SIR MICHAEL JOHN BERRIDGE

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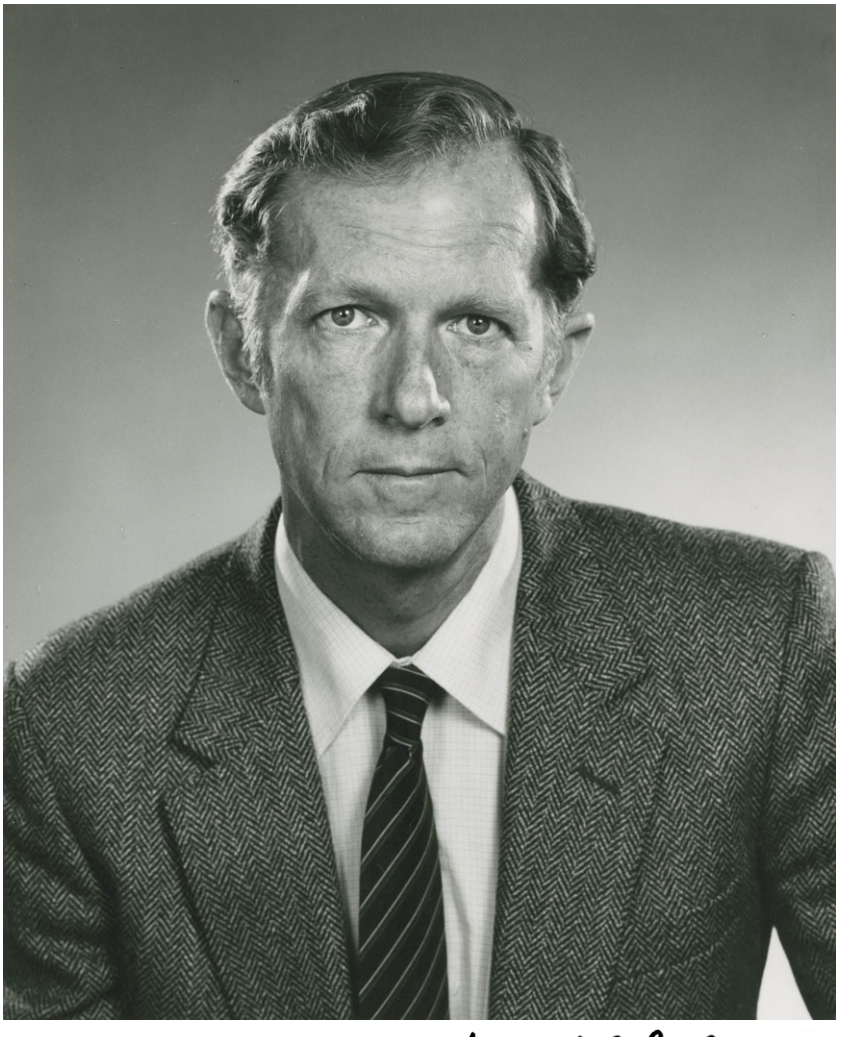

Michael J. Bowdy

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Elected FRS 1984

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Sir Michael Berridge was a giant in the fields of physiology and biochemistry who, by his discovery in 1983 of inositol 1,4,5-trisphosphate (IP_3) as the ubiquitous intracellular messenger releasing Ca^{2+} from intracellular stores, revolutionized our concepts of signal transduction mechanisms. Mike, as he was universally known, discovered one of the most important regulatory mechanisms in animal cells, involved in the control of virtually all bodily functions, including secretion, contraction and memory. Mike, working at the Department of Zoology at the University of Cambridge, personally identified IP_3 as one of the two initial products of hormone- or neurotransmitter-elicited inositol phospholipid breakdown and then, in collaboration with the research group led by Irene Schulz at the Max Planck Institute for Biophysics in Frankfurt, demonstrated directly the Ca^{2+}-releasing effect of IP_3 in pancreatic acinar cells. Subsequently, in collaborations with research groups in Switzerland and the USA, Mike confirmed the Ca^{2+}-releasing action of IP_3 in insulin-secreting cells, hepatocytes and photoreceptors. The IP_3–Ca^{2+}-releasing pathway became accepted amazingly quickly as one of the key elements of cellular signal transduction mechanisms and is now featured in virtually all textbooks of physiology and biochemistry. Mike was also a great synthesizer, who created an entirely new field, namely calcium signalling. Through his numerous, highly cited review articles, and his insightful keynote lectures at the most important biomedical congresses, he dominated this large and increasingly important research field for more than 30 years following his momentous 1983 discovery.

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Figure 1. Mike dissecting *Xenopus* with his teacher Pamela Bates at Jameson High School in Gatooma, Rhodesia (now Kadoma, Zimbabwe). (From the Berridge family collection.)

Early life and education

Mike was born on 22 October 1938 in Gatooma, a small town in South Rhodesia (now Kadoma, Zimbabwe) to George Kirton Berridge and Stella Elaine Hards. He went to Jameson High School in Gatooma, where he was a keen cricket player. In his autobiographical article published in *Annual Review of Physiology* (37),* he described his childhood love of nature and wildlife, elephants in particular, which stayed with him for his whole life, later resulting in numerous trips from England to Zimbabwe, including many safaris. Mike also developed an early interest in biology, inspired by an enthusiastic teacher, Miss Pamela Bates (figure 1), who encouraged him to pursue a scientific career. She managed to persuade his non-academic parents, who were initially unenthusiastic about Mike going to university, about the merits of a university education (37). He read zoology and chemistry at the newly founded University College of Rhodesia and Nyasaland (now University of Zimbabwe) in Salisbury (now Harare) and received his BSc (first class honours) in 1960. His initial interests were focused on big game ecology, but lectures by Dr Eina Bursell gradually shifted his interests towards physiology and he spent one of his vacations helping Dr Bursell with his research on water metabolism in tsetse flies. This became his entry into the field of insect physiology, and he applied for, and obtained, a Commonwealth Scholarship to do a PhD in the Department of Zoology at the University of Cambridge, supervised by Vincent (later Sir Vincent) Wigglesworth FRS, who is regarded as the creator of the field of insect physiology. Mike's project dealt with the excretion and control of excretion of water, ions and nitrogen from a red bug, the African cotton stainer. He obtained his PhD in 1964 and the work was subsequently (1965, 1966) published as four single-author papers in the *Journal of Experimental Biology* (1–4).

* Numbers in this form refer to the bibliography at the end of the text.

Figure 2. Sue and Mike. Charlottesville, Virginia, 1965. (From the Berridge family collection.)

