CLINICS IN ENDOCRINOLOGY AND METABOLISM

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AND BONE DISEASE

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Diabetes and Related Disorders
D. A. PYKE, MD, FRCP, *Guest Editor*

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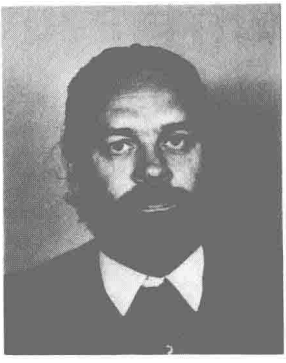

Foreword

In this first issue of *Clinics in Endocrinology and Metabolism* it may be useful to state the general editorial philosophy of the series. Each issue will be devoted to a specific aspect of the subject, and will constitute a review which will be complete in itself. The series is directed at clinicians specialising in endocrinology, and it is hoped that it will be useful to those in teaching hospitals as well as specialists in the non-academic environment. But the series is directed mainly at the latter group, who may not have as easy access to recent advances as their colleagues in teaching hospitals.

Our knowledge of medicine, as of the rest of biology, has moved forward at an accelerating rate in the last few years, and calcium metabolism is an area where advance has been most marked. This means that most textbook accounts of bone disease imply hopelessly outdated scientific concepts. I have therefore tried to ensure that the contributors to this volume on Calcium Metabolism and Bone Disease included both experienced clinicians and laboratory scientists.

It may seem strange that basic concepts form such a large part of a book for clinicians, but this was deliberate. Scientific advances have completely changed the theoretical framework of many clinical concepts about bone disease. Without an awareness of this fact, rational diagnosis and treatment of bone disease is impossible, and further clinical advances will be stultified. I hope that this book combines a modern clinical approach with the changed scientific basis on which it rests.

IAIN MACINTYRE

The Cellular Basis of Mammalian Calcium Homeostasis*

HOWARD RASMUSSEN

IONS AND EVOLUTION

Of all the alkaline earth cations, calcium and magnesium have by far the most important biological functions. In many respects the inorganic chemistry of these two metals is quite similar, but in certain key regards they differ (Gillard, 1970). This is even more true of their biological roles where their relationship may be that of antagonists, e.g. magnesium activates and calcium inhibits key glycolytic enzymes such as pyruvate kinase; that of serving as joint activators or inhibitors of the same process, e.g. phosphorylase b kinase; or of one or the other affecting a certain system which is little influenced by the other (Wyatt, 1964).

At first glance, it seems difficult to discern how such selectivity of ionic effects could be achieved in these biological systems. However, seen from its evolutionary point of view, the answer to this problem appears straightforward. To the best of our present abilities to reconstruct the nature of the milieu in which life first developed on this planet, it seems likely that this milieu or primodial sea was rich in K^+ and Mg^{2+}. If then the first simple proteins became catalysts under these circumstances, one of their likely attributes would be an ability to operate most effectively in a K^+ and Mg^{2+} environment.

With the advent of the limiting membrane and self reproduction, the first primitive cells appeared. We must imagine that the membranes of these cells did not (initially) possess ion pumps, such as the Na^+–K^+-activated ATPase so characteristic of most animal cells. However, very gradually over aeons of time, the composition of the sea shifted, becoming rich in Na^+ and Ca^{2+} and poorer in K^+ and Mg^{2+}, so the functions of the cell had to change

*Supported by funds from the United States Atomic Energy Commission (AT(30-1)3489) and the United States Public Health Service (AM-09650).

(Rothstein, 1964). Either they gave up their former internal sea, and their enzymes then adapted to the Na^+–Ca^{2+} milieu, or they developed ways of preserving their original internal sea. It became necessary either to limit the entry of these new ions, or to return them to the external sea after their entry, all the while retaining K^+ and Mg^{2+}. In the latter cases, this meant the maintenance of an asymmetric distribution of ions across the membrane which in turn meant the expenditure of energy.

The evolutionary solutions were the semipermeable cell membranes and its ion pumps which conferred upon the cell the additional attributes of excitability and of osmotic or volume, as well as ionic, regulation (Tosteson, 1964). Thus, from very ancient times Na^+ and Ca^{2+} and their respective pumps in the cell membrane have been key components of the process of excitation in animals cell. Hence the matter of the selection of certain ions for specific biological function is more an historical than a chemical selection. It depends upon the interaction of geological with biological evolution.

