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Viewing things the way the channel does: An interview with Karl Magleby

Transcript of a conversation between Elizabeth M. Adler and Karl L. Magleby[1]

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Transcript condensed and edited by EMA

EMA: Hi, this is Liz Adler; I'm the Executive Editor of *The Journal of General Physiology*. I'm in Miami speaking with Karl Magleby, Chairman of the Department of Physiology and Biophysics at the University of Miami.

Karl, thanks for agreeing to talk to me.

KLM: Well, Liz, it's certainly an honor to be asked to be immortalized on tape.

EMA: I think most readers of *JGP* are familiar with your work on the BK channel [the large-conductance calcium-activated K^+ channel]; but I actually am *more* familiar with your work on synaptic plasticity: how did you get interested in two such different areas?

KLM: Well, when I was a graduate student with Chuck Stevens in the Department of Physiology and Biophysics, University of Washington, we had a reading course, a Journal Club course, and we did a bunch of papers on synaptic physiology, and I just got interested from that.

Mallart and Martin had been studying neuromuscular junctions and had published a number of papers on [the] two components of facilitation, so that's how I got interested.

EMA: What's facilitation?

KLM: When you stimulate a synapse, the amount of transmitter release changes, depending on how fast you stimulate or how long you stimulate. In the old days, those were called "historical effects." Nowadays, it's called "short-term synaptic plasticity," or "STP."

We did a bunch of studies, Janet Zengel and I, building on Mallart and Martin's work; there are basically four processes that increase transmitter release:

Facilitation decays with a time constant of 50 ms; the second component of facilitation, maybe 300 ms; augmentation, which Janet and I discovered, decays with about a 7-second time constant; and then, potentiation, known before as post-tetanic potentiation [which decays with a time constant of tens of seconds to minutes]. So facilitation is just short-term synaptic plasticity, with a time constant of about 50 or 300 ms, that increases transmitter release.

EMA: You did most of that work at the frog neuromuscular junction? Is that right?

KLM: Yeah, we did it all at the frog neuromuscular junction. It's a great preparation.

EMA: If you look at the frog neuromuscular junction under physiological conditions of calcium and magnesium, you mostly see *depression*. But to unmask these processes you used high magnesium, right? Or was it low calcium?

KLM: We used high magnesium and low calcium; so, it was maybe 1/100th or 1/20th of the normal release. So it didn't get depleted of synaptic vesicles under those conditions.

There are central synapses that kind of fall in three classes: Classes like the neuromuscular junction at high magnesium, low calcium; then there are central synapses that are in between; and then central synapses that depress—that means if you stimulate repetitively, the release goes way down. And so we were studying release at low quantal content, so it wouldn't depress, and you could see the processes.

Later on, I did a study where we looked at release under conditions of low to normal to high; this was with Alice Holohean. And we found that the number of components of transmitter release actually just keeps decreasing as you keep increasing the amount of release. So it's a *very* complicated system.

EMA: The number of components decreases?

KLM: Yes. There's first and second component of facilitation, augmentation, and potentiation. As you increase the amount of transmitter release, if you just count the number of components, they decrease. In other words, they disappear.

EMA: *Really?*

KLM: Well, we can't *show* they're there. The way we tested that, is we had a quantitative model, probably too simple, of course. But we had depression be depletion [of synaptic vesicles] and we had facilitation, augmentation, and potentiation be increased probability [of release].

And so we would give trains of stimuli, all different patterns of stimuli, different frequencies. And then we would see if we could model the data, based on these very simple assumptions.

And what happens is, as the transmitter release increases, the components start disappearing. The rule of thumb explanation is that the four components of increased release are related in some manner to calcium—calcium buffering, mobile buffers, calcium in compartments, calcium stored in various pools and then pumped out. Now, that's a vast oversimplification; there are also many other things that affect it.

But you have all these things, and it says that if you *deplete*, it looks like some of those go away, or at least you don't need them in the model.

There is actually *much* more work that could be done there.

EMA: Do you think they have a function?

