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Sounds Physiological

Did Alan Hodgkin dream of calcium sparks? An interview with Eduardo Ríos

Transcript of a conversation between Elizabeth M. Adler and Eduardo Ríos[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 here in Baltimore, at the 59th Annual Meeting of the Biophysical Society, speaking with Professor Eduardo Ríos of Rush University.

ER: Hi.

EMA: Eduardo, thanks for taking the time to speak with me.

ER: My pleasure; you can throw whatever you want in my direction.

EMA: [*laughs*] Hopefully, nothing too awful. So, it's my understanding that you've been studying calcium signaling in muscle.

ER: For a very long time.

EMA: What got you interested in that? Was it the muscle, or the calcium, or both?

ER: Well, actually, it was something that was being done by a mentor in my alma mater in Uruguay, Venus Hermes Gonzalez Panizza. I was essentially making up a career in biophysics, because there was no formal career in that sense at that time in Uruguay. But the university system was totally free (and still is).

So, I went first to the School of Medicine, where in the Department of Biophysics I found this person doing calcium in cardiac muscle, and I got hooked.

Then I went to the School of Engineering, to learn the math, and I went to the School of Physics to learn, of course, physics, and also to get finally a degree. I remember I was something like 13 years, wandering to all those good schools.

And then I found Martin Schneider and Paul Horowicz, who were teaching in a course in Venezuela; they were in Venezuela for a short course, for a short international course. I went there as a student, and then next thing I was in Rochester, where Martin was a young assistant professor.

EMA: So you came to do a postdoc with him in Rochester?

ER: I came to do a postdoc, and I found a hotbed of what I would call post–Alan Hodgkin thinking. This line of work—the line of work that I engaged in—is an example of a long line of work that is predetermined by a big thinker—in this case, Alan Hodgkin—and then continued by fantastic disciples.

EMA: What do you mean by "post-Hodgkin thinking"?

ER: What Hodgkin and Huxley did at the core was recognize the importance of voltage as a determinant of the state of the channels that determine the action potential. So their success in modeling and understanding was based on that central realization of the importance of the voltage; in other words, the transmembrane electric force field.

EMA: Uh-huh.

ER: That it wasn't sodium, wasn't the current, or anything like that.

And then, Hodgkin got into skeletal muscle, and applied his same paradigm, and he and Paul Horowicz demonstrated again that the operative variable—physical variable—was transmembrane potential, to explain the contractile activation of muscle. Of course, the contractile mechanism itself was very obscure at the time, but they were the ones that figured out the importance of voltage.

My mentor, Martin Schneider, in turn, was mentored by Knox Chandler, who was mentored by Alan Hodgkin. So, here was Hodgkin influencing what was being done when I was a postdoc in Rochester in two ways. And Paul Horowicz, who was chair of the department, was working with Martin in a very humble role for a chair: He was dissecting the fibers, the muscle fibers, which was a most laborious thing.

EMA: So you were doing single fibers?

ER: They were doing single fibers; they had to be dissected from end to end, and Paul was the one who was doing that.

A few years before, Chandler and Martin, continuing the process that was kicked off by Hodgkin, had found the current caused by the movement of the voltage sensors that were needed in the theory of Hodgkin and Huxley. They had found them for the activation of muscle. They called it the intramembrane charge movement of skeletal muscle.

EMA: Yes.

ER: Of course, in '73 or '72, Bezanilla and Armstrong found the analogous current for the sodium channel of the squid giant axon, and they called it the gating current.

EMA: So it was found in muscle first?

ER: It was found in muscle first. That's well-known only in the muscle field. So I got into it there. It was a real hot-spot of basic physiological thinking, or biophysical thinking.

Then with Martin, we essentially figured out how to measure calcium in the cytosol.

And then I got lucky again; I had the luck to go to Chicago with my first job—which is the one I still have—in an environment that grew around Bob Eisenberg.

Bob Eisenberg was studying the roles of calcium in activation of contraction in muscle, of skeletal muscle. And Brenda Eisenberg, now Brenda Russell, was a distinguished microscopist of the field, and

Sue Donaldson was there doing fantastic work with the activation of peeled muscle fibers. All of them made important contributions at the time.

So, that's how I got into it, and that's how I made perhaps the most significant piece of work that I've made, which was to find the nature of the voltage sensor, the biochemical nature, so to speak.

EMA: So can you tell me what it is?

ER: Well, it's of course the dihydropyridine receptor of the plasma membrane and transverse tubules.

The molecule was very well known; but it was well known in the context of cardiac muscle function. It was known that there were these drugs, the dihydropyridines, the phenylalkylamines, and others that were called "calcium antagonists."

