Cell Calcium 72 (2018) 127–131

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Commentary

A personal journey

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My assignment for this essay, given to me by Shmuel Muallem, was to present "…a reflection on the history of SOCs and (my) career…" I must begin with an apology, as this will differ from some historical accounts I have already written [1,2] in being a (even) more "me-centric" account. So where should I start? My early education was unremarkable, small town high school (in Farmville, Virginia, class of '64), chemistry degree from the University of Virginia in Charlottesville (class of' 68). In fact, I am forced to admit that a good deal of my undergraduate education at Virginia was unfortunately bypassed in favor of social undertakings. Late in my third (junior) year I met with my organic chemistry professor to discuss my grade in the laboratory section. He asked me what I planned to do after graduation, and I related that I would like to pursue a graduate degree in some area of chemical or biological research (thinking that was what he would want to hear). However, on further discussion he learned that my grade point average at the time was a bare 2.0 (known at the time as the "gentleman's C"), the minimum required for graduation. With such credentials, he suggested my chances of admission to a reasonable graduate program were minimal.

The professor with whom I was discussing my future life was Alfred Burger, an internationally respected medicinal chemist, and Editor-in-Chief of the journal, Medicinal Chemistry. He had lately developed a keen interest in the growing discipline of pharmacology. At the end of our discussion, he made me a proposal. He outlined a course of study for my 4th year that included challenging course work: Physical Chemistry, Advanced Organic Chemistry, Genetics, and Molecular Biology. If I could emerge from this academic gauntlet with all A's and B's, he agreed to use his considerable influence to get me into a graduate program in Pharmacology. The short ending to this story is: I did it, and he did it. I was admitted to all three programs to which I applied, at Emory University, Vanderbilt University and the Medical College of Virginia in Richmond. The Vietnam War settled the issue of where I would go, as I obtained a slot in a National Guard unit near Richmond, so that I could immediately begin my graduate education while fulfilling my service obligation on a part-time basis.

My graduate work in the laboratory of a well-known toxicologist, Joe Borzelleca, focused on membrane transport of drugs from blood into saliva (for example, [3]). The combination of an excellent course in General Physiology together with prior training in Physical Chemistry laid the groundwork for my interest in membrane phenomena. Two members of the faculty in the Pharmacology Department at MCV were investigating the role of Ca^{2+} in smooth muscle function (Pat Hudgins and George Weiss), and through interactions with them I developed an interest in Ca^{2+} signaling. So, after graduation (1972), I took a post-doctoral position at the University of Pennsylvania in the laboratory of C. Paul Bianchi, an authority on Ca^{2+} signaling in skeletal muscle, and especially in the use and kinetic analysis of Ca^{2+} fluxes by use of $^{45}Ca^{2+}$ techniques. I only published one paper during my two years at Penn [4], but I was surrounded by some very smart people, and I learned a lot.

Younger readers may be surprised to learn that in "those days" it was common to find a tenure-track position after a two-year postdoc stint. I ended up (1974) in a newly formed Department of Pharmacology at Wayne State University in Detroit where I got my first NIH grant, and published my first independent papers. I also met, collaborated with, and married my wife Barbara. Despite my excellent training in skeletal muscle excitation-contraction coupling at Penn, I decided to try to combine my earlier training in salivary gland biology with my training at Penn in calcium signaling to establish a program in a somewhat less competitive field; the role of calcium signaling in salivary glands. This was hardly a crowded field, and I was somewhat successful in carving out a novel niche for myself. I have often advised students and young postdocs that they should try to do this: combine their different training experiences to create a research domain that is somewhat unique. Within three years I had two NIH grants, and was promoted to Associate Professor with Tenure.

