Sounds Physiological

Peering into the black box: An interview with Bertil Hille

Transcript of a conversation between Elizabeth M. Adler and Bertil Hille[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 San Francisco at the 58th Annual Meeting of the Biophysical Society, speaking with Bertil Hille. Thank you so much for taking the time to talk with me; I really appreciate it.

BH: It's a pleasure. Thank you for the invitation, Liz.

EMA: When you went to graduate school, it was unclear how ions crossed membranes, and you were quite sure that they passed through pores, and not only pores but ion-selective pores. What gave you that idea?

BH: The idea of pores was always there. That is, pores in membranes, first biological membrane like an epithelium and then, later, cell membranes. But it had never been connected with the electrophysiology side—the pores were more about moving water—and having selectivity based on radius for osmotic purposes.

EMA: Mmmhmm.

BH: I felt that proteins had all the functions that one could possibly ever want, and it had to be a protein of some kind that accomplished this very specific gating and selectivity aspect that Hodgkin and Huxley first revealed. And so, thinking about what a protein could do, it seemed very natural to think about the pores, but I think both Clay Armstrong and I had this idea almost instinctually, and we could have been totally wrong, both of us.

Both he and I were in laboratories…[in which] there was no predilection towards pores. Both laboratories knew about the Hodgkin–Huxley stuff and knew about voltage clamp—so all of the electrical sides were wonderfully present in both labs—but I think people felt that it was premature.

It would be like talking about consciousness 20 years ago. It was something that you could imagine, but it wasn't polite, even, to begin to speculate about what it was or how it worked.

EMA: So they just didn't even want to think about it at that point, because it felt too far into the future.

BH: That's correct. It could have been wrong.

EMA: It was starting to use substances like tetrodotoxin and TEA that led you to thinking about specificity and different types of proteins for different types of ions?

BH: Yeah, so Clay Armstrong had started with TEA; that was the way he got into the squid giant axon channel ideas, which he enunciated about half a year before I got really into this. And then I felt that I could take everything off the kitchen shelf—everything that was known to pharmacology, basically—and put it on nodes of Ranvier and ask, looking at the currents there, were they [*editor's note*: the drugs] somehow selective and were they somehow telling me about these things which I was sure would be proteins.

And it was the finding that tetrodotoxin *so* specifically removed sodium currents and that TEA *so* specifically removed potassium currents and several other agents shifted the voltage dependence—those kinds of findings confirmed to me that we were talking about separate devices.

In my thesis work I wrote that they would certainly be proteins, and they would be coming from genes, and they would be subject to diseases, and they would be subject to evolution, and all of those things which didn't come for another 20 years almost.

EMA: It must have been wonderful to see it all unfolding.

BH: It *was* wonderful. And the idea that everybody had then—and still has today—is that by doing biophysical experiments you could test the black box and it would tell you—if you were clever about it—something about what was inside that black box.

And Clay and I deduced that in the black box there was a hole.

And he showed that the gate on the hole was on the inside and I got its diameter by testing what would go through it.

EMA: Mmmhmm.

BH: I wrote about the size of the pore in the sodium channel in 1971, and we still don't know for sure—from a crystal structure—we don't have a calibration that says those ways of thinking are correct.

There is a structure from Bill Catterall's lab of a sodium channel from bacteria [*editor's note*: see Payandeh et al. (2011)], but I'm withholding celebration until something mammalian—or closer to the ones that I studied—is known.

EMA: How did you get interested in local anesthetics?

BH: Again, it was just the kitchen sink. So, I read a giant review [*editor's note*: see Shanes (1958a,b)] that went through several hundred agents that did something to action potentials—or to "conduction."

EMA: The nerve impulse.

BH: The nerve impulse, yes, and so I just read these things carefully to see if there was any reason to believe that they might do something to the conduction of the action potential, in which case it would be a good candidate for me.

EMA: Mmmhmm.

BH: Facilitating my work greatly at the time was that, in the laboratory next to us, which was Keffer Hartline's laboratory (he later received the Nobel Prize for his work on *Limulus* eye and vision), he brought the first digital computer—laboratory computer—into the Rockefeller University. This was 1962, maybe.

Fred Dodge had made a voltage clamp of nodes of Ranvier together with Bernhard Frankenhaeuser in Sweden—that was at a time when Rockefeller University encouraged the students to take a year abroad. So, Fred showed me how to use this voltage clamp, and when you wanted to record what you saw, you took pictures of the oscilloscope and you projected the negatives of the film by an enlarger and traced it on graph paper, and so you spent perhaps a week analyzing perhaps 10 minutes of original investigation. And that seemed incredibly boring and painful for me—I couldn't see that—and Fred Dodge—when I told him that—he suggested that, well, maybe this was something that could be done on a digital computer.

So with his help—considerable help—the computer was attached to the voltage clamp and, through Keffer Hartline, I was allowed to use the computer once a week—on Saturdays—to do my experiments.

And this was the first time that ever a digital computer had been used with a voltage clamp. Today, nobody would even *buy* a voltage clamp without a computer almost built into it … and so I had the facility for the first time to record these ion currents facilely.

