Meet the Champions

Pancreatology

Pancreatology 2009;9:323–326
DOI: 10.1159/000213711

Published online: May 18, 2009

'Now We Have to Use the Skills We Have Developed in Cell Physiological Studies to Attack the Most Crucial Problems in Pancreatic Pathology'

An Interview with Ole H. Petersen, Medical Research Council Professor of Physiology, University of Liverpool, UK

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Abstract

Dr. Ole Petersen is one of the world's leading physiologists working on signal-transduction mechanisms in pancreatic acinar cells. He discovered local apical Ca^{2+} signals as important regulators of acinar secretion. His work has been widely recognized, most importantly by his election as Fellow of The Royal Society in 2000 and more recently last year, when Queen Elizabeth II appointed him Commander of the Order of the British Empire (CBE) for 'Services to Science'. In this interview for *Pancreatology*, Dr. Petersen shares his life experiences as an innovative investigator of exocrine pancreatic function.

Prof. Ole H. Petersen

M.E.F.-Z.: What initiated you to work in pancreas research in the first place?

O.H.P.: I started research on salivary gland electrophysiology when I was a medical student in Copenhagen and actually published a series of papers on this topic in *Acta Physiologica Scandinavica* (now *Acta Physiologica*) before graduating. I continued this research when, after my final examinations in 1969, I was appointed Assistant Professor of Physiology at the University of Copenhagen. Before that, I had been invited to give a lecture at an international symposium on exocrine glands held in 1968 at The University of Pennsylvania. The organizers undoubtedly did not realize that I was still an undergraduate student! At this conference I met Arnold Burgen, Chairman of Pharmacology at the University of Cambridge in England. Arnold was the leading scientist working on secretion mechanisms in salivary glands and he kindly invited me to come to Cambridge to work with him. However, when I finally arrived in Cambridge in the autumn of 1971, it turned out to be too late. Arnold had

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just been appointed Director of the Medical Research Council's National Institute of Medical Research at Mill Hill and had moved into a new research field. He therefore suggested that it might be best for me to work with Keith Matthews in the Cambridge Pharmacology Department who, although principally interested in endocrine secretion, had started work on the electrophysiology of pancreatic acinar cells. I was keen to expand my knowledge of exocrine glands and because Keith's principal interest was in the insulin-secreting pancreatic islet cells, it was agreed that I could 'take over' the exocrine pancreas. I completed several studies in Cambridge, but by far the best known paper from that period is one that came about as a result of my collaboration with John A. Williams, who – like me – was a visiting research fellow in Keith's laboratory. This work, published in the *Journal of Physiology* in 1973 was the first in which electrophysiology, Ca^{2+} signaling and amylase secretion from pancreatic acinar cells were correlated. After my return to Copenhagen I decided to continue work on the exocrine pancreas and this focus was maintained during my years as Chair of Physiology at the University of Dundee in Scotland and subsequently as the successor of Rod Gregory, who isolated and sequenced gastrin, in the George Holt Chair of Physiology at the University of Liverpool.

M.E.F.-Z.: You have pioneered pancreas research in so many directions. At the end of the day, what has given you the most personal satisfaction?

O.H.P.: I would like to mention two different studies. The first concerns the discovery together with Yoshio Maruyama of Ca^{2+}-activated ion channels in pancreatic acinar cells in the early 1980s and the second is the more recent unraveling of the mechanisms responsible for the polarized Ca^{2+} signaling in these cells.

