Sounds Physiological

Getting from "This can't be right" to "Of course": An interview with Paul Greengard

Transcript of a conversation between Elizabeth M. Adler and Paul Greengard[1]

[1]Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10065

Transcript condensed and edited by EMA

EMA: Hi, this is Liz Adler, Executive Editor of *The Journal of General Physiology*. I'm here in New York at Rockefeller University, speaking with Nobel Laureate Paul Greengard.

Paul, thank you so much for agreeing to talk to me.

PG: My pleasure.

EMA: So, as I understand it, you've been interested throughout your research career in how neurons communicate with each other. Is that a fair way of putting it?

PG: That's accurate, yes.

EMA: What got you interested in that field?

PG: In high school and college I was particularly interested in mathematics and physics.

But after the Second World War, the only funds available for graduate school in physics were from the Atomic Energy Commission—and this was just a handful of years after dropping the atomic bombs on Hiroshima and Nagasaki. So, I just didn't like the idea of being supported by the Atomic Energy Commission, and possibly using my talents to develop more powerful weapons of destruction.

And I learned from the parents of my roommate in college, who were both research-oriented physicians, about this emerging field of biophysics and medical physics.

Anyhow, I did my thesis on the electrophysiology and biochemistry of degenerating axons.

EMA: Huh.

PG: While I was there, Alan Hodgkin came and gave a lecture about the work on the ionic basis of the nerve impulse. And I felt that all the measurements being done, virtually, were electrophysiological.

At that time, two types of people were working on the brain. One were what we'd call neurophysiologists, who studied the electrical properties of nerve cells, who were not interested in the underlying biochemistry. And the other group were biochemists who used nerve cells because the brain is extremely active—way more than virtually any other tissue—and they used the brain just as a source of enzymes. Nobody was really studying—or there was an extremely limited amount of study of—the biochemical basis of nerve cell function.

So I got interested in that.

And then, what got me particularly interested in communication between nerve cells were the studies of Earl Sutherland, who had discovered cyclic AMP and showed that it mediated the actions of the hormones norepinephrine and glucagon on carbohydrate metabolism in liver and muscle, and Edwin Krebs, who had shown that cyclic AMP worked through activating a protein kinase [*note*: phosphorylase kinase kinase], which then, in the case he studied, phosphorylated phosphorylase kinase, which phosphorylated phosphorylase and regulated glycogen breakdown.

So between those two studies, I wondered whether it was possible that nerve cells communicated using mechanisms similar to those which had been discovered by Sutherland to mediate the actions of glucagon and norepinephrine.

And, so I started to try to understand the communication between nerve cells and gradually built up a story showing that, although transmission in synapses involves like a distance of angstroms, and the hormonal system can be like a meter in distance between the sending cell and the receiving cell, nevertheless, the principles that had been elucidated were also true in the nervous system.

EMA: So the sort of mechanisms that hormones use to communicate with their target cells?

PG: Yeah.

EMA: So, at that time, most people were more oriented towards electrophysiology; were people resistant to the idea of that sort of "hormonal communication" between neurons?

PG: Yes, they were. And so the typical attitude was, "Poor Paul, he's making a big mistake, but he'll realize what a mistake he made sooner or later."

Which was great for poor Paul, because poor Paul was able to do a lot of work without the competition of a hundred other laboratories. By the time people realized the importance of what I had done, it was too late for them to jump in as competitors. It was now established dogma.

It was about 15 years from my first paper until there was really acceptance of the idea of a signaling cascade being involved in synaptic transmission.

EMA: What did the papers in those 15 years show?

PG: We showed that the neurotransmitter dopamine worked through raising the level of cyclic AMP in cells.

And we showed that there was a cyclic AMP–dependent protein kinase in the brain—which was *not* phosphorylase kinase kinase—which phosphorylated synaptic membrane proteins. We didn't know what they were. We called them protein I and protein II; they were actually what we later renamed synapsin I and synapsin II. And they were the first synaptic vesicle proteins known, phosphorylated or not phosphorylated. They were the first ones shown.

EMA: So, you think about synaptic transmission, you typically think about it as being pretty fast, which seems inconsistent with this type of signaling.

PG: The simple way to think about it is there are two kinds of transmission, which we call fast and slow synaptic transmission.