These drugs were known to block calcium channels; dihydropyridine receptors were named because it was realized that all these drugs, the phenylalkylamines, the dihydropyridines—there were other families—they were all hitting as targets the same protein of the membrane at that time of cardiac muscle cells and smooth muscle cells.

Therefore, those proteins were called dihydropyridine receptors; they were known to respond to these calcium antagonists by lowering, in general, the activity of cardiac muscle. Because, of course, cardiac muscle needs calcium inside that comes from the outside, an inward current, for activation. So it was understood that these molecules were calcium channels. Later, they were called "L-type calcium channels," or "high threshold calcium channels."

But, at that time, there were a few discoveries made more or less at the same time by different people, including these people I named in the department that pointed us in the direction of another function of the dihydropyridine receptor.

I remember a lecture by Wolf Almers, and Wolf Almers said very clearly that the richest source of these dihydropyridine receptors was skeletal muscle, a hundred-fold more abundant there than in cardiac muscle, which was supposed to be the target. So that was one piece of information.

Another piece of information was that these drugs were being used profusely in therapeutics without any noticeable effect on skeletal muscle. So I was wondering what do these dihydropyridine receptors do here [*Editor's note*: in skeletal muscle] that their function is not affected, and why is that?

And then, at the same time, Jeanne Powell and Kurt Beam had this strain of mice that they called dysgenic, that did not have dihydropyridine receptors, did not have the skeletal muscle isoform, the main subunit, and it could be demonstrated that they didn't have the L-type current, which was not surprising. And they also died at birth when they were homozygous, because they were paralytic; they couldn't breathe. So that was another interesting piece of information.

So what were these dihydropyridine receptors doing that were supposed to be channels, calcium channels?

It had been demonstrated some time before that calcium outside the skeletal muscle cell, was *not* needed for contraction, unlike cardiac muscle, where it was essential.

So you understand the confusion of the time. Right?

EMA: Yes, I do remember. I remember learning about L-type channels, and I knew they were blocked by dihydropyridines. Then all of a sudden, people were talking about it in muscle, and I didn't quite get what was going on.

ER: Right. So we were hot in pursuit of this question, and the other piece of the puzzle that just fell was by Bruce Bean. Bruce Bean had a very nice paper—he was a young assistant professor in Iowa. He demonstrated that dihydropyridine bound to the channel, to the L-type channel, but only when it was in the inactivated state.

So we said, "That's why you don't get any effect when you give nifedipine or verapamil to the people who need it for cardiovascular reasons, because the skeletal muscle is well-polarized, [and the channel is] not inactivated." It's the high threshold activation.

So we did some experiments where we applied nifedipine to skeletal muscle, slightly depolarized. By slightly depolarizing, we put some of the sensors in the inactivated state, and then we showed the effect in parallel on the calcium release flux and on the intramembrane charge movement of the voltage sensors.

And Bob Eisenberg had made a very nice finding at the time, which he called the "D600 paralysis." He had shown that if you applied a potassium, a high potassium depolarization, a contracture, a high potassium contracture to a muscle in the presence of this drug D600 . . .

EMA: What's D600?

ER: It's a phenylalkylamine. It's another one of the antagonists of the dihydropyridine receptor. You make this contracture in the presence of phenylalkylamine, and the muscle becomes paralyzed. It doesn't move any more, even if you remove the phenylalkylamine, the D600. That's because the big depolarization puts the sensors in a deeply inactivated state, and then the D600 hits it, and it becomes stuck there.

So everything came in nice agreement with our idea that the dihydropyridine receptor was, in fact, the voltage sensor for excitation–contraction coupling, for telling the underlying channels of the sarcoplasmic reticulum that they had to open.

It was also, I think, the first time, at least for me, that I knew of a protein that had two legitimate roles that were different. Because it's a channel, but it doesn't *have* to be a channel to do the other function of voltage sensing.

EMA: Right, so it doesn't rely on it . . .

ER: It doesn't rely on this current that comes through to do the voltage sensing.

Gustavo Brum was the postdoc with whom we did all of this initial work with on the dihydropyridine receptor.

So that was in the year 1987, I think. It was a memorable year for all of us, because at the same time, Sid Fleischer and Gerhard Meissner were describing identifying the ryanodine receptor as the calcium release channel of the sarcoplasmic reticulum.

EMA: Oh, really. So it was found at the same time.

ER: It all happened the same year.