After the PhD, Mike took up a postdoctoral fellowship, funded by the National Science Foundation, with Dietrich Bodenstein in the Department of Biology at the University of Virginia in Charlottesville, USA and started working on salt and water transport in the rectal papillae of the blowfly. He married Susan Graham Winter in 1965 (figure 2). She became his life-long bedrock and bore him a son (Paul) and a daughter (Rozanne). Sue and Mike moved to Cleveland, Ohio in 1966, where Mike took up another postdoctoral fellowship with Bodil Schmidt-Nielsen at Case Western Reserve University. Schmidt-Nielsen, daughter of the Danish Physiologist and Nobel Laureate August Krogh ForMemRS, was one of the world's leading kidney physiologists, who, together with Karl Ullrich, had made landmark discoveries concerning the kidney's mechanism of concentrating urine in the loop of Henle. Mike's indirect connection with Ullrich (who became Director of the Max Planck Institute for Biophysics in Frankfurt) proved important many years later. In Cleveland, Mike continued and expanded the work on the mechanism of urine formation in the Malpighian tubules of the blowfly (*Calliphora*), which he had initiated in Charlottesville. This work resulted in two substantial papers on the roles of potassium and sodium as well as phosphate and chloride in supporting urine production (5, 7). Of great importance for his future work, Mike also started experimentation on fluid secretion from the salivary glands of the blowfly (6). This became his central activity after his return to Cambridge in 1969 to take up a permanent appointment in John Treherne's Agricultural and Food Research Council (AFRC) Unit of Insect Neurophysiology and Pharmacology in the Department of Zoology at the University of Cambridge. Mike continued to work in the Cambridge Zoology Department until his move to the Babraham Institute in 1990.

Early work on fluid secretion by the salivary glands of the blowfly

In his initial work on secretion from the blowfly salivary glands, Mike provided evidence for 5-hydroxytryptamine (5-HT) being the physiological agonist activating fluid formation (6). At the time, the only known intracellular transmitter (messenger) of hormone or neurotransmitter action was adenosine-3′,5′-monophosphate (cyclic AMP), which had been discovered by Earl Sutherland and Ted Rall at Case Western Reserve University in 1956. This discovery had resulted in the award of the Nobel Prize in Physiology or Medicine to Sutherland in 1971. Mike interacted with Rall during his stay in Cleveland and it was therefore natural that he would want to test the hypothesis that the 5-HT-elicited activation of secretion was mediated by intracellular cyclic AMP. This appeared to be the case as exogenous cyclic AMP could by itself elicit salivary secretion (6). Later, Mike obtained results with various manipulations of the cyclic AMP level in the salivary gland cells that were compatible with the view that 5-HT simply acted via formation of cyclic AMP inside the cells (8).

However, Mike soon had to revise his own simple and therefore attractive cyclic AMP hypothesis. Electrophysiological studies showed that cyclic AMP did not reproduce all the effects of 5-HT. In fact, 5-HT and cyclic AMP, surprisingly, produced changes of the transepithelial electrical potential that went in opposite directions. Mike presented these results at a Royal Society Discussion Meeting in London, held in December 1970, on ‘Active transport of salts and water in living tissues’. The meeting was organized by Richard Keynes FRS, a close collaborator of Alan Hodgkin FRS, who had just become President of the Royal Society a few days before the conference started. This may have facilitated the arrangement of the meeting, which took place during a very difficult period in the UK, with frequent power cuts. When this happened, the slide projector was connected to a battery, so that presentations could continue, but with very poorly illuminated figures on the screen.

At the time of the 1970 Royal Society Meeting, it was generally assumed that cyclic AMP was the one and only intracellular mediator (messenger) of the action of hormones or neurotransmitters. This was clearly also Mike’s view (9). The principal new finding he presented to the conference—namely that the electrophysiological changes evoked by 5-HT and cyclic AMP were different, in spite of the fact that both agents elicited fluid secretion—was interpreted as a sign that 5-HT in addition to stimulating the formation of cyclic AMP had a direct membrane action (9). In view of Mike’s later work, it may now seem surprising that Ca^{2+} is not even mentioned in his published report from the conference. The 1970 conference raised many critical questions for Mike. The discussions at this and many later conferences played an extraordinarily important role in developing and shaping Mike’s thinking as well as formulating increasingly fruitful hypotheses.

First focus on calcium signalling

Mike’s initial neglect of Ca^{2+} was soon rectified by the encounter with a visitor to Cambridge from the USA, namely Howard Rasmussen, who, in a highly cited review article (Rasmussen 1970), had proposed that cyclic AMP in part acted by increasing the concentration of Ca^{2+} in the cytosol ($[Ca^{2+}]_i$), either by release from internal stores or by opening Ca^{2+} pathways in the plasma membrane. Rasmussen was not the first to propose a key role for intracellular Ca^{2+}

in the control of secretion. Bill Douglas (FRS 1983), in his Gaddum Memorial Lecture to the British Pharmacological Society, had formulated the concept of stimulus–secretion coupling in analogy with excitation–contraction coupling. He had proposed that intracellular Ca^{2+} acted as a link between an external stimulus to a gland cell and the secretory process itself (Douglas 1968). Rasmussen introduced Mike to the Ca^{2+}-signalling literature, and his own particular concept (Rasmussen 1970) strongly influenced their first joint paper on the role of Ca^{2+} in controlling secretion from the *Calliphora* salivary glands (10). The key finding that 5-HT elicits a fast and marked increase in the release of $^{45}Ca^{2+}$ from the pre-labelled gland has stood the test of time. A very similar finding, also published in 1972, was made in a study of a mammalian salivary gland (cat submandibular gland), but in that case the stimulants were acetylcholine or adrenaline, rather than 5-HT (Nielsen & Petersen 1972). Mike and Rasmussen (10) suggested that it was cyclic AMP that caused the intracellular release of Ca^{2+}, in agreement with Rasmussen's general hypothesis (Rasmussen 1970). However, the following year, Mike's view changed owing to experiments conducted with the Ca^{2+} ionophore A23187. In this further study carried out in collaboration with Rasmussen (11), it was shown that the Ca^{2+} ionophore, increasing both Ca^{2+} influx and efflux, elicited fluid secretion from the *Calliphora* salivary gland, mimicking the action of 5-HT, but with the important exception that it did not stimulate cyclic AMP formation. Furthermore, the rate of fluid secretion that could be achieved by stimulation with the Ca^{2+} ionophore was less than that obtained with maximal 5-HT stimulation, indicating that in addition to Ca^{2+}, cyclic AMP most likely did play a role in stimulating secretion.

Three years later, at the 1973 Alfred Benzon Symposium on 'Secretory mechanisms of exocrine glands' held in Copenhagen at the Royal Danish Academy of Sciences and Letters, it became clear that Mike had made remarkable progress and had discovered an important messenger role for Ca^{2+} (figure 3). He reported that in the *Calliphora* salivary glands, 5-HT stimulates fluid secretion via two intracellular messengers, cyclic AMP and Ca^{2+} (12). An increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) opens Cl^- channels in both the basal and apical membranes, whereas an increase in the cyclic AMP concentration activates a K^+ pump in the apical membrane (figure 3). It later became clear that 5-HT activates two separate membrane receptors (16). The 5-HT_1 receptor mediates the rise in $[Ca^{2+}]_i$, whereas the 5-HT_2 receptor activates adenylate cyclase, generating cyclic AMP. Mike's talk in Copenhagen, in the autumn of 1973, made a deep impression and was praised by Bill Douglas in the discussion immediately following Mike's presentation: 'Once again we have had a beautiful illustration of a very efficatious combination in biology: the combination of a simple preparation and a sophisticated investigator. That was a very beautiful, pellucid presentation.' (12). Although the evidence for a messenger role for Ca^{2+} was now clear, there was no clarity at all with regard to the mechanism that would link receptor activation to the release of Ca^{2+} from internal stores or opening of Ca^{2+} channels in the plasma membrane. The search for this link would be a constant theme at conferences dealing with cellular control mechanisms for the next 10 years.

