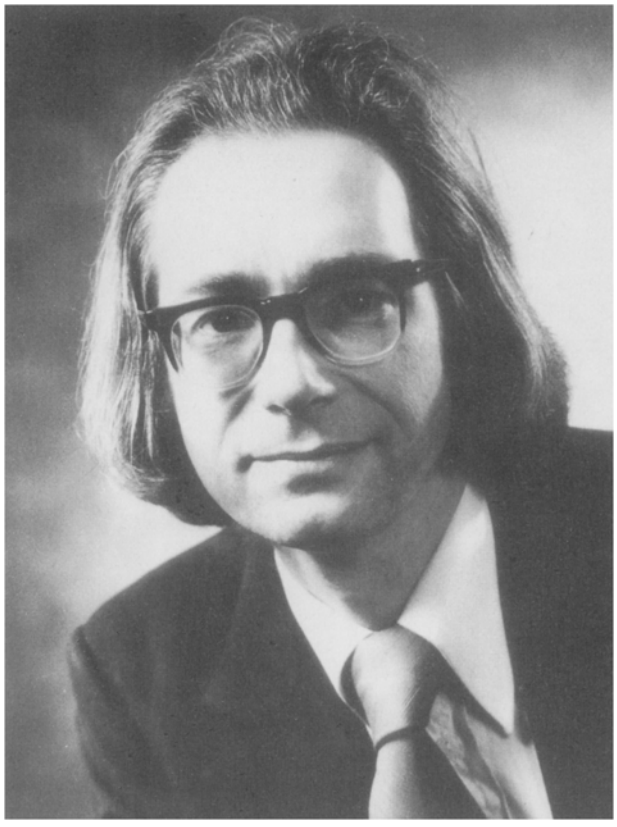
J. Membrane Biol. 103, 1–6 (1988)

The Journal of
Membrane Biology

In Memory of
Peter F. Baker



An Appreciation of Peter Baker

Peter Baker, who died suddenly on March 10, 1987, aged 47, served on the editorial board of The Journal of Membrane Biology for many years. His interests in the life sciences were diverse and his contributions in corresponding fields of research internationally acclaimed.

Peter was born in Lincoln, England, on March 11, 1939. He attended Lincoln School and from there won an open scholarship to Emmanuel College Cambridge where he read Natural Sciences, specializing in Biochemistry. His talent and enthusiasm for research were apparent at an early age when, before entering university, he undertook two research projects in the area of entomology, the results of which were subsequently published.

After graduating from Cambridge with a first, he began his graduate studies into phosphorous metabolism and ion transport in crab and squid nerve, under the supervision of Sir Alan Hodgkin. Although based in the Physiology Laboratory Cambridge, much of their research was conducted at the Marine Biological Association in Plymouth, and it was here that, together with Trevor Shaw, they showed nervous impulses could be conducted along a nerve in which the protoplasm had been removed and replaced by a solution containing low sodium and high potassium concentrations. This series of experiments led to several classical publications and refined the ionic theory of nervous conduction (Baker, Hodgkin & Shaw, 1962). The technique of replacing axonal protoplasm with a solution of defined chemical composition showed quite clearly that ATP was not needed for the conduction of nervous impulses. By physically removing axoplasmic ATPase activity, Baker and Shaw showed that plasmalemmal ATPase associated with the ouabain-sensitive Na efflux, i.e., the sodium-potassium pump, operated such that three sodium ions were transported per molecule of ATP split (Baker & Shaw, 1965).

His interests in ion transport moved on from the potassium-coupled sodium efflux to the component of the sodium efflux that was dependent on extracellular calcium (Baker, Blaustein, Hodgkin & Steinhardt, 1969). This, together with similar but independent findings by Reuter and Seitz in Switzerland using heart muscle, and also with the observations of his colleagues at Cambridge of a calcium efflux dependent on extracellular sodium, gave rise to the concept of a sodium-calcium exchange mechanism. The movement of calcium out of the cell coupled to the inward movement of sodium down its electrochemical gradient suggested a rather simple mechanism whereby cells were able to maintain their intracellular ionized calcium concentration at some one ten-thousandth of the extracellular level. Baker characterized the sodium-calcium exchanger in greater detail with Peter McNaughton (Baker & McNaughton, 1976) and in so doing revealed an uncoupled component of calcium efflux (the calcium pump). His interest in the sodium-calcium exchanger continued, and at the time of his death Baker was in the midst of organizing the first international conference on sodium-calcium exchange. The conference took place as planned at Stowe School, England, in July 1987 (Sodium Calcium Exchange, T.J. Allen, D. Noble & H. Reuter, editors. Oxford University Press, *in preparation* 1988). As the exchanger is electrogenic (the transport of 3 to 5 sodium ions being coupled to 1 calcium ion), its operation should be voltage dependent. Shortly before Baker died he, together with Jeff Allen, began to investigate the possibility that the voltage sensitivity of the exchanger could have some physiological role in the generation and control of cytosolic calcium. In particular, they were looking into the possibility that during an action potential the ionized calcium in squid axon could rise as a result of the exchanger operating in one direction, i.e., to exchange extracellular calcium with intracellular sodium, only to reverse direction after the action potential and so return the intracellular Ca^{2+} back to its original level (Allen & Baker, 1986).