And right after we identified the dihydropyridine receptor as the voltage sensor, Shosaku Numa cloned it, and then he and Kurt Beam, using the dysgenic mice as a context, demonstrated that this clone of Numa rescued the dysgenic mice from the paralysis and completed the demonstration of the nature of the voltage sensor.

Then the dynamic duo of Beam and Numa continued this to demonstrate a number of other properties, like isoform independence and the functional differences between the cardiac and skeletal isoforms of both dihydropyridine receptor and ryanodine receptor, and used that to learn a lot about structure–function relationships.

EMA: Are they very different?

ER: They are not *very* different, but that's an important—I think—motif of all that experimentation, which is that you can use intelligently the small differences, or big differences. In this case, the cardiac system still has the two molecules—in their own isoform—but they don't talk mechanically, they talk via calcium-induced calcium release, or CICR. Calcium comes through the L-type channel, and then activates calcium release; calcium itself activates calcium release from the sarcoplasmic reticulum. And so that was used making chimeras, and that kind of thing, based on the knowledge of the difference in sequence, to understand which were the portions of both molecules involved in the interaction.

EMA: So all of that was putting together a lot of chains of evidence, and figuring it out that it had to be the dihydropyridine receptor.

ER: Right, right, right. So yeah, I think what I am trying to say is that there were so many people who contributed over the years. But it's a long story, that took a long time to evolve, and then to develop.

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There was a lot of excitement in the field of calcium signaling in '93, when [Mark] Cannell, [Jonathan] Lederer, and Peace Cheng described the calcium sparks of cardiac muscle, and showed how these were the building blocks of the calcium response for contractile activation. So with Lothar Blatter—who, at that time, was a young professor at Loyola University in Chicago—we extended that demonstration to skeletal muscle. We showed two years later the calcium sparks of skeletal muscle.

EMA: Was that when you first got interested in calcium imaging?

ER: Exactly. Lothar, as part of his package, brought the first confocal microscope to the Chicago area, and we were planning how to use it even before he got there. So we did the first application of that. And my former mentor, Martin Schneider, who already was in Baltimore, in Maryland, he and Alain Lacampagne, and Jon Lederer himself, and Mike Klein, they went on to demonstrate that these sparks involved calcium-induced calcium release.

In other words, there were a bunch of ryanodine receptors that were interacting, keeping themselves open, by calcium-induced calcium release.

So we thought for a long time, for a few years anyway, that the excitation–contraction coupling in skeletal muscle would duplicate many aspects of the excitation–contraction coupling in cardiac muscle, including the sparks. In fact, they did, to some extent, in the muscle that was in vogue at the time, which was frog muscle—frog leg muscle.

But later, my former postdoc, Natalia Shirokova, demonstrated that it wasn't the case for mammalian muscle. Working on rat and mouse, she showed that activation there was not composed by sparks. There were no sparks, unless you did bad things to the cell.

And rapidly it came to be known that the frog had two types of ryanodine receptors, two isoforms, whereas the mammals had only one—the ryanodine receptor 1. So the ryanodine receptor 3 was present in the frog, but not in the mammal.

Later it came to be known that the position of the ryanodine receptor 3 was not the same, the location; that, while the location of the ryanodine receptor 1, which is common to the mammal, is good for direct interaction with the voltage sensor, the location of ryanodine receptor 3 is outside. So the natural hypothesis was that calcium-induced calcium release occurred—in other words, sparks, which represent calcium-induced calcium release, occurred—provided that the ryanodine receptor 3 is present. That was demonstrated to be the case in our lab by another brilliant postdoc, Sandrine Pouvreau, who is now in Bordeaux with her own lab, who successfully expressed ryanodine receptor 3 in mammalian adult mouse muscle.

EMA: Oh, really? And then did she get sparks?

ER: And then she got sparks, and she got sparks activated by depolarization.

So now we have this scheme of two-stage activation, where the voltage sensor first opens the ryanodine receptor 1, which is underneath them, and then the calcium that comes via those activates secondarily, via calcium-induced calcium release, the ryanodine receptor 3.

EMA: Are there functional implications as to why you'd have sparks in one system and not another?

ER: Yes, yes, there are. This CICR happens to make a big burst, and then essentially exhaust itself and terminate. So there is one characteristic of the calcium transient elicited by an action potential, or elicited by a voltage-clamp depolarization, which is that it starts with a big burst of flux, and then it decays spontaneously, even if the voltage sensor is still in the activated position. A part of it may be due to some kind of calcium-inducing activation of the channel; part of it may be due to exhaustion due to the big initial burst, especially when there is CICR.