KLM: These processes have been said to have functions in the central nervous system; they can reduce noise in synaptic transmission, and various things [and of course short-term memory].

But the neuromuscular junction . . . I mean, I can *speculate* function.

EMA: Sure.

KLM: In normal frogs, or humans under normal conditions, neuromuscular transmission is almost always one for one. So, if you stimulate the presynaptic terminal, you get an action potential in the muscle fiber.

The idea of this is, if you're going to be chased by a tiger, you don't want decisions made about what to do being made at the neuromuscular junction; you want the neuromuscular junction to *work*. So under normal conditions, these things do not increase or decrease the efficacy of transmitter release. So, in other words, you have this one-to-one function. But the truth is, physiologically, they probably have a function, because, as soon as you start stimulating, the [supply of] vesicles are depressed, and to make up for it, the facilitation two components and augmentation would increase the probability of release, and so it would maintain the release, even as the number of vesicles went down.

So that's a hypothesized function for these processes.

EMA: So, how did you get interested in BK?

KLM: Well, Chuck Stevens had been off on a sabbatical looking at the theory of how agonist-activated receptors would work. So he came back, and he had these *great* ideas to study neuromuscular transmission, look at acetylcholine receptors, the end-plate currents—what's going on. So in a 1-year postdoc, he and I did the so-called Magleby–Stevens papers, where we developed a quantitative model to describe the end-plate currents. And a simple conclusion from that is that the decay of the end-plate currents actually represents a closing of the agonist-activated channels [rather than the time course of removal of ACh from the synaptic cleft]. And we had a very simple model that could describe the end-plate currents, and then Chuck Stevens went on and did noise analysis to more or less confirm his model, but that got me interested in acetylcholine receptors.

And then, after I came to the University of Miami [in 1971], the lab studied both transmitter release and acetylcholine receptors . . . [In] about 1980, single-channel recording had just been done by Neher and Sakmann, so we built our own patch clamp, and we're studying acetylcholine receptor channels. Barry Pallotta was in the lab at the time, and he was recording from cultured rat skeletal muscle, and we were recording agonist-activated acetylcholine receptors. And so I ran down the hall to get John Barrett to come and see this, undoubtedly the first single-channel recordings in Florida, and John comes down, and then we're watching this, and then the trace on the oscilloscope would go off the top of the screen and then come back a little later, and then it would go off the screen again and come back a little later, and John said, "Oh, that's the calcium-activated K^+ channel." He had been studying it [calcium activated K^+ currents] using action potentials in these cultured muscle cells.

So we quickly did experiments to see if it was activated by calcium, and we found out it *was* and it was voltage dependent.

It had this *very* large, 300-picosiemens conductance, which made it very easy to study compared to the 40 or 50 picosiemens for the acetylcholine receptors, which is why the trace went off the top of the screen. So we built a second patch clamp—you had to build your own in those days, and they cost about $50 to build [instead of $12,000]—and we experimented for 2 weeks, my lab and John and his lab, and we wrote a *Nature* paper—so it's a 3-week *Nature* paper—and we got it published.

At the same time, Marty had been working with Neher, and Marty saw these BK channels in chromaffin cells, and Latorre was isolating BK channels from, I think, rabbit skeletal muscle. So the BK channel was basically discovered by three labs all at the same time, and then a couple of other labs had also discovered it, but they were a little bit later to get their work out.

So that's how we got interested in the BK channel—purely by accident, just one of those discoveries that you make if you just do experiments.

We also looked at [identified at the single-channel level] other channels—the SK channel, the fast chloride channel [by Andy Blatz]. But most of the time we've been studying the BK channel, because it's much easier to do experiments if you're the first doing them, because you don't have to read the literature. You know, you can spend all your time discovering stuff rather than confirming observations.

