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The endless puzzle of calcium signaling in the heart: An interview with Donald Bers

Transcript of a conversation between Elizabeth M. Adler and Donald M. Bers[1]

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Transcript condensed and edited by EMA

EMA: Hi, this is Liz Adler, Executive Editor of *The Journal of General Physiology*. I'm in Newry, Maine, speaking with Don Bers of the University of California at Davis. We've been attending back-to-back Gordon Conferences, first on Muscle Excitation–Contraction Coupling and now at Calcium Signaling.

Don, thanks so much for agreeing to talk to me.

DMB: Sure; my pleasure.

EMA: As I understand it, you've been involved in studying calcium and its role in regulating cardiac function for many years. Is that right?

DMB: Yes. From the beginnings in graduate school, I started studying calcium signaling in heart.

EMA: Was it heart that interested you first? Or was it calcium? How did you get interested in that field?

DMB: I think it was probably heart. In graduate school, one of the professors who was teaching a cardiovascular physiology course lit my interest, and I did a rotation in his lab and it took off from there. Glenn Langer was his name.

EMA: Was he looking at calcium?

DMB: Yes; he was a cardiologist and also a basic scientist at UCLA, and he was studying calcium in heart. He had a pet theory about how extracellular calcium was critical in activation of cardiac contractility. I was attracted initially to the role of calcium in activating contractile force, and how the calcium that entered the cell or [was] released from intracellular stores would activate the contraction, and how that would change under different stimulation protocols or during different pharmacological interventions, and how it might go wrong in heart disease.

EMA: Did you figure that out?

DMB: Well, we figured a *lot* out. So that's been a really fun and intellectually stimulating ride, to pick apart the pieces of the puzzle, as it were.

That's been taking me in lots of different directions, not just studying the calcium supporting contractions, but studying local calcium signaling involved in regulating other processes, including mitochondrial metabolism and transcriptional regulation.

EMA: Oh, you've gotten into transcriptional regulation.

DMB: My favorite part of it is the calcium end of it. How do calcium signals get transduced into regulating gene expression?

So there are calcium-dependent signaling pathways involving calcium-calmodulin, calcineurin, and NFAT translocation to the nucleus, and also, for calcium-calmodulin, the activation of CaM kinase, another kinase that we're quite interested in; *that* actually phosphorylates histone deacetylases, and causes their *export* from the nucleus.

So it's kind of a dynamic interplay of these parallel pathways that are at least *part* of the calcium-dependent transcriptional modulation.

EMA: Uh-huh . . . did you have a favorite project?

DMB: It's hard to pick. Because, you know, it's like having a favorite child.

EMA: Uh-huh . . . can you tell me about one of them?

DMB: Sure. Well, I can tell you one of the early ones that helped move me into more of a leadership position in understanding calcium balance and fluxes in the heart.

In different species, and under different stresses, the amount of calcium that activates the contraction comes from different places. That is, in skeletal muscle, for example, calcium is released from the SR and taken up by the SR, and it's almost a closed system inside the cell; not entirely, but almost entirely.

What we found was, in different species, some [cardiomyocytes] work kind of like skeletal muscle. For example, mouse and rat myocytes tend to have mostly this internal cycling where the SR release and SR uptake is totally dominating it, and only a tiny fraction of the calcium involved in contraction relaxation comes from outside the cell, and is extruded back out of the cell on each heartbeat. But, in larger mammals, the balance is quite different, and much more of the calcium involved in activating contraction comes from outside the cell, and then has to be extruded back out of the cell; and that made, in cardiac cells—as opposed to skeletal muscle—the dynamics of calcium regulation quite different. That means that the process that takes calcium out of the cell, that came in on each beat, is quantitatively much more important—this is the sodium–calcium exchange process that takes it out of the cell.

And so we very quantitatively analyzed how much calcium goes through which pathway: how much goes into the sarcoplasmic reticulum, how much is taken out of the cell by the sodium–calcium exchange, how much is extruded by the plasma membrane calcium pump, and how much is taken up by mitochondria. Working out those details gave us a really quantitative framework for not only the amount of calcium that moves around the cell and where it goes, but also what binds the calcium and what the cytoplasmic buffering was doing.

EMA: Uh-huh.

DMB: That was stimulating and rewarding in the sense that we got to be very quantitative and analytical, and understand where all the pieces are.

And what happens is, then, that the balance of fluxes changes.