A voltage-sensitive route for calcium entry into squid axon had been characterized in the early seventies when Baker, Hodgkin and Ridgway monitored intracellular Ca^{2+} with aequorin. They showed that, associated with a series of action po-

tentials, Ca^{2+} entered the cell, causing the intracellular Ca^{2+} concentration to rise. By using voltage clamp and potassium challenges to effect a step change in membrane potential, they showed that calcium entry could be divided into two phases, i.e., an early phase that was sensitive to TTX and a later one that was not. These two components of voltage-activated Ca entry were attributed to Ca entering through the sodium channel and also through a "late" calcium channel which inactivated more slowly (Baker, Hodgkin & Ridgway, 1971: Baker, Meves & Ridgway, 1973). These early findings were to focus attention for many years on voltage-operated calcium channels in excitable tissue.

Baker then considered the implications of the exchanger and the voltage-activated Ca channels in stimulus secretion coupling (Baker, 1972), and he subsequently investigated this with two of his students, Andrew Crawford and Tim Rink. In 1975 Baker and Crawford showed that, although inhibition of the sodium-potassium pump led to an increased transmitter release at the neuromuscular junction, it was unlikely that sodium-calcium exchange across the plasma membrane featured in the secretory process, and furthermore that if an increase in Ca^{2+} was triggered by an increase in intracellular sodium, it was through mobilization of Ca^{2+} from intracellular stores. Baker and Rink also presented evidence to support the idea that the exchanger did not feature predominantly in elevating Ca^{2+} to trigger secretion. However, they did suggest that voltage-activated calcium channels played a part in the stimulus secretion coupling (Baker & Rink, 1975). They showed that the transient secretory response seen when chromaffin cells are exposed to a maintained depolarizing medium was not a consequence of the exhaustion a releasable pool of transmitter from the cells, but rather of the transient nature of the channels through which calcium entered the cell. They suggested therefore that these voltage-activated, slowly inactivated calcium channels were similar to the late channels seen earlier by Baker in the squid giant axon.

By this time Baker had shown himself to be not only a very talented researcher but also an excellent teacher. He was appointed Halliburton Professor in Physiology at King's College London in 1975 and was elected a Fellow of the Royal Society a year later. Baker was able to pursue his research interests in the area of calcium homeostasis (Baker & Dipolo, 1984), and using the squid axon as an experimental model, branched out into other areas of physiology. Along with Tony Carruthers and Jeff Allen, he conducted studies on sugar and amino acid transport, cyclic nucleotide metabolism and signal transduction coupling. With Michael Whitaker, he also pursued another area of interest which had first attracted him at Cambridge, namely the mechanism underlying the elevation of the fertilization membrane of the sea urchin egg. This membrane appears to lift off away from the surface of an egg after fertilization and serves to prevent other sperm from reaching the surface. The mechanism through which this occurs involves exocytosis, i.e., the fusion of intracellular granules with the inner face of the plasma membrane and the discharge of their contents from the cell, via fusion pores. Baker and Whitaker adopted a technique, earlier introduced by Vacquier, to facilitate the preparation of fragments of plasma membrane with attached secretory granules and thereby to investigate the ionic requirements for the fusion and discharge of the granules with the plasma membrane. They showed, with others in America, that micromolar levels of Ca^{2+} were sufficient to trigger exocytosis, and presented evidence that suggested that the cytoskeleton was not involved in the secretory process (Baker & Whitaker, 1978; 1983).