Barry [Pallotta] recorded data; I started writing programs for single-channel analysis. Things were much different then; the computer had—don't drop dead—the computer had 16K. Not megabytes or gigabytes, but 16K of memory. It was a PDP-11E10. Even [though] the programs were written in machine language, they were still very slow; they couldn't sample fast enough. And so we'd record the data on tape, and then we'd slow the tape recorder down four times or eight times, and then we'd play that into the computer, and the computer could then keep up to measure the durations of the open and closed intervals. But because of the 16K memory, we had to stop, and then download the interval durations onto a hard drive, which contained 1 megabyte of space. So it would take all day just to download the data; sometimes *days*, if we had hundreds of thousands of intervals.

So we developed techniques to analyze and record data, and we developed some models to describe the BK gating. And then, when [Owen] McManus joined the lab, we developed stability plots, so we could look for stable single-channel data, and then we were able to record and analyze hundreds of thousands of events of single-channel data, which simply hadn't been done before.

This is kind of an interesting historical note: so, if you fit dwell-time distributions of interval durations with sums of exponentials, theoretically you get an exponential component for each state that the channel gates in. So you may not detect them all, but theoretically you'll get an exponential component in the distributions for each state. And it had been predicted for *decades*—ever since Hodgkin and Huxley—that channels would gate in large numbers of states.

And so, for the BK channel, Owen and I analyzed lots of data, and we found that the open dwell-time distributions were described by three to four open exponentials, and the closed dwell-time distributions by five to six exponential components.

So this was indirect evidence that the channel was actually gating in large numbers of states.

When this was first presented, it was met with basically disbelief by large numbers of people, that there would be so many states, even though they had been *predicted*.

And then Brad Rothberg came into the lab—I'm not going to have time to talk about *every* great person that's been in the lab—but when Brad Rothberg came into the lab we continued the analysis, and we developed ways to look at the correlations in the data, and found out that, basically, it has to be a two-tiered model—a row [tier] of open states and a row [tier] of closed states—to account for the data.

BK channels, as most people probably know, are activated by both voltage and calcium, and the voltage activation is similar to other K_V channels where there are four voltage sensors. But the channel can open with zero, one, two, three, or four voltage sensors up.

EMA: Oh, really?

KLM: Yeah. It can open with any number of voltage sensors activated. It can open without *any* activated.

EMA: I'm trying to think of how the different states that you're describing electrophysiologically correlate with structures. Do some of them have to do with how many of the sensors there are?

KLM: Yeah, to correlate with the structures, in the simple models when all the voltage sensors are deactivated, meaning they are basically—in the jargon, they're *down*—then, that would be one state. Then, if one goes up, that would be another state. Two up would be another state; three up, another state; four up, another state.

BK channels also have calcium sensors, two per subunit, that have been identified in a number of very elegant papers by other laboratories. And so you could have one or two calcium sensors activated on each subunit. So you can have all these combinations of one voltage sensor up, two calcium sensors activated; two up, five calcium sensors activated . . . If you do all the possible combinations, then a minimal model for BK channel gating is actually 50 states: 25 states on the upper tier and 25 states on the lower tier [when the two high affinity calcium sensors per subunit are modeled as a single calcium sensor. If the two high affinity calcium sensors per subunit are treated as separate sensors, then there would be a minimum of 125 states].

Cui, Cox, Horrigan, and Aldrich were working on this at the same time at the macro current level, pointing out all the combinations as well.

And then, what Rothberg and I did is, we made some simplifications and showed that a simpler model than a 50-state model could account for the gating, the voltage and calcium dependence of the gating. So, if this simplified model can account for the gating, then the more complicated model would be able to as well. So Rothberg and I showed that the [minimal] 50-state model [based on the structure] could account for the single-channel gating. Once again, all these experiments are *single* channel, not macro current.

So that's what we did in the BK channel.

When we started, we said there were three to four open states and five to six closed states; the structure of the channel wasn't known except that it was a tetramer. Actually, the core of the channel, the part in the membrane, that structure hasn't been done yet, but it's assumed to be similar to other K_V channels.

But the BK channel has this large gating ring, and in MacKinnon's laboratory, this has been crystallized, and also Jiang's laboratory.

