THE JOURNAL OF GENERAL PHYSIOLOGY

Sounds Physiological

Investigating calcium compartmentation and dynamics: An interview with Tullio Pozzan

Transcript of a conversation between Elizabeth M. Adler and Tullio Pozzan[1]

[1]Department of Biomedical Sciences, University of Padua, 35122 Padua, Italy

Transcript condensed and edited by EMA

EMA: This is Elizabeth Adler, Executive Editor of *The Journal of General Physiology*. I'm here in Nassau, Bahamas, at the FASEB Conference on Calcium and Cell Function, speaking with Professor Tullio Pozzan of the University of Padua.

Tullio, thank you so much for agreeing to speak with me.

TP: It's a great pleasure for me.

EMA: You're well known, of course, for your pioneering studies investigating calcium compartmentalization and dynamics in various intracellular compartments. Can you tell me how you got interested in that?

TP: It's been the topic that [I've studied since] I started to get involved in science, from the very, very beginning. I graduated in medicine a long, long time ago, 1973, and the thesis I wrote was concerned with mitochondrial calcium and effects of hyperloading mitochondria with calcium. It was a topic of great interest in the '70s. Leading figures in the field were Albert Lehninger, Britton Chance, Ernesto Carafoli, and others, and I contributed a few things while I was in Italy.

Then I moved to England, and I was lucky enough to meet Roger Tsien. We became friends, and, ever since, I've been involved in this business. So it's a quite continuous interest in my life. I've used different models, different problems, but they were *all* concerned with calcium and signaling in general.

EMA: Why is it so important to understand calcium dynamics in different compartments within a cell?

TP: For a while, the interest [was]—and still is, of course—was to understand how you can handle calcium in the cytosol—what are the targets of calcium changes in the cytosol—because so many events depend on the changes.

However, at some point, we realized that calcium in the cytosol depends very much on what the organelles can do: so release it or take it up.

But in the late '80s, when I started to address this problem directly, all the information we had was either based on pharmacology or indirect type of experiments.

And pharmacology is fantastic, but what I learned from my friends—pharmacologists—is, if a drug doesn't affect the process you are interested in, most likely that process is not involved; if it *does*, you don't know *why* it does.

EMA: Right. [laughs]

TP: So we knew nothing of the actual concentration of calcium within the organelles. How was it buffered? How much did it change? Etcetera, etcetera. And having a description, an understanding of how the different organelles can really handle calcium in *quantitative* terms was essential to understand the physiology of the cells and, after that, the pathology.

And so that was what moved me into developing a methodology, not for the method *itself*, but to understand in quantitative terms what this calcium signaling is doing within the organelles, and from the organelles to the cytosol, and the overall cell function.

EMA: One of the really interesting areas you explored had to do with communication between mitochondria and the endoplasmic reticulum. Can you tell me a little about that?

TP: That was totally accidental. As I told you, I was trained in bioenergetics and mitochondrial calcium, and at some point of my life I was totally fed up. I thought it was an unimportant problem. Actually, I even wrote a review in the middle '80s saying that it is a very interesting experimental artifact of isolated organelles. Because the mainstream [view] in those days was that mitochondria don't take up calcium under physiological conditions; they only do it at the last chance to survive.

So when we started with Sarino Rizzuto this new approach of developing a genetically encoded probe that at that time was aequorin, we thought, "Okay, we have a method. We have to look for an organelle that we *know* what it does." We *thought* we knew what it does—and it was simple. Also, the molecular biology of making a construct of aequorin to go into the mitochondria was the simplest one. So we did the mitochondria first, thinking, "Okay, this is a control, and then let's go. . ."

EMA: We know it.

TP: ". . . and look at something more interesting." And then, when we did the first experiments, we found ourselves in front of something totally unexpected: mitochondria, in response to "physiological" stimuli took up calcium *extremely* efficiently, *extremely* rapid[ly], with peaks that were at least an order of magnitude larger than we expected.

And so we said, "Well, the method is wrong, because everybody knows that that's not the case." And so we started investigating, and at some point, we ran the key experiment.

The key experiment was as follows:

We took the cell, transfected with aequorin, and we permeabilized the plasma membrane. So we let it equilibrate rapidly with the extracellular medium. And we added calcium as it should occur under a stimulated condition, let's say, a couple of micromolar at the peak of a calcium transient in cytosol.

EMA: Right.

TP: And we looked at what happened in the mitochondria, and the mitochondria took it up very sluggishly—very, very slow. Never reaching a peak. Completely different from what we saw in the intact cells. So, okay, so they're working as predicted.

EMA: Yes.

TP: And then, rather than introducing calcium in the cytosol and around the mitochondria by perfusing a calcium buffer, we added inositol trisphosphate (IP_3). So we were releasing calcium from the very place where it's coming from under physiological conditions.

And *then*, we reproduced the effect of an intact cell.