A model for insect salivary gland fluid secretion

In 1975, Mike and his collaborators published an important paper in the *Journal of Physiology* (13) in which they characterized and quantified the ionic conductance changes in response to 5-HT stimulation occurring at the basal and apical membranes of the secretory tubule of the

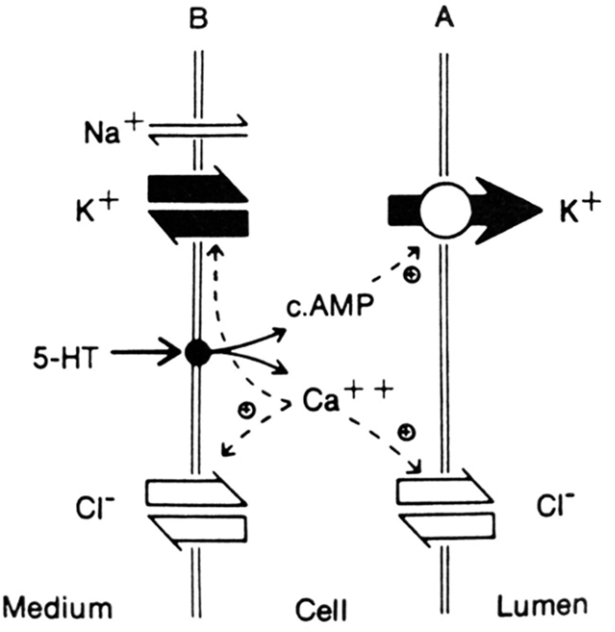

Figure 3. Mike's 1973 model showing the role of Ca^{2+} and cyclic AMP in the action of 5-HT on fly salivary gland cells. B, basal membrane; A, apical membrane. For further explanation, see text. (From: Berridge *et al*. (12), used with permission from Elsevier.)

Calliphora salivary gland. They presented evidence for an increase in Cl^- conductance across both the basal and apical membranes, which had been predicted by Mike earlier (figure 3). Mike's 1975 model of how 5-HT stimulates fluid secretion in the insect salivary glands (13) was the first complete account of the mechanisms underlying exocrine fluid secretion and its control. The most important finding was the demonstration of the 5-HT-induced, and Ca^{2+}-mediated, increase in the Cl^- conductance of the apical (lumenal) membrane. In later models, Mike avoided any mention of the Cl^- conductance change in the basal membrane (37). The specific location of this channel in the apical membrane became a key feature of all models of exocrine gland fluid secretion, although it took more than twenty-five years before it became technically possible to prove this directly by combining patch-clamp whole-cell recording of Cl^- current with local uncaging of intracellularly caged Ca^{2+} (Park *et al.* 2001).

Direct and indirect assessments of changes in the cytosolic Ca^{2+} concentration: discovery of agonist-elicited Ca^{2+} oscillations

Douglas's stimulus–secretion coupling concept (Douglas 1968) stated that stimulation of a gland cell elicits a rise in $[Ca^{2+}]_i$ and that it is this rise that initiates secretion. However, until 1980, there was no information about the level of the resting $[Ca^{2+}]_i$, nor of the magnitude of the rise in $[Ca^{2+}]_i$ upon receptor activation. Mike was the first to report direct measurements of $[Ca^{2+}]_i$ in gland cells, and he published these data in 1980 in the very first volume of *Cell*

Calcium (15). Mike's paper had the modest title 'Preliminary measurements of intracellular calcium in an insect salivary gland using a calcium-sensitive microelectrode', but the data have stood the test of time. Mike reported that the resting level of $[Ca^{2+}]_i$ was *ca* 0.1 μM or less and showed that 5-HT increased this to more than 1 μM. Two years later Roger Tsien (ForMemRS 2006), Tullio Pozzan (ForMemRS 2020) and Tim Rink, using Tsien's first-generation Ca^{2+}-sensitive fluorescent probe, quin-2, reported that the resting level of $[Ca^{2+}]_i$ in lymphocytes was *ca* 0.1 μM and that lectins could double this concentration (Tsien *et al*. 1982). Furthermore, an electrophysiological approach, using the Ca^{2+}-activated K^+ channel as an endogenous Ca^{2+} sensor (Petersen & Maruyama 1984), produced almost identical numbers to those reported by Mike (15).

It turned out to be difficult in practice to use ion-sensitive microelectrodes to routinely monitor changes in $[Ca^{2+}]_i$, so—in view of the early indications that Ca^{2+}-activated ion channels exist in the plasma membrane (9, 13), later confirmed directly (Petersen & Maruyama 1984; Park *et al*. 2001)—the idea of using membrane potential measurements as a means of assessing changes in $[Ca^{2+}]_i$ was attractive. Using this approach, in collaboration with Paul Rapp, Mike demonstrated agonist-elicited oscillations in the transepithelial electrical potential in the *Calliphora* salivary glands (17). This was the first, but indirect, evidence showing that at physiologically relevant agonist concentrations, the cellular response is not a sustained increase in the intracellular messenger concentration, but rather oscillations. Importantly, Mike showed that the frequency of these oscillations correlated with the physiologically relevant event, in this case fluid secretion, so that an increasing oscillation frequency was associated with increased secretion. In retrospect, it is surprising that this key paper, which essentially reported the first demonstration of agonist-elicited $[Ca^{2+}]_i$ oscillations in electrically non-excitable cells, made so little impact. Mike's article was so little known that when Ca^{2+} oscillations in electrically non-excitable cells were directly demonstrated in 1986, by Peter Cobbold and his collaborators, they were unaware of, and therefore did not cite, Mike's 1981 paper (17) in their article in *Nature* (Woods *et al.* 1986). Cobbold's paper, in contrast to Mike's, became massively cited. Later, after Mike had become famous and had received worldwide recognition, the situation reversed and, at several international meetings in the 1990s and later, I witnessed that the beautiful records of Ca^{2+} spiking in liver cells, published by Peter Cobbold in 1986 (Woods *et al.* 1986), were presented by other colleagues as having been done by Mike.