For example, in heart failure, what happens is the sarcoplasmic reticulum function tends to go *down* and the sodium calcium exchange tends to go *up*. So, in heart failure, there is more influx and efflux on a beat-to-beat basis. And that relates to energetics, because, in fact, if you were having calcium come in and get pumped out of the cell, it actually takes twice as much energy for each calcium ion to be pumped back *out* of the cell than to be taken up by the sarcoplasmic reticulum. So it adds another sort of energetic demand in an already-compromised heart.

EMA: So there are functional implications about the relative strengths of the different pathways. Are there functional implications for different species?

DMB: So, it kind of makes sense [in] that, in the smaller animals that have higher heart rates, the energetic costs are higher at those higher heart rates. So, it's actually more energy-conservative to have a cycle inside the cell, because it costs you less ATP to do it. On the other hand, it also changes the dynamics of the calcium transient in the cell. For example, it's much shorter in mouse and rat. Again, that might have a relationship to how short the cycle length is; those hearts are beating in mouse at 10 beats per second. And so, in larger mammals, where the heart rate's like, in man being one per second, or in other large mammals being about two per second, that changes the dynamics.

And, in disease, it's also become clear in the last 10 or 15 years that this calcium mismanagement that contributes to heart failure—that is, less calcium in the SR, partly because of this down-regulation of the SERCA pump, and up-regulation of sodium–calcium exchange—it's like a competition. So, that depletes calcium in the SR, and makes the contraction weaker. But it also sets up the substrate for arrhythmias, because the higher sodium–calcium exchange current is actually an inward current, and when you get what are called spontaneous calcium releases in waves in cardiac myocytes, that activates an inward current through the sodium–calcium exchanger as it's taking the calcium out, and that depolarizes the membrane. And, if it's big enough, it can create what we call a delayed afterdepolarization or a triggered action potential. And that's what can lead to premature ventricular contractions.

EMA: You've also been very interested in calcium domains . . .

DMB: I think one of the things that we have come to realize is that activation of every place in the cell at once, like in muscle contraction, is a really interesting phenomenon, but it's probably an unusual phenomenon with respect to the way calcium signaling works in cells. That is, I think *most* of calcium signaling is done in local microdomains, and that's something that I think allows the calcium signaling to do multiple jobs in the same cell at the same time, in different locations.

An example is some of our work on transcriptional regulation, where the calcium signal that seems to activate at least one of these pathways involving CaM kinase is calcium released from IP_3 receptors at the nuclear envelope.

EMA: Mmm-hmm.

DMB: And that's controlled by G protein–coupled receptors at the membrane, which cause IP_3 to be produced, and diffuse to *there*.

And what we showed was that activation of this calcium store–dependent CaM kinase and transcriptional regulation actually is, in effect, insulated from the calcium transients that are happening with every heartbeat. That is, stimulating the cells doesn't turn that pathway on at all, whereas the G protein–coupled receptor—like the alpha adrenergic activation or endothelin activation—can cause a robust activation of this nuclear translocation process.

And that's uninfluenced by even putting stimulation on top of it. So it's almost like you have this private local signaling of calcium at the nucleus that mediates that process. And I think that happens in many other domains in the cell.

EMA: It makes sense when you explain it that way, because if calcium were uniformly increased all over the cell, everything would be happening at once, and it wouldn't be able to do individual processes as needed.

DMB: Absolutely. Yeah; I think that's exactly right. And one of the hot spots for excitation–contraction coupling is the cleft where the L-type calcium channels bring calcium in, and it triggers calcium release from the SR. In that domain, the calcium may be extremely high for a very short period of time; so that may activate different processes than get activated in the cytosol. So the cleft calcium might go up to 100 µM during the peak of release, whereas the cytosol will barely get up to 1 µM in bulk cytosol.

So for certain signaling molecules, like CaM kinase, that works especially well in environments where there are very high calcium spikes. So CaM kinase is very important in, for example, the synapse, where it's involved in transducing these high local calcium signals to memory. And the same thing happens in cardiac myocytes where these clefts have very high local calcium fluxes, and the CaM kinase that's located in that domain is much more likely to get activated.

One of the puzzles that *that* leaves us with is how does CaM kinase that's *not* in such domains where calcium is *so* high ever get activated? So we do a lot of computational modeling, too, to try to understand how these pieces all fit together. We can do a lot of quantitative measurements, and the modeling helps in the sense that, if we understand enough of it, we can write equations out that explain those details. And if the equations don't tell us what we see experimentally, then we don't understand it well enough, and sometimes that helps us identify which is the part of the puzzle that we *don't* understand well enough. And that also then helps us devise new experiments to test those parts.