EMA: Yes.

BH: And I could just inject one drug after another after another—which, in the past, was not at all possible—and you could quickly assess changes of current, changes of time course, and so forth, so that was a great advantage for this kitchen sink approach.

EMA: I thought it was interesting that you studied use-dependent block *before* thinking about the implications for frequency-dependent activity.

BH: It was true; I studied use-dependent—or we discovered in my laboratory—use-dependent block, and analyzed it and published it, and only after its publication did some other publication came out from other people working independently [*editor's note*: see Hondeghem and Katzung (1977)], talking about cardiac arrhythmia and the same drugs being used and having the same use-dependent aspect as how to explain their antiarrhythmic effects, and only then did I realize that it had some medical application.

EMA: Hey. It's got some application.

BH: So, I was totally naive about medical aspects.

EMA: Well, that's really general physiology.

BH: It *is* general physiology.

And that's what we always say when they say "Well, what's the translational value?" and we say "you never know what outcome there might be, and we don't know if my work or the next person down the hall is going to be the one that suddenly makes a breakthrough. We just have to do it."

EMA: Can you tell me a little bit about the modulated receptor hypothesis?

BH: Yeah. So the idea of the modulated receptor hypothesis is that local anesthetics bind to ion channels by entering into the pore both from the cytoplasm when the gate is open and from the membrane when they're lipid-soluble molecules just slipping into the channel from the side, and that the binding to the channel affects gating of the channel, so, for example, it promotes inactivation, so that's the modulation part…

EMA: Mmmhmm.

BH: that the fact of binding also changes the energy associated with inactivation of the channel.

And so, you now bias the channel to be in more inactivated states; it's, of course, already blocked by the drug that's in there…

EMA: Mmmhmm.

BH: but the inactivated state, which would normally close the channel, proceeds to be favored, and the channel goes into that state, binds the drug more strongly so the drug has a harder time coming out and, if you stimulate the neuron repetitively, going through inactivated states several times, the drug sticks more and more strongly. So stimulation of the nerve intensifies the block from the local anesthetic.

And now, in retrospect, that's good for antiarrhythmia in the heart, where extra and premature heartbeats would be made impossible by a drug that just stayed there a little longer. And, in pain, in neurons, where high-frequency firing in pain fibers would be selectively blocked, compared to the slower low-frequency firing controlling the motor system, let's say.

EMA: Were you interested to learn about the fenestrations that Catterall found?

BH: Well, it was very exciting.

He said that there was a hole in the side of this prokaryotic sodium channel. And I had to stand back and be somewhat reserved about whether—could this prokaryotic channel have any evolutionary relationship to the ones we have in mammals or not?

So it would be very exciting if it were truly a homologue.

It would be quite interesting if it's a convergent solution that actually came to almost the same structure as the sodium channel that we know from mammals.

But it is quite exciting, and, just at this meeting, I saw a paper from Bonnie Wallace's lab, which showed a drug sitting in the [*editor's note*: lateral, lipid facing] fenestration of one of the prokaryotic channels and sticking into the pore—it was a fairly long molecule—and it sat in there, maybe 10 Å of it hanging in the pore itself, clearly blocking it, and the rest of it hooked into this hydrophobic hole from the side, which would give access from the membrane for the drug to go in [*editor's note*: see Bagnéris et al. (2014)].

EMA: Yes.

BH: And to go out.

EMA: So, later on, you started looking at G protein–coupled receptor–mediated regulation of ion channels. What led you to that pathway?

BH: After I wrote my book, I felt that I had now written about and summarized and done a lot of the work related to the biophysics of ion channels, telling us what they are, how they work, and that maybe it was time to move on.

Especially time to move on into more biological areas.

Biophysicists were getting very excited about "are there 48 states, or 37 states, or 64 states of the gating of an ion channel," and I thought that could be great biophysically, but it bears little relationship to what would be the biological functions of the channel.

So, I thought I had to find some other area to go into.

And so we explored various things and then we stumbled into the G protein–coupled receptor, because, as kind of a hangover from the past, I suggested to a student, Paul Pfaffinger, that we study more about the ion selectivity of the inward rectifier K channels that had not yet been characterized.

And so, I said we'll have to find first a cell—this was before you could buy the gene and just stick it into a cell—so we had to find a natural cell that had this, and I knew that Neil Nathanson down the hall was working with heart and that heart had an inward rectifier, so, Neil Nathanson had this, but it turned out he was interested in how acetylcholine made it open, and he was doing this with radioactive isotopes and I said, "You know, we can do that better just doing electricity."

And so we got these chicken embryo heart cells that he was making—and they had inward rectifiers—and we were immediately interested in the problem that he was interested in: how acetylcholine influences the channel. And the upshot of that study was that a G protein is involved in coupling the muscarinic receptor activated by acetylcholine to the opening of this inward rectifier K channel.

And you would not be at all surprised now. But, at that time, Bert Sakmann and his colleagues had proposed that the muscarinic receptor was probably just like the nicotinic receptor, something that had a channel in it and it opened right away when you added the drug.