The biochemical processes leading to Ca^{2+} signal generation

Long before the 1986 rediscovery of Ca^{2+} spiking or oscillations, Mike's interest had become focused on an even more fundamental problem, namely the nature of the link between agonist–receptor interaction at the outside of the plasma membrane and the generation of the Ca^{2+} signal in the cytosol. This field had been pioneered by Hokin & Hokin (1953), who had shown that acetylcholine markedly increased the incorporation of ^{32}P into phospholipids in pancreatic acinar cells. This was the first demonstration of receptor-activated lipid turnover, later often referred to as the PI effect. Following up on the work by the Hokins, Bob Michell (FRS 1986) (figure 4) had published a review article (Michell 1975), drawing attention to correlations between agonist-elicited Ca^{2+} signals and phosphatidylinositol (PI) breakdown in many different cell types. Michell proposed that PI breakdown, following receptor activation, might cause opening of Ca^{2+} channels in the plasma membrane, thereby allowing Ca^{2+} flow

Figure 4. From left to right: Mike, Bob Michell, Robin Irvine and Yasutomi Nishizuka. CIBA Foundation Symposium 122 on 'Calcium and the cell', London, 1985. (Copyright unknown.)

into the cells. However, the molecular mechanism was entirely unclear. Together with John Fain, Mike undertook an elegant study on his well-characterized salivary gland preparation, showing that PI breakdown triggered Ca^{2+} signal generation (14). However, there was still no mechanistic explanation for the linkage between PI breakdown and the rise in $[Ca^{2+}]_i$.

Two misconceptions, still firmly in the minds of many investigators at the end of the 1970s, stood in the way of further progress. The first was the belief that the primary biochemical event following receptor activation was breakdown of phosphatidyl inositol 4-phosphate (PIP) and the second was that cytosolic Ca^{2+} signal generation was mainly owing to opening of Ca^{2+} channels in the plasma membrane (Michell 1975) (18). Early in the 1980s, both Michell and Mike began to question the first assumption.

Focus on inositol 1,4,5-trisphosphate: a testable hypothesis

At a Royal Society Discussion Meeting in 1981 on 'The control of secretion', Michell proposed for the first time that the primary biochemical event upon receptor activation might not be breakdown of PIP, which had seemed the simplest assumption, but rather phosphatidyl inositol 4,5-bisphosphate (PIP_2) (Michell *et al*. 1981). Surprisingly, one of the products of this

reaction, namely inositol 1,4,5-trisphosphate (IP_3), is not explicitly mentioned in the published version of Michell's presentation. However, shortly thereafter, in a thoughtful review for *Cell Calcium* (18), Mike explicitly pointed to the likely generation of IP_3 as a result of receptor activation. However, he did not at that stage formulate a specific hypothesis with regard to its possible action.

At the very end of 1982, Mike submitted an important single-author paper to the *Biochemical Journal* that was published in June 1983 (19). Mike reported clear evidence for PIP_2 breakdown as a primary event following receptor activation and hence generation of IP_3. Specifically, Mike demonstrated the very rapid rise in the concentration of IP_3 following receptor activation and importantly found that it occurred earlier than the rise in $[Ca^{2+}]_i$. This allowed him to propose for the first time that IP_3 might be the long-sought-after messenger releasing Ca^{2+} from internal stores. It turned out to be an immensely fruitful hypothesis.

Considering that both Michell (1975) and Mike (18) had suggested that receptor activation mainly stimulated Ca^{2+} entry from the extracellular solution, it is interesting that Mike in his crucial 1983 paper (19) now focused on Ca^{2+} release from internal stores as the primary event. This was an essential step, because only the precise formulation of the hypothesis that IP_3 acts by releasing Ca^{2+} from an internal store (19) could have led to the critical experimental test, that proved so immensely successful (20).

Amsterdam 1982: a workshop that changed the signalling field

Mike was an invited speaker at a workshop held at the Royal Netherlands Academy of Arts and Sciences in Amsterdam from 6 to 10 December 1982. Crucially, as it turned out, Irene Schulz from the Max Planck Institute for Biophysics in Frankfurt was also speaking. In retrospect, it is clear that Schulz's talk about Ca^{2+} handling in permeabilized pancreatic acinar cells was the most important event at that meeting. It is no mere coincidence that, in the critically important paper (19) Mike submitted a few weeks after the conference, he for the first time cited an article by Schulz & Stolze (1980) that provided a careful and rather complete overview of Ca^{2+} transport and Ca^{2+} signalling in the exocrine pancreas.

Shortly before going to the meeting in Amsterdam, Schulz had submitted an original article about Ca^{2+} transport in permeabilized pancreatic acinar cells to the *American Journal of Physiology* (Streb & Schulz 1983) and she presented some of these data at the workshop. The basic idea behind using permeabilized cells was to assess Ca^{2+} transport in and out of intracellular structures and be able to use relevant pharmacological agents that could be added to the external solution and then easily enter the intracellular space through the holes in the permeabilized plasma membrane. The concentration of Ca^{2+} in the medium outside the cells, which because of the permeabilized plasma membrane was described as an 'extended cytosol', was measured with a Ca^{2+}-selective electrode. From Mike's perspective, Schulz's most important result was that muscarinic receptor activation elicited a transient rise of $[Ca^{2+}]$ in the extended cytosol. Mike immediately realized that Schulz's preparation of permabilized pancreatic acinar cells would be ideal for testing his IP_3 hypothesis, as the putative messenger could simply be added to the external solution and thus enter the intracellular space through the permeabilized plasma membrane. My abiding memory of the 1982 Amsterdam conference is of Mike explaining his hypothesis, and how to test it, to Schulz in almost every coffee and tea

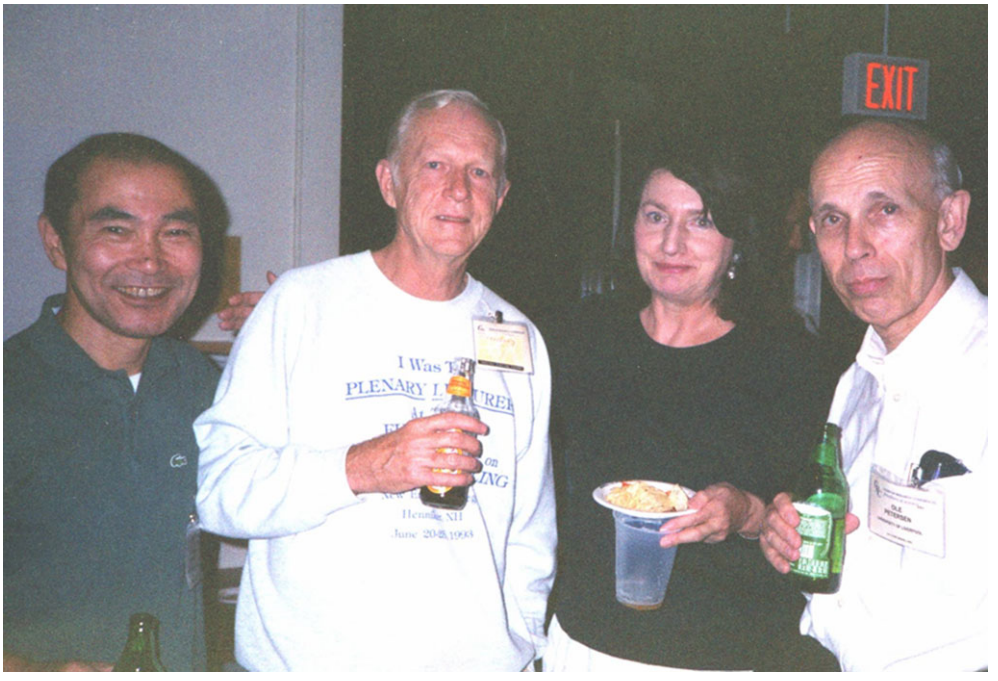

Figure 5. From left to right: Katsuhiko Mikoshiba, Mike, Irene Schulz and Ole Petersen. Gordon Research Conference on 'Calcium signaling', Hadley, Massachusetts, USA, 2003. (From Ole Petersen, private collection.)

break. However, the impression that he had successfully persuaded Schulz to give immediate priority to the project turned out to be over-optimistic.