I was quite happy in many respects with my situation at Wayne State, and I owe much to the Department Chair, Bernie Marks. Bernie was always very supportive and gave me a good deal of useful advice, about science and about succeeding as a scientist. I set out to investigate calcium signaling in salivary glands, but at the time calcium signaling research involved one of two indirect methods. Movements of radioactive calcium could give information on fluxes, but not on the key variable, the concentration of calcium in the cytoplasm. The latter was often inferred from measurements of parameters known to be dependent on cytoplasmic calcium, contraction or shortening in muscle for example. Salivary glands do not contract, but it was known that, like most secretory epithelia, they expressed plasma membrane potassium channels that are activated by calcium. Thus, one could infer the relative levels of cytoplasmic calcium by the rate of efflux of radioactive potassium, or usually, for technical reasons, the potassium surrogate rubidium. By using this approach, my first independent papers indicated that in salivary glands, calcium signals involved a release of intracellular calcium stores and entry of calcium across the plasma membrane [5]. This was not an earth-shaking discovery, as this basic concept had been known by smooth muscle physiologists and

https://doi.org/10.1016/j.ceca.2018.05.004
Received 14 May 2018
Available online 21 May 2018
0143-4160/ Published by Elsevier Ltd.

pharmacologists for some time [6,7]. In addition, I found that the calcium released intracellularly was largely lost to the extracellular space unless calcium was present in the medium. I concluded that the agonist-activated influx channels served to refill the intracellular stores [8]. A subsequent publication demonstrated that in a lacrimal gland preparation, calcium could enter the cell and refill the stores independently of receptor activation [9], but in so doing, no increase in cytoplasmic calcium was observed. These observations laid the groundwork for future ideas of store-operated calcium channels.

At some point during my tenure at Wayne State, I became aware of the experimental and theoretical work of Robert (Bob) Michell. Michell was a biochemist at the University of Birmingham (UK) studying the metabolism and function of inositol lipids. In work beginning in the 1950's, Mabel and Lowell Hokin had demonstrated that inositol lipid turnover was strongly accelerated upon activation of certain autonomic receptors [10,11]. Despite detailed characterization of this "PI" effect by the Hokins and others, the physiological significance of this phenomenon had remained a mystery for two decades. In his seminal review in 1975 [12], Michell highlighted two previous observations. First, earlier work by the Hokins [13] had shown that inositol lipid turnover did not require the presence of calcium, whereas other physiological responses of receptor activation (secretion, smooth muscle contraction for example) did. Second, an Israeli group had demonstrated that artificial introduction of calcium into cells by use of a calcium ionophore did not activate inositol lipid turnover [14]. From these two observations Michell reasoned that inositol lipid turnover represented a step upstream of, and causative of calcium signals [12]. Michell also surmised, from his own data, that inositol lipid turnover was initiated by the degradation of an inositol lipid (phosphatidylinositol he thought), and that this was the key reaction initiating calcium signaling.

Michell's idea was not widely accepted, but a few laboratories, my own included, began to think about an underlying mechanism. Strong support came from studies of Berridge and Fain [15] that showed a dependence on inositol for calcium signaling in a blowfly salivary gland preparation. It was around this time that I put forth the idea, with supporting experimental evidence, that a product of phosphatidylinositol breakdown, phosphatidic acid, could function as a calcium carrier or ionophore in the plasma membrane [16]. This of course turned out to be wrong, but I do not regret having made this proposal; I always liked to try to come up with testable proposals for cellular mechanisms, and it is not surprising that sometimes (often?) these turned out to be wrong. A mantra in my laboratory, as former students and postdocs will recall, is that it is OK to be wrong - but if so, we want to be the ones to show it. Indeed, that was the case for the phosphatidic acid idea, as I will point out later.