The fast transmission is used predominantly by glutamate, which is excitatory, and which causes the opening of ion channels, which allow sodium ions to go in and depolarize the cell, producing an excitatory response.

And then, the other class are receptors—mainly GABA receptors—which cause an opening of ion channels which allow chloride ions to go through, and which causes a hyperpolarization of the cell and are inhibitory.

And all the other hundred or so neurotransmitters, which we now know to work through slow transmission, are used to modify fast transmission. Because they *are* relatively slow, what they'll do is increase the probability or decrease the probability that glutamate or GABA released from the presynaptic terminal will or will not activate or inhibit a postsynaptic response.

EMA: So how does that happen?

PG: Well, one of the most common ways is that the enzymes involved in the fast transmission pathway get phosphorylated or dephosphorylated, and that determines the intensity of the response—of the fast response.

For example, phosphorylating a receptor can make it more sensitive or less sensitive to the neurotransmitter. Say, phosphorylating a glutamate receptor will alter its responsivity to glutamate, and, therefore, permit or inhibit transmission at that synapse.

EMA: Right.

PG: So, since it's modulating, modifying, fast transmission, the speed doesn't need to be as fast.

It was a problem that puzzled me for quite a while, exactly the question you asked, was: "How can this all happen in these cells?" And that's what people kept saying in meetings. "How do you explain that?"

EMA: How did they end up finally being convinced?

PG: I think it was just the sheer amount of evidence.

Somewhere in the early '80s people began to believe this story, because we had just such a fantastic array of data. I mean, you could put a neurotransmitter on a nerve cell preparation, get a rise in cyclic AMP; then you could add cyclic AMP and *mimic* the neurotransmitter—that was a pretty big breakthrough.

Cyclic GMP was another second messenger; we showed where certain neurotransmitters raise the level of cyclic GMP, and cyclic GMP mimicked those neurotransmitters in producing an effect on the target cells.

And then we went downstream from there.

At the time that we discovered the cyclic GMP–dependent protein kinase, people thought this was an artifact; it was really cyclic AMP–dependent protein kinase. They said cyclic GMP was a poor mimic of it. But we found preparations where there was no cyclic AMP, and the neurotransmitter raised cyclic GMP, and cyclic GMP activated cyclic GMP–dependent protein kinase. We discovered soluble guanylate cyclase and that it activated cyclic GMP–dependent protein kinase.

In the case of calcium, we discovered the calcium kinases: Our lab were the ones that discovered these Ca^{2+}-calmodulin–dependent protein kinases, which is the major way through which calcium works, CaM kinase I and CaM kinase II, and so on.

To go back to your question: The evidence was so *overwhelming*, that people went from "this can't be right" to "of course."

Now, in fairness to my colleagues, I found myself doing the same thing with new discoveries.

I think as a mental framework, especially in a field like the brain—the complexity is so overwhelming—that we all should be so humble about it, and not many people are. I'd like to think that I'm still very humble. I think we know one-zillionth of one percent of what's to be known.

EMA: Did you have a favorite project, of all of the research that you did?

PG: I'm very excited now about the work we're doing on major depressive disorder.

So, I guess my favorite project is that, and that's still very much ongoing, but I know that many old people are very excited about what they're doing, when I'm not so excited about what they're doing; I'm more excited about the things that they did earlier. I hope I have not reached that level of dotage yet.

EMA: [*laughs*] Tell me about what you're doing with depression.

PG: Well, we did studies looking for endogenous proteins that might control depression in the brain.

There are two people—very talented people—named Marc Flajolet and Per Svenningsson, and they, using yeast two-hybrid technology, took serotonin receptors and incubated them to see if anything might bind to them. They found this protein called p11 bound.

p11 was known—not in the brain, there was no evidence it played any role in the brain—but in the periphery it was shown to regulate certain signaling pathways.

p11 is a binding partner of a protein called Annexin A2: They form a heterotetramer, a dimer of p11 and two monomers of Annexin A2.

And what we've found over the intervening years is that—or what *they* found—if you gave an animal an antidepressant, the level of p11 went up. If you knock out p11 genetically, the animals are depressed; if you restore p11 to those animals, the animals behave in a normal way again.

EMA: This is a mouse model?