Not only was this original internal sea rich in K^+ and Mg^{2+} maintained as the normal intracellular ionic environment, but with the advent of the next major evolutionary change, that of the development of multicellular life, the younger but still ancient Na^+ and Ca^{2+}-rich sea was incorporated and maintained as the normal extracellular environment. In the process, the cellular mechanisms developed to maintain intracellular ion homeostasis were adapted and extended to maintain extracellular ion homeostasis.

CELLULAR IONIC HOMEOSTASIS

If this historical view is a correct one, it follows that maintenance of intracellular Na^+ and Ca^{2+} homeostasis became a critically important aspect of normal animal cell function. As the major cation in terms of extracellular concentration, Na^+ became the ion of major concern in membrane excitability. In addition, control of its intracellular concentration became of decisive importance in regulating cell volume (Tosteson, 1964). Because Na^+ forms complexes with few biologically important organic or inorganic anions, changes in its concentration are not greatly influenced by changes in relative anion concentrations within the cell. On the other hand, Ca^{2+} because of its lower concentration, and its marked propensity to form complexes with organic and inorganic anions, particularly phosphates, became of great importance as a regulator of intracellular systems following cell activation. As a consequence of these differences, the mechanisms for regulating cellular Na^+ and cellular Ca^{2+} homeostasis are quite different.

The major control of Na^+ metabolism takes place at the cell surface. The permeability of the cell membrane to Na^+ and the activity of the Na^+ pump of this membrane determine respectively the influx and efflux of Na^+ across the cell membrane, and thereby cellular Na^+ homeostasis. There is no evidence of Na^+ pumps as components of intracellular membranes with the possible exception of the nucleus.

In contrast, cellular calcium metabolism is considerably more complex (Figure 1). In addition to a leak and pump system in the cell membrane, there are similar systems in both mitochondrial and microsomal membranes

(Borle, 1967; Chance, 1965; Ebashi and Endo, 1968; Rasmussen, 1966). The latter, in muscle at least, can be viewed as an extension of the plasma membrane system, but the mitochondrial system is clearly different in its characteristics, function, and requirements. All three of the pumps are oriented in such a way that they actively remove calcium from the cell cytosol. This means that it is the concentration of cytosolic calcium which is most highly controlled and therefore probably of critical importance in the regulation of cell function. It follows from this supposition that regulation of cytosolic calcium concentration may and does differ considerably from cell type to cell type, depending upon the relative calcium transport activities of the three membrane systems (Bianchi, 1969); and furthermore that control of calcium fluxes across intracellular membranes is an important part of the mechanisms underlying cellular calcium homeostasis.

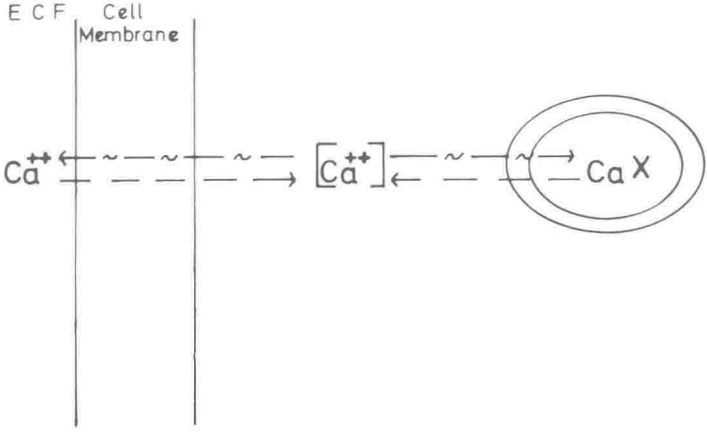

Figure 1. A stylised model of the basic systems involved in cellular calcium homeostasis. In addition to a leak and pump system in the cell membrane, there is a similar system in one or more intracellular membranes particularly mitochondria and microsomes. —~— represents active transport processes, — — — passive transport reactions, and CaX bound calcium.