And we showed that you needed the G protein—you needed GTP and it could be stopped by ADP ribosylation through pertussis toxin, and so forth, which were all criteria that I had no idea about—it all came from Neil Nathanson.

And that was before many receptors had been cloned…probably rhodopsin and the β-adrenergic receptor had been cloned. So we didn't really have a comprehensive idea that there was this big family of receptors, and the muscarinic receptor wasn't yet coupled into this concept.

EMA: Yeah.

BH: And I think we helped to make that connection.

And from then on, we just went from one channel to another and asked about receptors that were G protein coupled—how do they work?

It's been very interesting: Twice, because we're electrophysiologists, we got into ideas that had not come yet from the field of G protein–coupled receptors. And the first one was that the—this was mostly David Clapham's laboratory, and Diomedes Logothetis.

What we concluded was it was a part of the G protein that acted on the channel and there was no other second messenger—none of the cAMP or calcium things were needed.

They showed that it was specifically βγ [*editor's note*: see Logothetis et al. (1987)] And that was a kind of new idea, especially that βγ subunits could do anything. It was very strongly opposed by several other laboratories.

So, that was a first: Something that you couldn't discover without doing voltage clamp, or doing patch clamp. You would never have guessed that an ion channel was influenced by the G protein unless you were an electrophysiologist.

Then the second one was that later, the turning off of a potassium channel that was called the M current, now called KCNQ, was mediated by a muscarinic receptor—a different muscarinic receptor—that coupled to phospholipase C. And the reason that the channel was inhibited was *not* because phospholipase C makes a calcium signal, not because it makes diacylglycerol and a protein kinase C signal, which all seemed very logical, but instead because the substrate, PIP_2, phosphatidylinositol 4,5 bisphosphate, is decreased by activating phospholipase C, and the channel needs PIP_2 just to do the work.

So that's another mechanism of regulation, which the biochemical side of G protein–coupled receptors—they wouldn't have found it. It required electrophysiology to get to that. So, by bringing electrophysiology into fields that are more cell biological and more biochemical we were able to add new results to those fields.

EMA: Did you have a favorite project?

BH: Well, I suppose the ion selectivity, way back when I first became a faculty member…

EMA: Uh huh.

BH: was the most interesting to me. I had tested probably 15 different cations that could go through sodium channels.

EMA: Uh huh.

BH: And there was a very puzzling aspect of why some of them couldn't go at all and other ones could. Those other ones that could go seemed rather large. And the ones that couldn't go at all seemed rather small.

EMA: Yeah.

BH: And what could it be? And it took me several years of just sitting on the results and having to think about the sizes and shapes of cations from crystal structures and things like that, and their interaction with oxygens and other things that I presumed were in the pore.

In the end, I came up with a hypothesis that, by being able to make hydrogen bonds, some rather large-seeming things "look" small because the distance between them and the oxygen gets shorter than you expect. Whereas something like a methyl group, which is no bigger than an amino group but can't make these hydrogen bonds, the oxygen cannot get as close to that because the hydrogen bond allows the oxygens to penetrate as it were into oxygens and nitrogens.

EMA: It's one of those things that makes sense once it gets explained to you.

BH: It only makes sense [laughs]…and of course there was no one to explain it to me. You know, I had to work through a number of ideas before finally I thought this was quite a reasonable idea.

But now, that's exactly the issue—the thing that I said was so far uncalibrated, that was a speculative idea, and, to this day, we don't know if that was a correct thing, and maybe I didn't think it through correctly and maybe it's quite different.

EMA: Is there anything else that you'd like to talk about that hasn't occurred to me to ask?

BH: There's a difference between the arts and the sciences. Each one of us can remember five or more authors or artists from each of the previous centuries—composers—and we cannot do that for scientists; we can remember Newton and Einstein and a few others. And we haven't had the direct exposure to those scientists' products. We know the theories that came from their work, but we haven't read their papers, whereas with the artists, we've actually seen the plays, and we've read the books, and we went to museums and saw the individual creations.

So, arts are kind of about each individual creating something that then is a human statement that lasts forever, whereas sciences are not that at all. It's a product of a group of people, and we know that, within a few years, some corrections will be made and therefore each paper will become a little bit archaic. And so if you've read papers from previous centuries, you know you can expect rather archaic and quaint things, not things that speak to you so directly any more.

Sciences are this collective. Scientists spend a lot of our time being critics: so we do peer review of grants and papers. And so we're tuned into trying to find the detailed difficulties of each other person's contributions, which we will want to improve upon, or we will criticize them, or ask them to improve, and that's quite different from the arts, I think.

So, scientists are not used to having good things said about themselves, because all we are supposed to do, in a way, is to be the critic and to find the loopholes that still exist and point them out. So, I'm saying that we should all be kind to each other and remember that we are human beings and we do good things—and praise our students.

We will be forgotten.

So, it has to be now, if we're going to have any of this pleasure of being recognized: it's now and it's not after we die.

Those papers will be erased by the new generation that will say "such and such was done, but now we're going to do better."

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