The permeabilized cell preparation used by Schulz and her postdoctoral fellow Hanspeter Streb could only be useful for Mike if he thought that IP_3 would act inside cells to release Ca^{2+} from internal stores. If the hypothesis had been that IP_3 would act on the plasma membrane, the permeabilized preparation would be useless. However, at the time of the Amsterdam meeting, Schulz did not believe that receptor activation caused Ca^{2+} release from the endoplasmic reticulum (ER) or any other organelle inside the cell and therefore she did not believe that an intracellular messenger mediating the cytosolic Ca^{2+} signal was needed. She favoured the hypothesis that receptor activation in a more direct way would release Ca^{2+} bound to the plasma membrane: 'As it is difficult to imagine the action of a second messenger in these open cells, this result suggests that secretagogues release Ca^{2+} directly from the plasma membrane' (Streb & Schulz 1983). It is to Mike's credit that he was not confused by this, but clearly realized what had to be done to test his hypothesis. It is nevertheless intriguing that the principal outcome of the crucially important 1982 workshop in Amsterdam was that the task of proving Mike's hypothesis fell to Schulz, who did not at the time believe in the need for a second messenger.

IP_3 IS THE Ca^{2+}-releasing intracellular messenger

The scene was now set for the actual experimental test of Mike's hypothesis in the Schulz laboratory at the Max Planck Institute for Biophysics in Frankfurt, but this did not happen immediately. In spite of the long conversations between Mike and Schulz at the Amsterdam workshop, and the mutual agreement to do the experiments, neither Schulz nor Streb gave this task a high priority. Robin Irvine (FRS 1993) (figure 4), who was Mike's main collaborator in Cambridge, provided suitable quantities of IP_3, which were sent to Schulz's laboratory, but the material stayed in the fridge. Naturally, Mike became impatient and decided to call Karl Ullrich, the Director of the Institute (as already mentioned, there was an indirect connection between Mike and Ullrich through Schmidt-Nielsen that may well have been helpful). Ullrich was an outstanding Max Planck Director, who took a strong interest in all research programmes at his Institute, even those that were far away from his own personal expertise. Mike carefully explained the rationale behind the planned experiments and, having understood their potential importance, Ullrich persuaded Schulz of the need to start the experimental work immediately. Ullrich continued to encourage the development of the project and his help is rightly acknowledged in the 1983 *Nature* paper (20).

The beautiful records obtained by Streb showed clearly that IP_3, but not IP_2 and not IP_1, evoked a clear increase in $[Ca^{2+}]$ in the extended cytosol. When IP_3 had elicited a maximal increase in $[Ca^{2+}]$, a subsequent application of the muscarinic agonist carbachol had no further effect. When carbachol elicited a sub-maximal response, a subsequent addition of IP_3 caused a further increase to the maximal level seen with IP_3 alone. The sum of the responses to carbachol and IP_3 was constant. Inhibition of mitochondrial function did not inhibit the response to IP_3 (ATP was always present in the external medium and therefore also inside the permeabilized cells), but inhibition of the Ca^{2+}-activated ATPase by vanadate caused a markedly reduced response to IP_3. The conclusion that IP_3 released Ca^{2+} from a non-mitochondrial intracellular pool was clear. The paper describing these data (20) was submitted to *Nature* towards the end of June 1983, accepted at the end of August and published on 3 November 1983. The importance of the results was recognized almost immediately throughout the world. The Ca^{2+} signalling community understood that the field had changed fundamentally. A new, and important, intracellular messenger had been found.

Nevertheless, there were of course critics. It was pointed out that the method of permeabilizing cells described in the paper (20), namely to expose cells to a nominally Ca^{2+}-free solution, was unorthodox and generally did not result in membrane permeabilization, but this was quickly forgotten. However, this issue resurfaced many years later at a Gordon Research Conference (GRC) on Ca^{2+} signalling. A talk about electrophysiological recordings from pancreatic acinar cells immersed in a Ca^{2+}-free solution was interrupted by Roger Tsien who, in his characteristically forceful style, pointed out that the recordings were non-sensical as the membrane would have been permeabilized by the Ca^{2+}-free external solution. However, it was indicated that the cell, in the absence of external Ca^{2+}, responded to acetylcholine with a marked increase in the transmembrane current, owing to opening of ion channels permeable to Cl^-, so the plasma membrane was clearly not permeabilized. Tsien then turned to Mike in the audience and said that then there must be something wrong with Mike's famous 1983 paper! Mike did not have a good answer, because it is still a mystery how the pancreatic cells used in the Schulz laboratory became permeabilized. However, they were permeabilized (most

likely owing to mechanical damage), because the result that IP_3 elicits Ca^{2+} release from intracellular stores was quickly reproduced in other laboratories in experiments on several other cell types. Mike and Irvine tested the effects of IP_3 in liver cells in collaboration with Jim Putney (21), in insulinoma cells with Claes Wollheim (22), and in photoreceptors with Alan Fein (23) and with Joel Brown (24).

Given the importance of the discovery of IP_3 as a universal Ca^{2+}-releasing messenger, it seemed that the time was ripe for writing a big review and to publish it prominently. Mike proposed this to *Nature*, but the initial response was not encouraging. Then, in April 1984, a review article appeared in *Nature* written by Yasutomi Nishizuka (ForMemRS 1990) (figure 4) focused on diacylglycerol activation of protein kinase C (Nishizuka 1984). Because diacylglycerol and IP_3 are the two intracellular messengers that are produced when receptor activation causes breakdown of PIP_2, Mike was able to point out to *Nature* that it was unreasonable for the journal to highlight the functional importance of only one of these two messengers. *Nature* then relented and the famous IP_3 review article authored by Mike and Irvine duly appeared on 22 November 1984 (25). This article summarized very effectively all the evidence showing that IP_3 was a genuine intracellular messenger, generated by a phosphodiesterase catalysing the breakdown of PIP_2 into diacylglycerol and IP_3, the latter then acting to open pathways in the ER membrane permeable to Ca^{2+}. The immediate impact of the 1984 review was enormous and it very quickly became immensely influential. Overall, 1984 was a remarkably successful year for Mike. His 1983 landmark discovery had been reconfirmed in collaborations with several laboratories across the world and published in four papers in *Nature* and, to crown it all, he had been elected a Fellow of the Royal Society.