In 1980, Erwin Neher and Bert Sakmann (Physiology/Medicine Nobel Prize 1991) invented the high-resolution patch-clamp technique, which for the first time allowed the very tiny currents that flow through single ion channels in the plasma membrane to be recorded. This revolutionized electrophysiology and caused a dramatic revival of interest in ion channel physiology and pharmacology. It was my good luck to have been invited by Erwin to give a seminar at the Max Planck Institute for Biophysical Chemistry in Göttingen in the autumn of 1980, where I was generously given access to the still unpublished data and techniques concerning the patch-clamp technique. I had the further luck to have an exceptionally talented and able post-doctoral fellow in my laboratory at that time (Yoshio Maruyama – now Professor of Physiology at Tohoku University School of Medicine in Sendai, Japan), who personally constructed our first patch-clamp amplifier (such amplifiers only became commercially available later). We were therefore able already in 1982 to publish in *Nature* our first two papers on Ca^{2+} activation of single-channel currents recorded in pancreatic acinar cells. These were in fact the very first single-channel current studies of epithelial cells. Combining results from isolated patches of membrane with those from intact cells, we showed that the actions of ACh and CCK on pancreatic acinar cells are mediated by intracellular messengers and that the final messenger is Ca^{2+}. We published three further *Nature* papers on this topic in 1983/84 and in the last of these articles we presented the first coherent model for Ca^{2+}-mediated regulation of acinar fluid secretion in exocrine glands [Calcium-activated potassium channels and their role in secretion. *Nature* 1984;307:693–696]. This paper became an ISI Citation Classic in 1993. It is still to this day my most highly cited paper.

At almost exactly the same time, Irene Schulz – one of the most distinguished physiologists working on the exocrine pancreas – discovered the function of inositol 1,4,5-trisphosphate (IP_3) as an intracellular messenger releasing Ca^{2+} from intracellular stores in permeabilized pancreatic acinar cells. Her paper, published in *Nature* in 1983, caused a sensation and was very quickly followed up by numerous reports of studies on other cell types confirming the discovery of a new and extremely important intracellular messenger – perhaps even more important than cyclic AMP.

Physiological Ca^{2+} signals do not occur as a sustained elevation of the intracellular Ca^{2+} concentration, but rather as trains of discrete short-lasting Ca^{2+} spikes. This was explained by the hypothesis that sustained stimulation with hormones or neurotransmitters would generate pulsatile IP_3 production; each pulse causing a single Ca^{2+} spike. We tested this hypothesis in experiments on internally perfused single cells, but found that a steady intracellular infusion of IP_3 generated repetitive cytosolic Ca^{2+} spikes [Pulsatile intracellular calcium release does not depend on fluctuations in inositol trisphosphate concentration. *Nature* 1989;339:317–320]. This paper was initially controversial because it went against what was at that time the favored hypothesis, but our principal conclusion, that IP_3 oscillations are not required for Ca^{2+} oscillations, has been confirmed by Akihiko Tanimura and collaborators, in a paper published in March this year in the *Journal of Biological Chemistry*. By combining measurements of the intracellular IP_3 and Ca^{2+} concentrations, they showed that a constantly elevated intracellular IP_3 concentration can elicit repetitive Ca^{2+} spikes.

At a meeting in Göttingen, shortly after the publication of our 1989 *Nature* paper, I was told that there was

something wrong with our experiments, in which we had recorded the Ca^{2+} spikes electrophysiologically by monitoring the Ca^{2+}-activated Cl^- current. The Göttingen colleagues told me that they had done similar experiments, but had used a Ca^{2+}-sensitive fluorescent probe to monitor the cytosolic Ca^{2+} concentration and had found that intracellular infusion of IP_3 did not evoke any changes in this parameter! The only way to resolve this controversy was to simultaneously record the Ca^{2+}-activated Cl^- current and the cytosolic Ca^{2+} concentration with a fluorescent probe. This was not a trivial task at that time, but we managed to do these difficult experiments, which turned out to be extremely interesting.

In our initial experiments, using an intracellular fluorescent Ca^{2+} probe, IP_3 infusion actually failed to elicit Ca^{2+} spike responses, but we noticed that in this case the Ca^{2+}-activated Cl^- currents had also gone. The Ca^{2+}-sensitive probe, which by definition is also a Ca^{2+}-binding agent, had buffered the cytosolic Ca^{2+} concentration so well that no fluctuations could be observed. This was a clear demonstration of a frequently encountered general problem with scientific measurements, namely that the act of measuring a particular parameter interferes with this very parameter. However, by 'trial and error' we refined the measuring conditions by reducing the concentration of the Ca^{2+}-sensitive probe, thereby diminishing the Ca^{2+} buffer capacity, but of course taking care not to reduce the probe concentration so much that it became impossible to record signals. By finding a reasonable compromise, we were finally able to observe Ca^{2+} oscillations in response to stimulation with ACh and CCK as well as intracellular IP_3 infusion.