In other words, the CICR through the second batch of channel continues to amplify that peak; it's an amplifier. We all wondered why the frogs have it and the mice don't.

EMA: Yes. Why would a frog and a mouse heart have it?

ER: And here comes our dear colleague Beth Stephenson to one of our posters where we were showing this, and she says, "Do you know, Eduardo, why this is necessary in the frog? Because the frog only has one T-tubule, one system of release channels per sarcomere, and it happens to be on the Z-line, and the Z-line is the farthest place you can be from the area of filament overlap where the target troponin-C is and does its job, signaling job, for contraction."

So, in the mammal, by developing a system of two transverse tubules and two sets of triads per sarcomere: First you have two; also, they move much closer to the area of overlap. And then, you know the diffusion of calcium is a very ineffective way of reaching far away. So the mouse has gained a lot . . .

EMA: So you no longer need to need to amplify the signal . . .

ER: You no longer need to amplify, and everything is more under control.

EMA: Okay, that makes sense.

ER: Right now, the action generally in the field is with the other proteins of what we call the couplon, which is the system of chained proteins that are involved in this job, of which only two, by the way, are essential for life, which are the voltage sensor and the release channel. The other ones, you can knock them out and the animal survives. Sometimes barely, but survives.

So, we became interested in regulation of the release process from within the sarcoplasmic reticulum.

EMA: So regulation of the ryanodine receptors?

ER: Regulation of the ryanodine receptors. Regulation of the whole release process. There is now evidence even of regulation of the *dihydropyridine* receptor from within the SR. Skipping the ryanodine receptor. It is a protein discovered by Francesco Zorzato and Susan Treves called JP-45, that links directly outside the SR to the dihydropyridine receptor and inside the SR to calsequestrin, which is my favorite protein these days.

Because there is lots of it. It is a protein that stores; it's a low affinity calcium-binding protein. It is linked to a number of sites and important proteins by other proteins, so it is linked to the ryanodine receptor—calsequestrin is linked to the ryanodine receptor via two proteins. And it's linked, as we now know, to the dihydropyridine receptor, and maybe linked to SERCA 4, but I'm not sure about that.

EMA: So calsequestrin is your favorite protein.

ER: Calsequestrin is in the SR, so it does at least the function of buffering of calcium, low affinity buffering. So it has to release it when calcium is released, and vice versa. And there is lots of it. But it has many other functions. Clara Franzini-Armstrong has demonstrated that it essentially shapes the SR, so [if] there is less calsequestrin, the SR changes, the terminal cisternae especially change their shape, and become smaller and convoluted, and greater membrane, lower volume. The SR doesn't want to be empty, so it shrinks, and calsequestrin is the main protein that fills it inside.

But it's even more interesting than that, because it's been known for a long time, almost since the discovery, but especially after 2003–2004, thanks to the work of the person that first crystallized it, Chul Hee Kang, who works in Washington State University, that calsequestrin polymerizes as calcium is increased, as the free calcium concentration is increased.

It does so because calcium serves as interface, as glue for the increasing polymerization. It goes in between in the interfaces between the protomers. So what happens then is that calsequestrin becomes a better calcium store as the need grows, because as calcium increases . . .

EMA: Because it's polymerizing it, yes.

ER: . . . it, relatively to the amount of calcium *and* of calsequestrin, captures more calcium. So the implicit inference is that when muscle releases its calcium, well, maybe, calsequestrin [polymer] crumbles, and that might be a way by which it tells something to the ryanodine receptor. So calsequestrin may be telling the ryanodine receptor how much calcium there is. So that's my favorite project.

EMA: That's cool.

ER: That's what keeps me going these days.

EMA: Is there anything else that you'd like to tell me that I haven't asked you?

ER: I am very grateful to the American taxpayer, of course, but also to Uruguay because of these 13 years of studies, free studies, and this free-flowing university system that they have. And I was very happy to see that lately, in the last few years, this university system that was largely concentrated in the big city, Montevideo, has now expanded and has plenty of schools in the interior of the country, in small places like Tacuarembó, the place where I was born. So now the young people don't have to go to Montevideo.

At the end of the year, this year, they will have the first Regional Biophysics Congress in Salto, Uruguay, which is where a big branch of the School of Medicine is installed. So I get to go there and help them organize this big event.

EMA: When is it scheduled for?

ER: This is in November 2015. But it's going to be extraordinary, because they will also hold it, they told me, in the Horacio Quiroga Hotel and Resort, which is on the shores of the beautiful Uruguay River. Uruguay River is a big river that goes north–south; it separates Uruguay from Argentina. The environment there is beautiful.

So I am excited about that.

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