases. Won, I agree with you and have made that point in our earlier publications. Aβ peptides are produced throughout the lifespan and in many, if not all, cell types.

Ben Albensi: All, so is it just the induction of LTP that is affected, or later stages, as well?

Bruce Kagan: Ben, I believe it is just the induction, but I could be mistaken. Dave, do you know?

Tom Fagan: All, how many monomers does it take to form the simplest channel, in theory even, if there is no experimental data?

Ratnesh Lal: Tom, a model presented by Durrel et al. [11] suggests that it can be anywhere from tetramer to octamer and more (perhaps up to 12).

Tom Fagan: Ratnesh, is a tetramer big enough to form a pore and span the membrane?

Bruce Kagan: Tom, Aβ42 tends to form hexamers, and those would be excellent candidates for the smallest channels.

Charlie Glabe: It is not clear that it has to span the bilayer in the traditional sense. How do you explain the fact that polyQ permeabilizes membranes in the same fashion?

Tom Fagan: Charlie, I do not know. Any ideas?

Charlie Glabe: It may be the same reason that highly charged, polar peptides like HIV tat and antennapedia can cross the bilayer with large passenger molecules attached to them [12].

Tom Fagan: Charlie, are you suggesting we abandon "the channel" hypothesis and call it something else?

Charlie Glabe: I do not know; this is something we are still trying to sort out.

Ratnesh Lal: There is a model by Mobley et al. [13] suggesting that monomers may or may not span the whole membrane. If they do not, it can still form an ion channel (like porins or others); otherwise, one would need an additional four monomers from the other membrane leaflet (and in that case, it would be eight monomers total), or six or more, depending upon whether they form tetrameric, pentameric, hexameric, or higher-order oligomeric channels.

Bruce Kagan: Charlie, the polyQ story is striking in that there is a minimum length for pathology, yet this minimum varies from illness to illness. For Aβ peptides, we have shown that the minimum length to obtain channels is nine residues, which would span the hydrophobic core of the bilayer in beta-sheet form.

Wonmuk Hwang: How about the pure dimensional argument? The hydrophobic region of a lipid bilayer is about 3 nanometers, and if the channel diameter is about 2 nanometers, the oligomer channel must have about 36 nanometers^2. Given the size of Aβ, we can

guess how many monomers are needed to span a bilayer like a channel. But as suggested above, even if the oligomers occupy only one leaflet, it is still possible for there to be a hole, through fluctuation of the lipids. In this case, the hole will be highly dynamic rather than statically open.

Ratnesh Lal: Wonmuk, the pore diameter of 2 nanometers is quite big for any channel. The outer diameter of the oligomeric channel diameter would be consistent with what we had published earlier [8]. The inner (pore) diameter will be considerably smaller ($\sim$1–1.5 nanometers).

Wonmuk Hwang: Ratnesh, I was referring to Lashuel, Lansbury, and colleagues' work on torroids; their electron microscopy (EM) images suggest that the inner diameter is about 2 to 3 nanometers [14].

Ratnesh Lal: Wonmuk, Lashuel's work is done on annular pores; peptides were never associated with any membrane (either before or after). For their actual channel conformation, they refer to our work [8].

Charlie Glabe: If peptides that do span the bilayers are extended beta structures, you would expect that they would have to form H bonded beta barrels, which typically have about 20 strands.

Bruce Kagan: Charlie's point is well taken. This would predict very large channels, indeed.

Dave Teplow: Caution to all–there is a distinct possibility that Lashuel's work, while relevant in *in-vitro* systems, is not representative of what occurs *in vivo*.

Tom Fagan: Charlie, I was just going to ask about molecular models that would have a pore. If the pore idea is correct, then would it not be possible to estimate how many monomers would be needed, and how that would fit with current ideas on trimers, dodecamers, and so on, that seem most toxic?

Charlie Glabe: Uli Aebi [15] tried to visualize pores or channels by AFM using preparations and conditions that caused dye leakage, and he did not observe "donuts." Instead, he saw defects in the membrane radiating from the oligomers.

Ratnesh Lal: Charlie, there is a serious difference between Aebi's work and other channel work. In his work, amyloid was added to membranes that were preformed and adsorbed on a substrate, a common practice for AFM work. On the contrary, when you reconstitute in liposome or bilayer and then image with AFM, you do see channels [8].

Wonmuk Hwang: In relation to Tom's question, what is the shortest length of a model peptide tried that forms channels?

Bruce Kagan: There is a report of an Aβ31–35 fragment forming channels [16]. I do not know what to make of this unless there is one peptide in each leaflet of the bilayer.

Tom Fagan: Charlie, Bruce, have either of you examined the effect of different lipids on leakage? Sorry, I am not remembering the data off the top of my head.

Charlie Glabe: Yes, not much specificity. Yuri can jump in on this if I have misspoken.

Bruce Kagan: Tom, cholesterol inhibits. Negatively charged lipids are required for activity. There is not much other specificity.

Ratnesh Lal: There are some publications from the Arispe group on the effect of cholesterol on amyloid insertion and channel activity [17].

Bruce Kagan: Charlie and Yuri, does membrane fluidity affect your results?