The surprising result of this work was that at low levels of stimulation, resembling physiological conditions, we could only observe Ca^{2+} oscillations electrophysiologically, but not with the fluorescent probe. However, at high stimulation intensities both types of measurements gave exactly the same result, reporting a sustained elevation of the Ca^{2+} concentration. We reasoned – correctly as it turned out – that low level stimulation generated Ca^{2+} spikes close to a region of the plasma membrane where Ca^{2+}-activated Cl^- channels are clustered, that is too small to make an impact on the overall intracellular Ca^{2+} level, whereas high intensities of stimulation caused global elevations of the cytosolic Ca^{2+} concentration. Thus the important concept of local and global Ca^{2+} signaling was borne and we published this work in the *EMBO Journal* early in 1990.

A completely convincing demonstration of local and global Ca^{2+} oscillations could only be made when high-resolution digital imaging enabled us to visualize these phenomena. This development enabled us to show directly that physiological stimulation generates repetitive local Ca^{2+} spikes that are mostly confined to the granule-containing apical region of the pancreatic acinar cells, whereas hyperstimulation evokes Ca^{2+} waves starting in the apical pole, which then spread to the base. Our paper reporting these data was published in *Cell* in 1993 and has become one of my most highly cited articles [Local and global Ca^{2+} oscillations in exocrine cells evoked by agonists and inositol trisphosphate. *Cell* 1993;74:661–668]. In 1999, in a paper published in the *EMBO Journal*, we were able to explain the mechanism that normally prevents spreading of the local apical Ca^{2+} spikes. This is due to a dense mitochondrial belt, acting as a Ca^{2+} buffer barrier, which separates the granule-containing part of the acinar cell from the basolateral region containing the nucleus.

Finally, there was another controversy in which I became involved. The importance for human biology and medicine of the data we had obtained concerning CCK-elicited Ca^{2+} signaling was questioned because of a study indicating that human pancreatic acinar cells, in contrast to acinar cells from mice, rats and guinea pigs, do not possess functional CCK receptors. However, isolation of fully functional live pancreatic acinar cells, from tissue obtained during surgery, is inherently difficult. The leakage of powerful proteases from a few damaged cells can be a very serious problem. Negative results are therefore not necessarily conclusive. In close collaboration with our surgical colleagues in Liverpool, we managed – over time – to build up considerable material based on well-functioning isolated human pancreatic acinar cells. Our work, published in *Gastroenterology* in 2008, showed clearly that low picomolar concentrations of CCK8 – as well as the probably more physiological CCK58, which we obtained from Joe Reeve at UCLA – evoked oscillatory Ca^{2+} signals, stimulated mitochondrial metabolism and elicited amylase secretion. Fortunately, for us and other pancreatologists, the acinar cells from man are not fundamentally different from those of other species!

M.E.F.-Z.: Based on your experience as mentee and mentor, can you comment on the value of mentorship for the development of a new investigator?

O.H.P.: The ideal mentor has to be both encouraging and critical and the balancing act is delicate! When I started out in research as an undergraduate medical student at Copenhagen University, the world of science was rather different from now. I had no research supervisor, but worked independently from day one and generated my own research project. I did nevertheless have good advice and support from two senior investigators, who worked in different fields from mine. Christian Crone was a very dis-

tinguished capillary physiologist, strongly rooted in quantitative biophysics and immensely critical. There was little encouragement from him, but a lot of very good criticism. Niels Thorn, an internationally known investigator of the function of the neurohypophysis, on the other hand, was immensely encouraging and also provided crucial practical advice with regard to grant applications and paper writing. I shall always remain grateful to these two men, who were under no obligation whatsoever to help me.