So you have this large gating ring comprised of eight RCK domains. You have two domains from each subunit, each with a high affinity calcium-binding site [actually two high affinity sites] hanging underneath the channel in the cytoplasm. There'd been postulated the gating ring does the calcium activation of the channel. So we *tested* this; there's the linkers that go from the RCK-1 to the S6, part of the channel in the core, and Niu and Qian and I changed the linker length, and we found that the response was consistent. But a really interesting finding was, in the absence of calcium, this gating ring acts as a passive spring to increase the channel opening probability. And then, when you add calcium, it turns into an active machine to pull on the linkers. Anyway, the data is consistent with that model. So you change the linker length, and it changes the way one would predict for that type of model.

And in more recent studies, [Yanyan Geng and myself] working with the Salkoff laboratory; Salkoff figured out a way to take the gating ring off, so we could sever the entire gating ring; we showed that removed all calcium and magnesium sensitivity, which would tie that to the gating ring. And, interestingly, if you took the *gating* ring off, the channel activity was *way* less. Way less. The open times were

less. The resting open probability was less, suggesting that the gating ring does act as a passive spring to open the channel, because if you take it off, the probabilities are less. Of course, there are other explanations. The gating ring could be contacting the core of the channel in certain places, and increasing the probability.

EMA: So it still shows voltage sensitivity?

KLM: Yes, its voltage sensitivity basically is not changed, but it's *shifted*. It's shifted because the gating ring doesn't put this passive force on the channel.

There are a number of very good laboratories out there working on the BK channel; I think we're making great progress. Of course, we need the structure of the core, so we know exactly what we're studying. But my guess is within 5 or 10 years we'll actually have a very good picture about how this highly complicated channel gates.

EMA: It sounds like a pretty complicated channel.

KLM: Well, what I try to tell people is that it *is* complicated.

But for the *channel*, it's very simple. You see, even though you have a 50-state model, the channel is only in one state at any time. So, whatever state it's in, it just looks at the probabilities of transitioning out of the state, and the probabilities of going to connected states. So, for the channel, it doesn't actually *know* that it's complicated. It just moves around the various states.

I think that's the way that we should think about it, too, because when you see the state diagrams it's actually pretty depressing, but if you just look at the way the channel looks at it, one step at a time, then that's what the channel does, if we think of it. But we do have the capacity to study these large, two-tiered, multistate models. One can make assumptions in terms of how the models work, so the number of parameters are realistic to study the gating.

EMA: So you're still continuing your work on BK.

KLM: Yeah, we're still continuing the work on BK. There's an awful lot of work left to be done; it's modulated by many factors.

EMA: It's got auxiliary subunits, and it's subject to splicing . . .

KLM: It has the β subunit; there are many spliced versions; it's modulated by hydrogen ion, fatty acids . . . there's plenty of work to go there. I kind of say, if the BK channel threw up its voltage sensors, and said, "This is too complex; I can't gate anymore," then we'd be in serious trouble. Because BK channels are in almost every tissue in the body. In the brain, they're expressed with some of the auxiliary β subunits; in skeletal muscle there aren't subunits; in smooth muscle they're expressed with β1, which changes the properties.

They're expressed in the auditory system. Depending on the animal, the BK channels can have different properties as you go down the spiral cochlea thing.

EMA: Really?

KLM: Yes. They can alter the tuning in some preparations. Some of that's through alternative splicing. There's only one gene for BK channels. It's not like sodium channels, where you have many different genes; different genes in different tissues. BK channels, it's the same gene, but you have alternative

splicing, and then all these auxiliary subunits to change the properties of the channel to match the tissue.

EMA: Is there anything else you'd like to talk about?

KLM: Well, I would certainly like to say that what makes the whole business worthwhile, I think, is all the super-intelligent graduate students and postdocs, and talented—not only intelligent, but also highly skilled and talented—who basically I've worked with all these years, who have made it so rewarding to be able to work with these excellent people over the years.

Many now have their own laboratories out there, working away.

There are 20 other things I could talk about, but I guess I won't. I think that gives you at least an idea of kind of what we've been doing for all these years.

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