So after demonstrating that IP_3 was not acting on mitochondria, we put forward the hypothesis that the reason why, when it [calcium] was released from the right place, the mitochondria took it up so rapidly, was that they were not seeing the *bulk* cytosolic or medium calcium, but they were seeing hot spots close to their calcium carrier. And these hot spots were much higher in calcium concentration only transiently, and that allowed this very fast uptake.

Then there was all the morphology showing this connection between the two organelles that had been known for *ages* from electron microscopy. People thought these are artifacts of fixation, or maybe they are to exchange lipids between ER and mitochondria, because phospholipids are made in the ER and then transferred to mitochondria—which is probably *true*—but in *addition* to that, these close contacts are probably the site—and then we showed it many, many years later, directly—the site where these hot spots are formed and the mitochondria take it [calcium] up extremely rapidly [*editor's note*: see Rizzuto et al., 1992, 1993, 1998].

So that was how we got it. But totally unexpectedly. We were thinking of doing just the control experiments.

EMA: That's really funny. I guess it shows the importance of doing controls.

TP: Yes. As usual.

EMA: As usual. Did you have a favorite experiment, or a series, or a project?

TP: I think that *that* very experiment is one of my favorites.

Of course, if I have to mention another one, I go back to the first experiment we did together with Roger Tsien and Tim Rink in the early '80s, when we first were able to measure calcium in the cytosol with the prototype of these calcium indicators—quin2 [*editor's note*: see Tsien et al., 1982a,b].

I remember vividly when I was working in the lab, and Roger came and said, "Why are you wasting your time doing this experiment? Come on, bring your cells; we'll do the experiment with one of my new dyes."

And that was late in the evening, and there were the three of us, and we put lymphocytes that we loaded with this indicator, and then we added polyclonal mitogen.

EMA: Uh-huh.

TP: And as soon as we did it, we saw it, ooooOOOOOO, going up. And that was really an emotional moment.

EMA: Oh, that must have been so exciting.

TP: Very exciting. Very exciting. So these are the two experiments, I think, really. The one with quin2 and the experiment in which we saw this very clear response of mitochondria to inositol trisphosphate that was dependent on this close apposition between the two organelles, which makes sense. The cell is a very complicated and extremely organized structure, and the interactions between organelles,

between structures—of course, between proteins, but even between structures—are absolutely *essential*. It's not a bag of water, where organelles are moving around.

EMA: It's interesting how our views have evolved along those lines. I remember getting the picture of it as a bag of water. . .

TP: Absolutely. Absolutely.

EMA: And then, well, there were these organelles, but they were in isolation. And then there were channels, but where they were didn't really matter.

TP: Yeah. And think about, for example, the mitochondria. All the textbooks, I think even today, they describe mitochondria as *beans*. This is simply a misinterpretation of electron microscopy data, because you don't have the three-dimensional structure.

Another experiment I remember—I think this is the third experiment—is when we transfected the cell with GFP that was targeted to the mitochondrial matrix. And we looked at the cell, and it was like *spaghetti* . . . very long strings of tubules [*editor's note*: see Rizutto et al., 1995]. And I said, "Are these *mitochondria*?" And in fact, they are. Essentially, they are not those beans. They are long, long tubules. There is a long interconnected tubular network very similar to the ER. The idea of these little beans is simply that when you cut a slice in electron microscopy, you see mitochondria that is coming in or is coming out, and then you cut it transversely, and that's what you see.

EMA: What are you working on now?

TP: Well, at the moment, more than 50% of my time is dedicated to running the Department of Biomedical Sciences of the National Research Council of Italy. The department has about 1,500 people working throughout Italy—and then another 1,500 approximately that are associated with this institute—so I have an organization "political" role. I still have a lab that's smaller than I used to have, in Padua, and, at the moment, our main interests are concerned with—on the one hand—calcium and pathology, particularly in neurodegeneration. There is plenty of indirect evidence that calcium may play a role.

EMA: Mmm-hmm.

TP: Maybe even *causative* in some cases. But how it does, what is the real target, where and why it's altered, is not clear.

If you consider what a neurodegenerative disease of this type is, it's a disease that occurs, in the worst possible case, after 40 years of life. And that means that the alteration in the metabolic pathway must be really very, very minor, and it's only this extra input that occurs over *such* a long-long-long-long period of time that accumulates and eventually leads to pathology.

We are studying particularly, these days, mutations in proteins that lead to Alzheimer's disease.

EMA: Mmm-hmm.

TP: These people have Alzheimer's at the age of 45, and before that they are *perfectly* fine. So even with a mutation, the disease takes such a long time. You need accumulation of hits for a long period. So they are really minor changes. And it has to do with mitochondria, I think, but that's my bias [*editor's note*: see Zampese et al., 2011].

EMA: Of course.

TP: The other thing that I am particularly interested in is this recent discovery of ours: the existence of a cyclic AMP signaling within mitochondria that is totally autonomous.

EMA: Yes. That's interesting.