Consolidation of the IP_3 story

In the following years (1985–1990) a number of important new elements were added to the IP_3 story. Although Mike and Schulz had shown that IP_3 releases Ca^{2+} from the ER (20), the nature of the Ca^{2+}-releasing pathway had not been disclosed. In an elegant biophysical analysis of transport pathways in ER vesicles from hepatocytes, Shmuel Muallem and George Sachs demonstrated that IP_3 opens ion channels in the ER membrane. They showed that in parallel to the electrogenic Ca^{2+} pump accumulating Ca^{2+} in the ER lumen, originally discovered by Setsuro Ebashi (ForMemRS 1977) and Fritz Lipmann (ForMemRS 1962) (Ebashi & Lipmann 1962), IP_3 opens a conductance pathway in the membrane that allows Ca^{2+} to escape into the cytosol (Muallem *et al*. 1985). In 1986, Andras Spät and Jim Putney produced evidence for the presence of a saturable IP_3 receptor on the ER membrane of hepatocytes and neutrophils (Spät *et al*. 1986) and subsequently the IP_3 receptor was sequenced by the laboratories of Katsuhiko Mikoshiba (figure 5) (Furuichi *et al*. 1989) and Thomas Südhof (ForMemRS 2017) (Mignery *et al*. 1989). From a functional point of view, it was critically important that Sol Snyder and his collaborators could demonstrate that purified IP_3 receptors mediated Ca^{2+} flux in reconstituted lipid vesicles when activated by IP_3 (Ferris *et al*. 1989). These studies from other laboratories provided hard biophysical and molecular data proving the existence of an ion channel permeable to Ca^{2+} in the ER membrane that could be opened by the direct binding of IP_3 to the channel protein. These data were essential for the rapid general acceptance of Mike's concept for the intracellular messenger role of IP_3.

Revisiting the Ca^{2+} oscillation mechanism

In this period it also became clear that the Ca^{2+} signalling events mediated by IP_3 consisted of oscillations or repetitive spikes rather than sustained elevations of $[Ca^{2+}]_i$. In a single-author paper published in 1988, Mike followed up his 1981 observation (17) that 5-HT evoked oscillatory changes in the transepithelial potential in *Calliphora* salivary glands—an early indication of agonist-elicited oscillations in $[Ca^{2+}]_i$. In the 1988 study (27), Mike injected IP_3 into oocytes and showed that this induced membrane potential oscillations owing to repetitive activation of Ca^{2+}-sensitive Cl^- channels. At that time, it was generally considered that cytosolic Ca^{2+} oscillations must be the result of oscillating IP_3 levels. However, the following year it was shown that IP_3 could evoke regular and repetitive spikes of Ca^{2+}-activated Cl^- current and that this also happened when an active IP_3 analogue was employed that could not be metabolized (Wakui *et al.* 1989).

Having provided the first, albeit indirect, evidence for hormone- or neurotransmitter-elicited intracellular Ca^{2+} oscillations (17), Mike was naturally keen to understand the nature of these events. Together with the theoreticians Albert Goldbeter and Genevieve Dupont in Brussels, Mike produced a minimal model for signal-induced Ca^{2+} oscillations. It was published in *PNAS* in February 1990 (30) and became immensely influential. In this two-pool model, IP_3 elicited a steady release of Ca^{2+} from one pool into the cytosol, which then at a certain $[Ca^{2+}]_i$ threshold triggered a Ca^{2+}-induced Ca^{2+} release (CICR) response from a second IP_3-insensitive Ca^{2+} pool (30). After the second pool had been emptied, there would be a pause during which this pool would be recharged with Ca^{2+} from the IP_3-sensitive pool until it again would be capable of Ca^{2+} release. The CICR concept came from earlier studies on muscle cells (Endo *et al.* 1970), reflecting properties of the ryanodine receptor, an IP_3-insensitive Ca^{2+} release channel, but there was no experimental evidence for CICR in non-muscle cells or in any electrically non-excitable cell type. Unknown to Mike, direct experimental evidence for his concept was being obtained during the very period in which he, Dupont and Goldbeter were developing their model. Yuri Osipchuk and Makoto Wakui showed by patch-clamp whole-cell recording of the Ca^{2+}-sensitive Cl^- current, with simultaneous fluorescent $[Ca^{2+}]_i$ measurements, that intracellular infusion of Ca^{2+} into pancreatic acinar cells could mimic the effect of intracellular IP_3 infusion, generating repetitive cytosolic Ca^{2+} spikes (Osipchuk *et al.* 1990). This paper, published only a few weeks after the appearance of the Goldbeter–Dupont–Berridge article, provided the necessary experimental evidence for the essential, and still completely valid, part of Mike's theoretical model, namely that the IP_3-elicited Ca^{2+} oscillations are because of CICR. The following year (1991), single-channel recordings of current through isolated IP_3 receptors showed that they are Ca^{2+}-sensitive in a biphasic manner, so that a small elevation of $[Ca^{2+}]_i$ triggers increased opening of the IP_3-sensitive ion channels, whereas a larger rise inhibits opening (Bezprozvanny *et al.* 1991), indicating that a simple one-pool model, but still based on CICR, might be sufficient to explain Ca^{2+} oscillations.

Mike continued to be fascinated by Ca^{2+} oscillations and agreed to chair a CIBA Foundation symposium on this subject in London in April 1994 (figure 6). As with all CIBA Foundation symposia, the discussions were recorded and fully reproduced in a book published the following year (Bock & Ackrill 1995). Mike steered the discussions gently, but also firmly. In spite of the concentration at that time on understanding the mechanisms underlying Ca^{2+}

Figure 6. Mike, second seat from right in front row, as Chair of CIBA Foundation Symposium 188 on 'Calcium waves, calcium gradients and oscillations', London, 1994. (Copyright unknown.)

spiking or oscillations, Mike insisted, quite rightly, that we also had to focus on the functional implications of these phenomena, which in many cell types were far from clear. In Mike's *Calliphora* salivary glands, he had of course immediately, from the time of the very early discovery of these oscillations, been able to show that the oscillation frequency determined the intensity of secretion, thereby establishing their functional importance (17).

First evidence for local subcellular Ca^{2+} signal generation

Mike's 1988 paper on IP_3-evoked Ca^{2+} oscillations in the oocyte (27) also showed that one part of the cell was much more sensitive to IP_3 than other parts, indicating the possibility of IP_3 receptors being concentrated in a sub-compartment of the cell. The following year, Mike followed this up in a study carried out in collaboration with Bob Burgoyne in which it was shown directly that an IP_3-mobilizing agonist could evoke a localized Ca^{2+} signal near the nucleus in chromaffin cells, whereas caffeine—activating a different Ca^{2+} release channel, namely the ryanodine receptor—elicited a broader response in another part of the cell (29). This was the first convincing demonstration of a subcellular concentration of IP_3 receptors, a theme that became crucially important in the following decade for understanding the control of many key cellular functions. Following direct demonstrations by other groups of local Ca^{2+} spikes in pancreatic acinar cells (Osipchuk *et al*. 1990) and cardiac cells (Cheng *et al.* 1993), Mike's laboratory, with Martin Bootman as the key investigator, engaged in detailed experimental analysis of local Ca^{2+} spiking events, and their assembly into global responses, in a variety of cell types (33–35). Mike summarized many of these findings effectively in

what became a highly cited review article (36), written together with Martin Bootman and Peter Lipp.