Yuri Sokolov: Bruce, we did not study the effect of membrane fluidity. But recently we found very strong lipid dependence of amyloid-induced conductance in bilayers.

Tom Fagan: So what about other channels besides Aβ ones? What have we learned from those? Do they clarify anything or just make things more confusing?

Bruce Kagan: I am struck by how similar the channels are that we see from a variety of unrelated peptides. Their physiologic properties are nearly identical. They are all "leakage" channels.

Charlie Glabe: I second that. All amyloids are pretty similar.

Larry Nault: Interesting hypothesis. Clearance of building channel blocks may be the key to defeat of

progression. Building channels over time and diving then through the bilayer is a process that might be interrupted with agents like zinc or by clearing essential oligomer building blocks.

Ratnesh Lal: There is published work on many other amyloid proteins showing channel-like activity (Laboratories of Kagan, Arispe, and Kourie), and we have structural data from AFM study to confirm channel-like structures.

Tom Fagan: Ratnesh, what do you mean by "channel-like."

Ratnesh Lal: Tom, as the AFM work is purely structural and we do not do electrical recording simultaneously (I mean not on the same structures because we do not have an appropriate recording system), we take a conservative view that it is channel-like. However, in parallel studies on the same batch of specimen, electrical recording shows channel activity.

Bruce Kagan: Tom, in the case of our work and that of Arispe and Kourie, these are clearly channels by all the standard criteria. Charlie's work clearly shows permeability without "channels."

Tom Fagan: Ratnesh, I thought you were referring to a channel-like structure rather than activity.

Ratnesh Lal: Tom, we do both: image the three-dimensional structure and in parallel do electrical recording. We are hoping to do both together someday soon.

Bruce Kagan: Tom, one thing we have learned from complement and toxin studies is that "holes" that one sees in EM do not always correspond to the electrical pore pathways.

Tom Fagan: All, we are nearing the end of our hour. I would like to thank you all for coming, and Bruce for agreeing to host this chat.

But before we go, what are the crucial experiments that need to be done to advance our understanding of what is going on?

Bruce Kagan: I think there are two crucial experiments to be done. First, specific channel blockers need to be developed and tested in cell and animal models. Second, channels (or permeability changes) need to be found in animal models of disease.

Dave Teplow: Bruce, are there any meta-studies that could be done examining patients taking channel blockers for other diseases that would answer your question?

Bruce Kagan: Dave, that is a great idea. However, for the easiest ones – the calcium channel blockers – we have already tried them without success.

Tom Fagan: Bruce, tried them *in vitro*?

Bruce Kagan: Tom, right, we tried them *in vitro* against our Aβ and other channels.

Tom Fagan: All, we talked about channel blockers, but are there other strategies to stop the leakage of these "channels"? Also, are there synthetic channels that could be made to mimic the natural ones, and how might that be useful?

Ratnesh Lal: Antibodies do block, and one can use peptides to block, as well (a practice useful in blocking gap junction ion channel activity).

Bruce Kagan: Tom, yes, one could devise treatments that make the bilayer less susceptible to channel insertion. Arispe did this recently with robust effects on toxicity [17]. He stiffened the cell membrane with cholesterol.

Yuri Sokolov: Jim Hall and I found that an increase of bilayer thickness inhibits the effect of amyloid-β in bilayers.

Bruce Kagan: Yuri, what is the latest on what ions and other molecules can go through your Aβ-treated membranes?

Yuri Sokolov: Bruce, looks like everything we tested. HEPES, Tris, TEA....

Tom Fagan: Yuri, do you do this by using longer-chain lipids?

Yuri Sokolov: Tom, looks like with longer chains we have only preliminary data. But definitely the effect of amyloid-β is completely blocked by the saturation of bilayer with decane. We are not going to use decane as a treatment, but it is good enough to study the mechanism.

Tom Fagan: Yuri, yes, for sure, anything that sheds some light on what is going on.

Bruce Kagan: Tom, there are other ways to increase membrane thickness, such as changes in dietary lipids.

Tom Fagan: Bruce, that is where I was headed. But you said that you tried different lipids and saw no specific effects, right?

Bruce Kagan: Tom, yes, we did try many lipids and did not see major effects except of charge and stiffness, but we did not try to alter membrane thickness (one needs lipids with long tails). We should go back and do that.

Tom Fagan: Or you could use shorter ones to see if the problem is any worse.

Bruce Kagan: Right. I know these manipulations to change lipid composition can be done in animals, but I do not know if they have ever been tried in humans. It is interesting to note that increased membrane thickness would inhibit standard channels as well as Yuri's permeability increase.

David Corbin: Bruce, have you also considered the systemic effects in Alzheimer's of impaired calcium transport by fibroblasts [18]?

Bruce Kagan: David, I am not familiar with this paper. Do you think it might be evidence of Aβ channels in the fibroblasts?

Ratnesh Lal: At least in *in-vitro* studies, we have shown Aβ-channel-mediated toxicity in fibroblasts [5].

David Corbin: Bruce, I do not know. I was just looking at some of the early research on cell membrane abnormality in Alzheimer's to see what others have observed, such as decreased microviscosity.

Tom Fagan: I think we should probably wrap up. Thanks, Bruce – most interesting. Thanks to everyone for joining in.

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