TP: It has nothing to do with the cytosolic one; it's an autonomous mechanism of production, degradation, and sensing of cyclic AMP within the matrix of mitochondria. From what we can see, it's a cross talk between calcium and cyclic AMP signaling in the mitochondria, too [*editor's note*: see De Benedetto et al., 2013, 2014]. So that's the two main topics: Alzheimer['s] and cyclic AMP in mitochondria.

EMA: Is there anything else that I haven't asked that you feel moved to say?

TP: You know, I made the decision of staying in Italy. And I did it reasonably well, also, living in a country where financing, where difficulties for science are much more important than in other places.

Now, as I told you, I took this other job because I think, at my age, it's probably the time to do something for the country, to try to change a little bit. And if you want to do that, you have to work at the national level, and I think that, if this job is done by someone who knows what science is about, what lab work is, I think it's much better than the classical politician can do.

But of course, scientists in general don't *want* to get involved, because they *love* staying in the lab. I *love* staying in the lab. I *love* looking at the microscope still. I still do it sometimes. But I think it is my duty trying to do something for the country, and the country *is* in trouble. As in any other country, money is decreasing, but in Italy, we started from much lower levels so decreasing from a low level means going close to nothing.

And most important of all, we have to motivate the young generation, convince them that you *can* do science in our country, that there is the possibility, and we should create the condition for them, for the *best* of them, to be able to do it in the country, and not running away.

I have a daughter who is in the States, however. [laughs]

EMA: Is she a scientist?

TP: She is a scientist in a totally different field. She is in linguistics.

And now she got a position in Australia, in Sydney—she thought she was not far enough from home!

No, I am joking. No, she got a good offer there, and she decided to go. And I am accompanying her to set up a new lab in a few weeks.

EMA: That sounds great. Well, thank you. Thank you so much.

TP: It was a pleasure.

ARTICLES CITED AND SUGGESTIONS FOR FURTHER READING

Rizzuto, R., A.W. Simpson, M. Brini, and T. Pozzan. 1992. Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin. *Nature*. 358:325–327. http://dx.doi.org/10.1038/358325a0 PubMed

Rizzuto, R., M. Brini, M. Murgia, and T. Pozzan. 1993. Microdomains with high Ca^{2+} close to IP_3-sensitive channels that are sensed by neighboring mitochondria. *Science*. 262:744–747. http://dx.doi.org/10.1126/science.8235595 PubMed

Rizzuto, R., P. Pinton, W. Carrington, F.S. Fay, K.E. Fogarty, L.M. Lifshitz, R.A. Tuft, and T. Pozzan. 1998. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science*. 280:1763–1766. http://dx.doi.org/10.1126/science.280.5370.1763 PubMed

Landolfi, B., S. Curci, L. Debellis, T. Pozzan, and A.M. Hofer. 1998. Ca^{2+} homeostasis in the agonist-sensitive internal store: Functional interactions between mitochondria and the ER measured in situ in intact cells. *J. Cell Biol.* 142:1235–1243. http://dx.doi.org/10.1083/jcb.142.5.1235 PubMed

Tsien, R.Y., T. Pozzan, and T.J. Rink. 1982a. T-cell mitogens cause early changes in cytoplasmic free Ca^{2+} and membrane potential in lymphocytes. *Nature*. 295:68–71. http://dx.doi.org/10.1038/295068a0 PubMed

Tsien, R.Y., T. Pozzan, and T.J. Rink. 1982b. Calcium homeostasis in intact lymphocytes: Cytoplasmic free calcium monitored with a new, intracellularly trapped fluorescent indicator. *J. Cell Biol.* 94:325–334. http://dx.doi.org/10.1083/jcb.94.2.325 PubMed

Rizzuto, R., M. Brini, P. Pizzo, M. Murgia, and T. Pozzan. 1995. Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells. *Curr. Biol.* 5:635–642. http://dx.doi.org/10.1016/S0960-9822(95)00128-X PubMed

Di Benedetto, G., E. Scalzotto, M. Mongillo, and T. Pozzan. 2013. Mitochondrial Ca^{2+} uptake induces cyclic AMP generation in the matrix and modulates organelle ATP levels. *Cell Metab.* 17:965–975. http://dx.doi.org/10.1016/j.cmet.2013.05.003 PubMed

Di Benedetto, G., D. Pendin, E. Greotti, P. Pizzo, and T. Pozzan. 2014. Ca^{2+} and cAMP cross-talk in mitochondria. *J. Physiol.* 592:305–312. http://dx.doi.org/10.1113/jphysiol.2013.259135 PubMed

Zampese, E., C. Fasolato, M.J. Kipanyula, M. Bortolozzi, T. Pozzan, and P. Pizzo. 2011. Presenilin 2 modulates endoplasmic reticulum (ER)-mitochondria interactions and Ca^{2+} cross-talk. *Proc. Natl. Acad. Sci. USA*. 108:2777–2782. http://dx.doi.org/10.1073/pnas.1100735108 PubMed