EMA: What are you working on now?

DMB: A lot of different things. My lab has grown, and we have a lot of different interests. It's still focally on calcium regulation, and I would say our sort of focal level is the cardiac myocyte. Most of our work is done on adults' ventricular myocytes, and trying to understand the way these processes work in that cell type, and how they interact. That includes studying different ion channels, and we characterize how calcium channels and sodium channels and potassium channels and sodium–calcium exchange are regulated in the cellular environment. And sometimes that means we use heterologous systems to express a channel to get more control and express things that modulate it, and that helps us to understand better how that modulation may be functional in the cardiac cell, where it's in a different environment.

EMA: Is there anything that you would like to say that hasn't occurred to me to ask you?

DMB: Oh . . . I guess what I would say is that doing science and exploring stuff that is driven by your own scientific curiosity, and being able to interact with other scientists who bring totally different perspectives to answering similar questions, I think makes the job of doing science, as we do—not like a job—it's more like fun. So I think it's been a really delightful career to be able to figure out some of these puzzles that have interested me and, in some way, impacted the progress of science in these areas.

EMA: It sounds like you've never run out of puzzles.

DMB: It's a maxim about scientific research, too, that you answer one question and it raises 10 new questions, and I think that is almost always the case. The fun part, then, is identifying, "Well, which one do we think is the most *important* one, and how shall we prioritize the different aspects we want to pursue next?" It's an endless puzzle.

Also, the picture gets filled in, every year, in more detail. And it really gets driven, I think, by this fundamental curiosity, not just for me, but all of the other people who bring different angles together, and then we integrate that information into a marvelously better understanding of how cells in general work.

In my case, the interest is in cardiac cells and how they work in the whole heart and the whole organism. It's fun to be part of a giant team that pushes this aspect of science forward, and I think that the underlying fundamental science is still the driver for understanding how disease occurs, how we might treat disease, and, in my mind, I think, it's imperative that we, as a society, manage to still support this fundamental mechanistic discovery-type science, because in the end, that's going to be crucial for developing the kind of understanding we need for treatment of diseases.

EMA: It sounds like you've been interested in solving fundamental problems in terms of the puzzles, even while being aware that they had implications in terms of disease.

DMB: Yeah. I think an example is when we were doing this very quantitative work I was telling you about, about how the calcium fluxes all work in the myocyte. Then we started to say, "Well, okay, there is this controversy about what's going on in heart failure. Well, we should go in there, and we'll be able to clean it up in no time."

Well, it turns out disease is complicated, and it took a long time. But we've made big inroads in helping to understand how that process goes. And so always with kind of the fundamental basis in mind that's led us to these discoveries about what goes wrong in heart failure and arrhythmias that have changed how people think of it.

Just this business about how calcium mismanagement in cardiac myocytes is actually now a really *central* player in our understanding of how arrhythmias occur. It used to be the cardiac electricians talked about electricity, and only about ion channels, and the calcium people just talked about calcium. Well, in fact, they are intimately intertwined, and many of the kinds of arrhythmias we know now are, at least in substantial part, due to calcium mismanagement of the cells. So, I think that's the sort of thing that only grows from building at the bottom up, and also talking to other people and integrating the information.

EMA: You've also used a lot of different approaches.

DMB: I also encourage students and postdocs—and even junior faculty—to do that, be driven by the *question* and find the tools you need that answer the *question*, and not just to apply *your* tool to looking around for questions because I think it's the individual's commitment and thirst for answering that question that gives you the perseverance that you need to be able to *find* the answer to the question, and use whatever tools you need to do. And on the positive side for science, I think that also encourages people to develop collaborations where they may not know how to do the type of experiment that's required for that. So either they can go to another lab and learn it or, in fact, collaborate with somebody who has that expertise. Then you not only have learned that new technology, but the scientific way that that other lab or person thinks about it influences *your* thought, and then there's this synergistic process of thought integration that I think makes the modern kind of collaborative science so

much more powerful than when *I* started out, where it was a student and his mentor, and they used one technique in a paper, and we made progress, but I think the progress is accelerating.

Some of my colleagues who are traditional bemoan that, okay, now there are 10 authors on this paper. But I kind of embrace it, because I think that means you have multiple people's different way of thinking about it; you can test your hypothesis in five different ways instead of two.

And so I think it enriches the science, and I think encourages us to think more broadly in general.

EMA: Well, thank you very much for talking to me.

DMB: My pleasure. Nice to talk to you, Liz.

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