PG: Yeah, in mice. And then in postmortem brain tissue, looking at people who had suffered from major depressive disorder, the level of p11 was lower in the depressed people.

All along, we've been able to validate in human brain tissue the things we've been finding in the mouse, to a remarkable extent.

EMA: What made them start out looking with the serotonin receptor?

PG: The reason is that the major antidepressants in use today, by far, are the SSRIs—they're serotonin reuptake inhibitors.

So we, along with everybody else, believed, and still believe, that the serotonin pathway of synaptic transmission plays a major role in whether we're depressed or not.

So the idea was that, if serotonin signaling is so important, maybe there's an endogenous protein in the brain which modifies the performance of serotonin receptors, maybe by affecting its location or its sensitivity or something like that. Which has turned out to be the case.

There are two major things we know about p11 so far.

One is, when you put serotonin onto a cell, the level of p11 goes way up. And the p11 *recruits* serotonin receptors to the plasma membrane. We're trying to determine whether it's actually a recruitment of the receptors to the membrane or an inhibition of endocytosis.

EMA: Right.

PG: I actually think it's likely to be an inhibition of endocytosis.

EMA: Huh.

PG: Just because we've found now many different types of receptors, the endocytosis of which is regulated by p11. So it seems, then, that serotonin, through a series of signaling steps, raises p11—we're still working on how that happens.

EMA: Right.

PG: And that raised p11 then causes an increased localization of the receptors at the plasma membrane, which would increase serotonergic signaling.

In other words, it's kind of like, here's the serotonin pathway saying "I'm trying to get through to you" and so the cells, the responsive cells, are *helping* it by putting more detectors on the membrane.

EA: It's kind of interesting. Because serotonin signaling is making serotonin signaling more effective . . .

PG: Right.

EA: . . . rather than a sort of negative feedback type of thing . . .

PG: You know, I'm a big fan of negative feedback; but this is positive feedback.

And then we found that p11—in conjunction with the Annexin A2 and the formation of this heterotetramer—this heterotetramer, we looked for binding partners for it, and found a bunch of them, five in particular, that look very interesting.

Some of them we now know are transcription factors. For example, there's one called SMARCA3. This is a chromatin-remodeling factor. And what it does is, when the p11–Annexin A2 complex sees SMARCA3 it forms a—actually, instead of a heterotetramer, it forms a heterohexamer—where two molecules of SMARCA3 are added: There are two p11s, two Annexin A2s, two SMARCA3s. This heterohexamer causes a relocation of the p11 and Annexin A2 to the chromatin, to the DNA.

We know that, if you knock that SMARCA3 out, you don't get the antidepressant response. So, if you knock out p11, or Annexin A2, or SMARCA3, you lose the antidepressant effect. You still get some other effects that are presumably not related to depression.

And now we're trying to find out, by looking at the myriad of genes which are controlled by SMARCA3, which ones are involved in mediating the responses to the antidepressant. So we know the antidepressant causes the p11 to go up, which causes it to bind to the Annexin A2, and that heterotetramer then binds to the SMARCA3, and that complex then binds to the DNA and causes alterations in gene transcription. We're now going through to see which genes are transcribed, and hopefully get gradually to understanding the biochemical basis by which antidepressants work and by which we become depressed.

So that's why I'm excited.

EMA: Oh, sure.

So, leaving depression aside, you and your wife used your Nobel Prize winnings to set up an award for women scientists [*note*: The Pearl Meister Greengard Prize].

PG: Yes.

EMA: Can you tell me what motivated you to do that?

PG: Well, my mother died giving birth to me, and so I never knew her. And when I got the Nobel Prize, I thought it would be nice to do something in honor of my mother for women, and I thought about various options. And the one that seemed to be the most attractive was to have a prize for outstanding women in biomedical research.

And we had talked about it on and off, but I didn't come to any decision before getting the Nobel Prize. And then, the day I got it, all the press were here, CBS, NBC, ABC, and we were walking from my office over to the auditorium for the ceremony, and I suddenly had that idea.

I said, "What would you think if we spend the money on that?" and she loved the idea; she was very supportive.

EMA: Did your mother have any interest in research?