I joined Baker soon after he moved to King's College, and in those early days we attempted to introduce Ca^{2+}-sensitive dyes into small adrenal medullary cells in the hope of quantifying the intracellular Ca^{2+} necessary to trigger exocytosis in these cells. The adrenal medullary cell was of particular interest as it represented both endocrine and neuronal tissue. After unsuccessful attempts to microinject dyes into the cells, we adopted the electroporation technique used earlier by Ulrich Zimmermann and his colleagues in Germany. The method involved breaching the plasma membrane of isolated cells by exposing them to intense electric fields of brief duration. The aim was to diffuse extracellular solutes, such as Ca^{2+}-sensitive dyes, through the electrically induced lesions and to trap them inside by resealing the plasma membrane. Although it proved possible to render the plasma membrane leaky and freely permeable to extracellular solutes of up to 1000 daltons, we could not induce the pores to reseal. The technique therefore unexpectedly provided a secretory cell with stable, localized lesions in the membrane and across which solutes could equilibrate. Thus we had before us a preparation in which we could control the chemical environment at the intracellular site of exocytosis (Baker & Knight, 1978). By such manipulation of the chemical content of the cytosol we soon showed that, provided ATP was present, micromolar levels of Ca^{2+} triggered secretion of catecholamine (Knight & Baker, 1982). The site of action of Ca^{2+} and the role of ATP was still not clear, however. If phosphorylation or dephosphorylation was involved, then the kinetics of release suggested that 2

Ca^{2+} ions were involved in each exocytotic event, and that they mediated a reaction in which the rate of phosphorylation (or dephosphorylation) regulated the rate of exocytosis. Together with Verena Niggli from Switzerland, we attempted to identify a key protein that was phosphorylated, but as there were so many phosphorylated proteins our results were inconclusive.

The leaky cell was in many ways akin to the skinned muscle preparation and allowed various intriguing hypotheses to be tested, the most interesting of which was the chemiosmotic hypothesis suggested by Harvey Pollard and his colleagues in America. In short, this hypothesis suggested that the electric and proton gradients across the membrane of the secretory granule were in some way a potential energy source necessary for exocytosis. As our preparation allowed us to measure these variables we were able to perform experiments to test the hypothesis directly. Our results showed quite clearly that there was little, if any, correlation between these gradients and the ability of the cell to participate in Ca^{2+}-dependent exocytosis (Knight & Baker, 1985).

Shortly before these experiments, a role for protein kinase C in secretion was suggested by Yasutomi Nishizuka and his colleagues in Japan. They had isolated this enzyme from secretory tissue and showed that, in the presence of phosphatidyl serine, it was activated by Ca^{2+}, the affinity for this cation being modulated by the presence of diacylglycerol or various phorbol esters. Nishizuka and his colleagues showed that secretion from platelets was triggered by these same agents and in a way that suggested secretion was much less dependent on Ca^{2+}. By introducing phorbol esters directly into electropermeabilized chromaffin cells we were able to show that they did indeed modulate secretion by increasing the affinity to Ca^{2+} of the secretory process (Knight & Baker, 1983). Since then we, and many others, have shown that other activators of protein kinase C increase the affinity to Ca^{2+} of the secretory process in various tissues.

Different preparations, however, respond in different ways to activators of protein kinase C, and Peter Baker suggested that three of the varying types of response could result from kinase binding Ca^{2+} and diacylglycerol in differing orders (Baker, 1986).

Even though protein kinase C seemed to be the most likely site of action of Ca^{2+} and ATP, the possibility that a calmodulin-dependent reaction could be involved in secretion from chromaffin cells was strongly suggested by the results of many workers, including those of Jose Trifaro and his colleagues in Canada. It seemed possible, for example, that the underlying Ca^{2+} activation curve for secretion could be a calmodulin-dependent process, whereas the modulation seen in the presence of diacylglycerol and phorbol esters could be mediated by protein kinase C. Shortly before Peter's death we embarked upon a series of experiments to dissect, pharmacologically, these two limbs of the secretory pathway. We were unsuccessful, however, in so far as we could not selectively inhibit the phorbol ester-sensitive component. Although the results of these experiments were not conclusive, Peter remained very excited and optimistic about future possibilities.

His plans involved not only a more detailed examination of the mechanism of exocytosis and of calcium homeostasis but also extended into other areas of life sciences. Had Peter not suffered a sudden heart attack it is certain he would have continued to make major contributions in these areas. The analysis of his work presented here is, of course, not complete and does not adequately appraise his gifts and talents, for in all he published over 120 papers and contributions to books.

The demands on Peter Baker were very great. He served not only on the editorial board of the Journal of Membrane Biology, but also as an editor for the Quarterly Journal of Experimental Physiology and Cell Calcium, and earlier as an editor for the Journal of Physiology. He was a member of various national research council committees and, as well as taking a keen interest in his own research, was the Head of a Department at a time when many colleges and departments were under constant threat of closure or reorganization. Nothing permeates the structure of a department so quickly and so thoroughly as bad management. It is a measure of Peter's qualities of leadership that over this difficult period the morale of the department was kept high and many of those within it were protected from this atmosphere of uncertainty and instability, and allowed to pursue their research without distraction. His unwavering enthusiasm for all that he did never failed to stimulate, and he was thus able to create an environment in which research and achievement flourished. His resilience and strength came not only from his unwavering beliefs, but also from the love and support of his wife and four children.

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