As mentor, I have tried to combine critical and encouraging attitudes. To abstain from serious criticism, as is common with the current overemphasis on encouragement, is in my opinion to let down a young emerging investigator. On the other hand, it is also important for the mentor not to be too intrusive. We learn most from our own errors and the current tendency to, for example, 'over-supervise' PhD students may be effective with regard to producing papers, but is not necessarily the best way to allow independent development.

M.E.F.-Z.: What is the best advice you have received during your career? What is your advice to the young investigators that are beginning in the field of pancreas research?

O.H.P.: Niels Thorn advised me to find my own niche and be patient. I had originally intended to study kidney cell electrophysiology, but Niels pointed out to me that there was strong competition in that field and that it might be better to choose a less crowded field, for example salivary glands. This approach may not always be the best, since a field that is too esoteric and in which very few people work may not allow you to grow sufficiently. I was lucky that various developments meant that a very small and rather uncompetitive sub-field later became an intensely competitive area of cell biology. This could not have been foreseen in the mid 1960s. You have to follow your intuition, but you do need to find an area where you have clear ideas about how to advance the field. When you have found your own niche, you need to focus on solving some outstanding problems and stay with them until you have been successful. I am always reminded of a scene in some unimportant B-film, the title of which escapes me, in which a police officer admits that he and his colleagues are perhaps not very clever, but they never give up! As a scientist, I have often felt that way. Progress is painfully slow. In my own case, it took about 30 years from the start of my Ca^{2+} signaling investigations of pancreatic acinar cells in Cambridge until the work in Liverpool finally allowed us to provide a coherent and detailed explanation for the control of Ca^{2+} signal generation, and therefore regulation of secretion, in pancreatic acinar cells.

M.E.F.-Z.: What do you think are the big questions that need to be answered in pancreatology?

O.H.P.: We are now into the age of serious pathophysiology. Of course we do not fully understand all aspects of normal pancreatic physiology, but the law of 'diminishing returns' is already beginning to set in. Now we have to use the skills we have developed in cell physiological studies to attack the most crucial problems in pancreatic pathology. For me the critical question is: How do the insults that initiate acute pancreatitis activate intracellular trypsin? We, and many others, have of course already been working on this problem for many years, but it is only recently that new and apparently fruitful investigative paths have opened up. We already know that trypsin activation is a Ca^{2+}-dependent process and that it is initiated by excessive global and sustained cytosolic Ca^{2+} elevations, in contrast to the physiologically important local Ca^{2+} spiking events. In the case of alcohol-related pancreatitis, we also know that the critical agents inducing these toxic signals are fatty acid ethyl esters together with fatty acids. Our 2006 paper in *Gastroenterology* reported some of these developments. Furthermore, our 2007 *PNAS* paper showed that the critical trypsin activation occurs in post-exocytotic, endocytic vacuoles. However, we still do not know what links critical release of Ca^{2+} from particular intracellular organelles to the often fatal trypsin activation.

M.E.F.-Z.: What do you think is the major need that a journal like *Pancreatology* should fill?

O.H.P.: *Pancreatology*, as well as other good scientific journals, is in the business of disseminating new and important information as quickly and effectively as possible. It is important for the field of pancreatic research that the journal is able to create excitement about major new progress and that it provides a valid and helpful general perspective. I think *Pancreatology* is doing a good job in these respects. As for all ambitious journals, critical peer review is essential; both for the sake of the readership and the authors. However, although peer review is the least bad method we know for research evaluation, it is not a good method! In this increasingly competitive world, some referees cannot resist the temptation to further their own interests. They ask themselves not whether a particular paper is good or bad, but whether publication is in their own interest! Our 'system' ultimately works, because there are so many journals competing for the best papers. A certain self-interested 'regulation' of reviewers by journal editors also helps. Nevertheless, we have to be aware that our publication system is beginning to 'creak' and is somewhat wasteful in time, since so many papers have to be rewritten and resubmitted repeatedly.