PG: No. I mean, people didn't in those days . . . it was like, did my mother have any interest in walking on the moon? It was not something you thought about. I mean, she was—from what I heard, a brilliant woman—and she had a job as a secretary, which was practically all women could do in those days. You could be a teacher or you could be a secretary—you know, a respectable girl. She had a job as a secretary until she and my father got married. So I don't know whether she . . . I think she probably would be very pleased with it. She'd probably be happy her little boy won a Nobel Prize.

EMA: I'm sure! Is there anything else that it hasn't occurred to me to ask you that you'd like to have recorded?

PG: I can't think of anything else; but this has been very nice talking to you.

EMA: It's been really nice talking to you, too. Thank you.

ARTICLES CITED AND SUGGESTIONS FOR FURTHER READING

Greengard, P., F. Brink Jr., and S.P. Colowick. 1954. Some relationships between action potential, oxygen consumption and coenzyme content in degenerating peripheral axons. *J. Cell. Physiol.* 44:395–420. http://dx.doi.org/10.1002/jcp.1030440304

Sutherland, E.W. 1972. Studies on the mechanism of hormone action. *Science*. 177:401–408. http://dx.doi.org/10.1126/science.177.4047.401

Krebs, E.G. 1993. Nobel Lecture. Protein phosphorylation and cellular regulation I. *Biosci. Rep.* 13:127–142.

Miyamoto, E., J.F. Kuo, and P. Greengard. 1969. Adenosine 3′,5′-monophosphate-dependent protein kinase from brain. *Science*. 165:63–65. http://dx.doi.org/10.1126/science.165.3888.63

Kuo, J.F., and P. Greengard. 1970. Cyclic nucleotide-dependent protein kinases. VI. Isolation and partial purification of a protein kinase activated by guanosine 3′,5′-monophosphate. *J. Biol. Chem.* 245:2493–2498.

McAfee, D.A., M. Schorderet, and P. Greengard. 1971. Adenosine 3′,5′-monophosphate in nervous tissue: Increase associated with synaptic transmission. *Science*. 171:1156–1158. http://dx.doi.org/10.1126/science.171.3976.1156

Kebabian, J.W., and P. Greengard. 1971. Dopamine-sensitive adenyl cyclase: Possible role in synaptic transmission. *Science*. 174:1346–1349. http://dx.doi.org/10.1126/science.174.4016.1346

Johnson, E.M., H. Maeno, and P. Greengard. 1971. Phosphorylation of endogenous protein of rat brain by cyclic adenosine 3′,5′-monophosphate-dependent protein kinase. *J. Biol. Chem.* 246:7731–7739.

Ueda, T., H. Maeno, and P. Greengard. 1973. Regulation of endogenous phosphorylation of specific proteins in synaptic membrane fractions from rat brain by adenosine 3′:5′-monophosphate. *J. Biol. Chem.* 248:8295–8305.

Miyamoto, E., G.L. Petzold, J.F. Kuo, and P. Greengard. 1973. Dissociation and activation of adenosine 3′,5′-monophosphate-dependent and guanosine 3′,5′-monophosphate-dependent protein kinases by cyclic nucleotides and by substrate proteins. *J. Biol. Chem.* 248:179–189.

Greengard, P. 1978. Phosphorylated proteins as physiological effectors. *Science*. 199:146–152. http://dx.doi.org/10.1126/science.22932

Schulman, H., and P. Greengard. 1978. Stimulation of brain membrane protein phosphorylation by calcium and an endogenous heat-stable protein. *Nature*. 271:478–479. http://dx.doi.org/10.1038/271478a0

Walter, U., S.M. Lohmann, W. Sieghart, and P. Greengard. 1979. Identification of the cyclic AMP-dependent protein kinase responsible for endogenous phosphorylation of substrate proteins in synaptic membrane fraction from rat brain. *J. Biol. Chem.* 254:12235–12239.