Despite the positive professional environment at Wayne State, this small town southern boy eventually tired of the cold winters and tiresome population density of a large midwestern city. After 6 years or so, and armed with respectable credentials, I began to apply for positions farther south. By coincidence, I ended up back at MCV in the Pharmacology Department (1980) which had now grown considerably. In addition to strong programs in toxicology and CNS pharmacology, there was a Division of Cellular Pharmacology which included two other faculty interested in calcium signaling, Ron Rubin and Suzanne Laychock. A bright postdoctoral fellow in my laboratory, Gillian Burgess, developed a permeable hepatocyte preparation that she used to sort out differing calcium concentration dependencies of mitochondria and endoplasmic reticulum, and their relation to the activation by calcium of glycogen phosphorylase [17]. She presented these results at the FASEB meeting (now called EB meeting) in Chicago. In attendance was Michael Berridge, from Cambridge University, who had proposed that the soluble breakdown product of phosphatidylinositol 4,5-bisphosphate, inositol 1,4,5-trisphospate (IP_3) might function as a second messenger to activate the intracellular release of calcium. Gillian's permeable cell preparation, allowing access of the membrane impermeant IP_3, would be an excellent system to test this hypothesis. In fact, Berridge had already entered into a similar collaboration with Irene Schulz, who had developed a permeable preparation of pancreatic acinar cells. These collaborations depended on the participation of Robin Irvine, at the Babraham Institute who had purified IP_3 to be used in the permeable cell preparation. The first publication verifying the efficacy of IP_3 as a releaser of stored calcium came from Irene Schulz [18]. This was followed by our own, similar findings with hepatocytes [19], and a publication from a Geneva group showing release of calcium from microsomes by IP_3 [20].

It is hard to overstate the significance of Berridge's discovery of the messenger function of IP_3. For technical reasons, all of the early experimental work was carried out with permeable cells or microsomes, so only the ability to release calcium stores was evaluated. Initially, I (and others I am guessing), invoking Occam's razor (https://en.wikipedia.org/wiki/Occam%27s_razor), assumed that IP_3 might also activate the plasma membrane calcium channels, thereby accounting for both the release and entry phases commonly associated with receptor-dependent calcium signaling. But shortly two papers appeared indicating that this was not likely the case, one involving measurements of transmembrane calcium movements in *Xenopus* oocytes [21], and another showing that while IP_3 readily released calcium from an endoplasmic reticulum microsome fraction, it failed to do so from purified plasma membrane vesicles [22]. Prior to the discovery of the signaling function of IP_3, Casteels and Droogmans [23] had published a study of calcium pools in smooth muscle, essentially reproducing the results of our earlier paper on lacrimal glands [24], and had proposed a privileged pathway from the extracellular space to the sarcoplasmic reticulum that did not involve calcium traversing the cytoplasm. The combination of these observations led me to propose the capacitative calcium entry model whereby the empty state of the endoplasmic reticulum somehow set in motion a mechanism to activate plasma membrane calcium channels, thereby refilling discharged intracellular calcium stores [25]. The term "capacitative" indicated the idea that the pathway of calcium from the extracellular space to the cytoplasm involved transfer into the endoplasmic reticulum and then release to the cytoplasm, that is, in the manner of a serial arrangement of a resistance and a capacitance. As such, the model was similar to the one I proposed in 1977 [8], except the release would come from endoplasmic reticulum (as clearly demonstrated by work with IP_3 in permeable cells and microsomes) rather than from plasma membrane binding sites. I chose to publish this idea in Cell Calcium in part because they had advertised their willingness to publish acceptable hypothesis-based papers, and because at the time I was at a loss as to how one might experimentally test this idea.

It was also at about this time that another revolution in the calcium signaling field was taking place. In 1982, the late Roger Tsien published his first paper describing the use of a fluorescence-based calcium indicator, Quin-2, that could be loaded into living, functioning cells [26]. Shortly thereafter an improved version, Fura-2, was developed [27] which is still widely used today. Soon turn-key instrumentation became available allowing investigators to obtain real-time intracellular calcium measurements in living cells, initially in suspension (i.e., in cuvettes) and eventually with microscope systems at the single cell level. In 1986, I was recruited by the late Martin Rodbell to a position at the National Institute of Environmental Health Sciences (one of the National Institutes of Health) in Research Triangle Park, North Carolina. Resources were made available to purchase state-of-the art instrumentation for Fura-2 measurements in cell suspensions. Also at this time, the late Haruo Takemura came to my laboratory from Sapporo, Japan. Haruo was keen to gain experience with fluorescent calcium indicators, and so we designed a simple project to get started. Basically, Haruo's assignment was to attempt to reproduce the experiments carried out at Wayne State showing that calcium could refill the intracellular stores independently of receptor activation, and without a global rise in cytoplasmic calcium. The protocol, similar to what we had