Attempts to understand the mechanism and regulation of Ca^{2+} release-activated Ca^{2+} entry

Although the mechanism by which agonists elicit intracellular Ca^{2+} release had been clarified, there was still an important unsolved problem. Right from the very earliest days of Ca^{2+}-signalling studies in epithelial cells, it was clear that following the initial agonist-evoked intracellular Ca^{2+} release there would be active Ca^{2+} extrusion owing to activation of the plasma membrane Ca^{2+} pump by the elevated cytosolic Ca^{2+} concentration. In order to compensate for this Ca^{2+} loss, a delayed Ca^{2+} entry from the extracellular fluid had to occur (Nielsen & Petersen 1972), but the mechanism underlying this process and its regulation were completely unknown.

Having established the mechanism by which the initial Ca^{2+} release occurs, Mike was naturally keen also to nail down the mechanism responsible for the subsequent Ca^{2+} entry. In collaboration with Irvine, it was shown that a significant part of the IP_3 generated by PIP_2 breakdown was phosphorylated to IP_4 (26). The kinase catalysing this process is a Ca^{2+}-dependent enzyme, so an attractive hypothesis presented itself, suggesting that whereas IP_3, formed initially after PIP_2 breakdown, activates Ca^{2+}-permeable channels in the ER, the secondary production of IP_4 might activate the Ca^{2+} entry process from the extracellular space. This is what was proposed in Mike's and Irvine's second major review article published in *Nature* in 1989 (28). The IP_4 hypothesis was immensely attractive, partly because of its simplicity and partly because it was seen as a natural extension of the 'IP_3 story'. It also appeared to explain key features of the Ca^{2+} entry process, namely its absolute dependence on an initital intracellular Ca^{2+} release and its delay as compared with the timing of the primary intracellular release. However, experimental evidence for a critical role of IP_4 in the control of Ca^{2+} entry was limited. Although there were a few relatively IP_3-insensitive cell types in which IP_4 was shown to permit or markedly enhance IP_3-dependent Ca^{2+} signals (28), no confirmation of such results in other cell types was forthcoming. Therefore, the model for IP_4 action proposed in the 1989 *Nature* review article (28) did not gain general acceptance. It gradually became clear that real progress would depend on first clarifying the biophysical nature of the Ca^{2+} entry process. In this period (1980s), patch-clamp single-channel current recording had enabled successful characterization of the different types of voltage-gated Ca^{2+} channels, particularly in cardiac cells and neurons (Reuter 1983; Nowycky *et al*. 1985; Fedulova *et al*. 1985), but the problem about the nature and control of the Ca^{2+} release-activated Ca^{2+} (CRAC) entry channels in electrically non-excitable cells was much more difficult to solve. It was only much later, in the 2000s, that work from many other groups finally provided a reasonably complete understanding of what turned out to be a rather complicated process (Parekh 2010).

In spite of limited resources and with relatively little expertise in molecular biology, Mike did make a brave attempt, together with his PhD student Carl Petersen, to identify the proteins encoding what became known as the CRAC channels, also often referred to as store-operated Ca^{2+} channels. One potentially interesting candidate was the *Drosophila trp* gene, which had been shown by Roger Hardie (FRS 2010) and Baruch Minke to encode a Ca^{2+}-permeable ion

channel activated downstream of phospholipase C (Hardie & Minke 1992). Minke & Selinger (1992) had even suggested that this channel might be directly activated by the IP_3 receptor. However, *trp* was thought to encode a *Drosophila* eye-specific protein (Montell & Rubin 1989), and there was no evidence for vertebrate homologues. Mike and Carl Petersen set out to clone *trp* homologues from cDNA of a variety of species and found evidence for both mouse and *Xenopus* versions of *Drosophila trp* (32). The most important results of this project showed that expression of the *trp* gene product in *Xenopus* oocytes led to significant increases in Ca^{2+} entry, but only when the intracellular Ca^{2+} stores were depleted. This was strong evidence indicating that trp channels could mediate the delayed Ca^{2+} entry following IP_3-elicited intracellular Ca^{2+} release (32). However, by the time Carl Petersen's and Mike's paper appeared, it was already clear that the relatively non-selective trp channel was not the universal CRAC channel everyone was looking for. In 1992, Markus Hoth and Reinhold Penner, at the Max Planck Institute for Biophysical Chemistry in Göttingen, had discovered a highly Ca^{2+}-selective CRAC channel in immune cells (Hoth & Penner 1992) that was later shown to be encoded by Orai1 (Feske *et al*. 2006). Through the work of many groups, including those led by Michael Cahalan, Stefan Feske, Richard Lewis, Tobias Meyer, Anant Parekh (FRS 2019), and Anjana Rao, the Orai1 channel is now generally recognized as the principal store-operated Ca^{2+} entry channel (Parekh 2010). Nevertheless, the *trp* gene family has been found to have many and important functions across a wide variety of biological tissues (Venkatachalam & Montell 2007) and, in some systems, trp channels may also function as store-operated Ca^{2+} entry pathways together with Orai1 channels. The work from Mike's laboratory (32) certainly encouraged the cloning of many further *trp* homologues over the next years in many different laboratories and this considerably advanced what became an extremely active research field (Venkatachalam & Montell 2007).

Enjoying phenomenal success

Although Mike was an accomplished and versatile experimenter, his principal interest—after his great discovery of IP_3 as a universal Ca^{2+}-releasing messenger—became focused on the 'big picture' of intracellular Ca^{2+} signalling and particularly on its relationship to physiological regulation. Increasingly, he also became interested in the dysregulation of Ca^{2+} signalling that led to many disease processes. Although his group at the Babraham Institute made many original contributions to our knowledge about the physiology and pathophysiology of Ca^{2+} signalling in many different cell types, it cannot be denied that the greatest impact of Mike's work after the discovery of the Ca^{2+}-releasing function of IP_3 was achieved through his many highly cited review articles and his frequent keynote lectures at the most important international biomedical conferences. Mike was a formidable lecturer, and this was a major reason for his international dominance of what became a large and expanding research field. He spoke with enormous authority, as well as exceptional clarity, and was therefore one of the most sought-after plenary lecturers at symposia and congresses throughout the world.

In spite of Mike's enormous success, he always remained a modest individual and never displayed even the slightest sign of arrogance. This was reflected in one of our many late-night discussions, when he told me that he did not regard himself as particularly clever. He did admit that one of his strengths was to constantly draw new cell-signalling models, helping

him to clarify his mind about the most critical issues. This made Mike a great teacher because he was able to present and explain these models in a way that could be understood also by non-specialists, both in his remarkably successful review articles as well as in the numerous plenary lectures he was invited to deliver.