Kennedy, M.B., and P. Greengard. 1981. Two calcium/calmodulin-dependent protein kinases, which are highly concentrated in brain, phosphorylate protein I at distinct sites. *Proc. Natl. Acad. Sci. USA*. 78:1293–1297. http://dx.doi.org/10.1073/pnas.78.2.1293

Palfrey, H.C., W. Schiebler, and P. Greengard. 1982. A major calmodulin-binding protein common to various vertebrate tissues. *Proc. Natl. Acad. Sci. USA*. 79:3780–3784. http://dx.doi.org/10.1073/pnas.79.12.3780

Walaas, S.I., D.W. Aswad, and P. Greengard. 1983. A dopamine- and cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions. *Nature*. 301:69–71. http://dx.doi.org/10.1038/301069a0

Kennedy, M.B., T. McGuinness, and P. Greengard. 1983. A calcium/calmodulin-dependent protein kinase from mammalian brain that phosphorylates Synapsin I: Partial purification and characterization. *J. Neurosci.* 3:818–831.

De Camilli, P., R. Cameron, and P. Greengard. 1983. Synapsin I (protein I), a nerve terminal-specific phosphoprotein. I. Its general distribution in synapses of the central and peripheral nervous system demonstrated by immunofluorescence in frozen and plastic sections. *J. Cell Biol.* 96:1337–1354. http://dx.doi.org/10.1083/jcb.96.5.1337

De Camilli, P., S.M. Harris Jr., W.B. Huttner, and P. Greengard. 1983. Synapsin I (Protein I), a nerve terminal–specific phosphoprotein. II. Its specific association with synaptic vesicles demonstrated by immunocytochemistry in agarose-embedded synaptosomes. *J. Cell Biol.* 96:1355–1373. http://dx.doi.org/10.1083/jcb.96.5.1355

Huttner, W.B., W. Schiebler, P. Greengard, and P. De Camilli. 1983. Synapsin I (protein I), a nerve terminal–specific phosphoprotein. III. Its association with synaptic vesicles studied in a highly purified synaptic vesicle preparation. *J. Cell Biol.* 96:1374–1388. http://dx.doi.org/10.1083/jcb.96.5.1374

Navone, F., P. Greengard, and P. De Camilli. 1984. Synapsin I in nerve terminals: selective association with small synaptic vesicles. *Science*. 226:1209–1211. http://dx.doi.org/10.1126/science.6438799

Greengard, P., J. Jen, A.C. Nairn, and C.F. Stevens. 1991. Enhancement of the glutamate response by cAMP-dependent protein kinase in hippocampal neurons. *Science*. 253:1135–1138. http://dx.doi.org/10.1126/science.1716001

Greengard, P. 2001. The neurobiology of slow synaptic transmission. *Science*. 294:1024–1030. http://dx.doi.org/10.1126/science.294.5544.1024

Svenningsson, P., K. Chergui, I. Rachleff, M. Flajolet, X. Zhang, M. El Yacoubi, J.M. Vaugeois, G.G. Nomikos, and P. Greengard. 2006. Alterations in 5-HT1B receptor function by p11 in depression-like states. *Science*. 311:77–80. http://dx.doi.org/10.1126/science.1117571

Warner-Schmidt, J.L., M. Flajolet, A. Maller, E.Y. Chen, H. Qi, P. Svenningsson, and P. Greengard. 2009. Role of p11 in cellular and behavioral effects of 5-HT4 receptor stimulation. *J. Neurosci.* 29:1937–1946. http://dx.doi.org/10.1523/JNEUROSCI.5343-08.2009

Alexander, B., J. Warner-Schmidt, T. Eriksson, C. Tamminga, M. Arango-Lievano, S. Ghose, M. Vernov, M. Stavarache, S. Musatov, M. Flajolet, et al. 2010. Reversal of depressed behaviors in mice by p11 gene therapy in the nucleus accumbens. *Sci. Transl. Med.* 2:54ra76. http://dx.doi.org/10.1126/scitranslmed.3001079

Oh, Y.S., P. Gao, K.W. Lee, I. Ceglia, J.S. Seo, X. Zhang, J.H. Ahn, B.T. Chait, D.J. Patel, Y. Kim, and P. Greengard. 2013. SMARCA3, a chromatin-remodeling factor, is required for p11-dependent antidepressant action. *Cell*. 152:831–843. http://dx.doi.org/10.1016/j.cell.2013.01.014

Svenningsson, P., Y. Kim, J. Warner-Schmidt, Y.S. Oh, and P. Greengard. 2013. p11 and its role in depression and therapeutic responses to antidepressants. *Nat. Rev. Neurosci.* 14:673–680. http://dx.doi.org/10.1038/nrn3564