In 1993, Mike's most highly cited review article with the simple title 'Inositol trisphosphate and calcium signalling' appeared in *Nature* (31). The triumph of IP_3 as a ubiquitous Ca^{2+}-releasing messenger was complete, but at that time it had also become clear that it was not the only messenger capable of generating intracellular Ca^{2+} signals. Following the original discoveries of Hon Cheung Lee, Anthony Galione (FRS 2016), who had done his PhD with Mike, had shown that cyclic ADP-ribose could function as a liberator of stored Ca^{2+} (Galione *et al.* 1991) and later made progress on working out the mechanism of action of yet another Ca^{2+}-releasing messenger, also discovered by Hon Cheung Lee, namely nicotinic acid adenine dinucleotide phosphate (NAADP) (Churchill & Galione 2001). In spite of these interesting developments, IP_3 continues to be generally accepted as by far the most important messenger linking receptor activation to intracellular Ca^{2+} release.

As the undisputed leader of the Ca^{2+}-signalling field, Mike created much of the context for the evolution of the Ca^{2+}-signalling research field, which exploded in the 1990s and early 2000s. This led to the establishment of the GRC on 'Ca^{2+} signaling' and the inaugural conference was held in New Hampshire in June 1993. It was not a great surprise that Mike was chosen as the inaugural plenary lecturer and at subsequent GRC 'Calcium signaling' conferences he always wore the T-shirt he had been given at the 1993 meeting showing that he was the first plenary lecturer (figure 5). Mike's enthusiasm for and loyalty to the GRC 'Ca^{2+} signaling' conference was impressive. Considering the numerous award and plenary lectures he was constantly asked to give to huge audiences at large international congresses, his presence throughout the whole week of almost every GRC 'Ca^{2+} signaling' conference in the years 1993–2013, often held in rather uncomfortable settings, was remarkable. Mike always remained a true scientist committed to understanding all aspects of his discipline. There was never any 'show'. Unlike so many others, Mike never asked questions to show off his brilliance or to humiliate others, but always tried to learn more. He was particularly engaged in small group discussions in coffee and tea breaks and would often contribute very effectively by making small model drawings that illuminated the most critical points. He was also an eager participant in poster sessions, engaging younger generations in vivid discussions.

In Mike's later years he became increasingly interested in vitamin D and its role in Ca^{2+} transport and Ca^{2+} signalling and this was reflected in the talk (38) he gave at a Royal Society Discussion Meeting held at Chicheley Hall in Buckinghamshire in March 2016 on 'Evolution brings Ca^{2+} and ATP together to control life and death'. That year, Mike also published his last 'magnum opus' in *Physiological Reviews* (39) on 'The inositol trisphosphate/calcium signaling pathway in health and disease'. Owing to the peculiar arrangement for scientists engaged as civil servants in the UK, Mike had to retire when he became 65, but not before the Babraham Institute had organized a magnificent retirement symposium in 2003, ending with an impressive firework display featuring Mike's name on the evening sky. Mike nevertheless continued to work at the Institute as an Emeritus Babraham Fellow as well as speaking and receiving awards at many international meetings.

Mike received, throughout his working life, fantastic support from his wife Sue (figures 2 and 7). He was always notably happier at international meetings when accompanied by Sue, who drew out his best human characteristics. Mike was generous with his time, freely gave

Figure 7. Sue and Mike meeting Shimon Peres (Nobel Peace Prize 1994) in Jerusalem on the occasion of Mike receiving the Wolf Prize in Medicine in 1995. (From the Berridge family collection.)

away ideas and was always willing to provide support when asked. His worldwide influence on everyone working in the Ca^{2+}-signalling field was profound, and his help, advice and direct support will not be forgotten.

Awards and recognition

1984	Fellow of the Royal Society of London
1984	Feldberg Prize
1986	King Faisal International Prize for Science
1986	Louis-Jeantet Prize for Medicine
1987	Abraham White Scientific Achievement Award (George Washington University School of Medicine)
1988	Gairdner Foundation International Award
1988	Royal Society of London Croonian Lecture
1989	Albert Lasker Basic Medical Research Award
1989	Baly Medal (Royal College of Physicians, London)
1989	Member of Academia Europaea

1990	Rita Levi Montalcini Award Lecture (Fidia Research Foundation, Washington DC)
1990	Dale Medal (Society for Endocrinology)
1991	Royal Medal of the Royal Society of London
1991	Foreign Member of the Belgian Royal Academy of Medicine
1991	CIBA-GEIGY/DREW Award in Biomedical Research
1993	Doctor Honoris Causa (Hasselt University, Belgium)
1994	Dr H. P. Heineken Prize for Biochemistry and Biophysics
1994/1995	Wolf Prize in Medicine
1996	Massry Prize in Nephrology, Physiology and Related Fields (The Meira and Shaul G. Massry Foundation, USA)
1996	Physiological Society Annual Review Prize Lecture
1998	Knighthood (Knight Bachelor)
1998	Fellow of the Academy of Medical Sciences (Founding Fellow)
1999	Ernst Schering Prize
1999	Foreign Associate of the US National Academy of Sciences
1999	Foreign Honorary Member of the American Academy of Arts and Sciences
2004	Honorary Member of the Biochemical Society
2005	Shaw Prize in Life Science and Medicine
2007	Honorary Degree, University of Liverpool

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I thank Susan Berridge for helpful information and photos. I have previously, together with Carl Petersen, written an obituary (Petersen, O. H. & Petersen, C. C. H. 2020), which, although less detailed, inevitably contains sections that are similar to some of those found in this account.

The frontispiece portrait photograph was taken for the Royal Society in 1984 and is copyright © Godfrey Argent Studio.

Author profile

Ole H. Petersen CBE FMedSci FRS

Ole Petersen is Professor of Physiology at Cardiff University. In 2010, he succeeded Sir Martin Evans FRS as Director of the Cardiff School of Biosciences. Before that, he held the George Holt Chair of Physiology at the University of Liverpool (1981–2009), succeeding Roderick Gregory CBE FRS. Ole graduated in Medicine from the University of Copenhagen in 1969 and was subsequently Lecturer and then Associate Professor in Copenhagen until his appointment as Symers Professor of Physiology and Head of the Physiology Department at the University of Dundee (1975–1981). He was a Wellcome–Carlsberg Travelling Research Fellow in the Pharmacology Department at the University of Cambridge, 1971–1972. Ole's research work has been focused on the physiology and pathophysiology of mammalian exocrine glands. He pioneered patch-clamp single-channel and whole-cell current experiments on epithelial cells, published in a series of *Nature* papers in the 1980s, and discovered local Ca^{2+}-signalling events in pancreatic acinar cells in the early 1990s, published in a series of articles in *Cell*. He has received many awards, including the Novo Nordisk Foundation's Jacobaeus Prize, the Czech Science Academy's Purkyně Medal, the American Physiological Society's Walter B. Cannon Award, the Academia Europaea Gold Medal and, most recently, the International Association of Pancreatology George E. Palade Medal and Prize (Kyoto, 2022). He is an elected member of several European science academies, including the German National Academy of Sciences Leopoldina (2010), the Royal Danish Academy of Sciences and Letters (1988), and the Hungarian Academy of Sciences (2004).

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