A later evolutionary event of great importance for the development of higher life forms was the advent of the calcified endoskeleton. By the time of this development, the means for regulating cellular and extracellular calcium homeostasis had become quite highly developed. Thus it was likely that the new needs for maintaining extracellular calcium homeostasis in an internal environment containing a calcified endoskeleton were met by an adaptation and extension of those already highly developed mechanisms for maintaining cellular calcium homeostasis. This likelihood is supported by recent experimental evidence. Of all this evidence, that of central importance to an understanding of the cellular basis of mammalian calcium homeostasis concerns the interrelated roles of calcium and cyclic AMP in cell activation (Namm, Mayer, and Maltbie, 1968; Rasmussen, 1970; Rasmussen and Tenenhouse, 1968).

CALCIUM AND CYCLIC AMP IN CELL ACTIVATION

Following their original detection of cyclic AMP as an intermediate in the activation of hepatic glycogenolysis by epinephrine, Sutherland and co-workers (Sutherland and Rall, 1960; Sutherland, Øye and Butcher, 1965; Robison, Butcher and Sutherland, 1968; Sutherland, Robison and Butcher, 1968) went on to establish that this cyclic nucleotide is found in nearly all animal cells, and that it is an intermediate in the activation of many different kinds of animal cells by specific extracellular stimuli. These findings led to the proposal of the second messenger hypothesis (Figure 2). In this model of cell activation the specific extracellular or first messenger interacts with the adenyl cyclase on the cell surface leading to an increase in the rate of synthesis

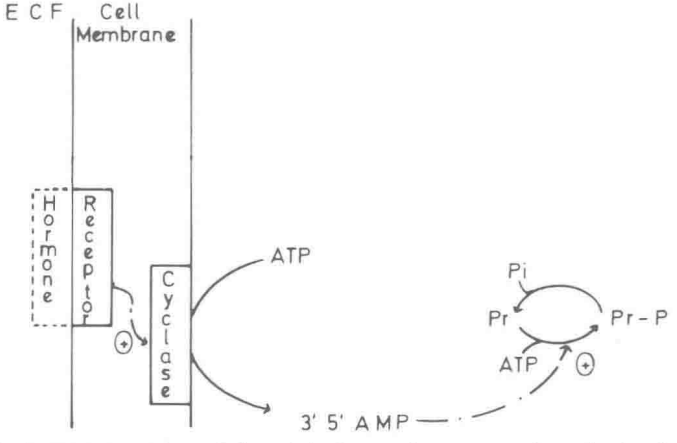

Figure 2. A current version of the original second messenger hypothesis of Sutherland, Øye and Butcher (1965). Hormone–receptor interaction leads to an activation + of membrane-bound adenyl cyclase, and the rise in intracellular 3′,5′-AMP activates a group of enzymes, protein kinases, which in turn phosphorylate one or more proteins (Pr–P) and thereby determine the physiological response of the cell. (Pi is inorganic phosphate.)

of cyclic AMP from ATP, resulting in an increase in the intracellular concentration of this cyclic nucleotide or second messenger. In order to account for the diverse physiological consequences of first messenger action on different cell types, it was originally proposed that the second messenger exerted very different effects in different tissues (Sutherland et al, 1968). However, more recently a class of cyclic AMP-dependent enzymes have been found in nearly all cells, the protein kinases, which catalyse the phosphorylation of specific proteins utilising ATP as the phosphate donor, and it has been concluded that this is the mode of second messenger action (Corbin and Krebs, 1969; Walsh, Perkins, and Krebs, 1968; Kuo and Greengard, 1969).

In its present form the second messenger hypothesis can be pictured as an initial activation of adenyl cyclase by first messenger, a rise in intracellular 3′,5′-AMP concentration, an activation of one or more protein kinases with the resultant phosphorylation of one or more specific proteins which are in turn the eventual mediators of the cellular response (Figure 2).

However attractive this model may be, it does not take into account a number of other facts concerning cell activation. In particular, calcium ions also have a widespread role in the physiological expression of first messenger action (Table 1), and not all the cellular effects of first messengers are easily accounted for in terms of adenyl cyclase activation (Shanfeld, Frazer, and Hess, 1969; Langstet and Øye, 1970).

Table 1. *Cellular Systems in which both Ca^{2+} and 3′,5′-AMP have been Observed to Play a Role in Cell Activation*

Cell	Stimulus	Response	Ca^{2+} required	3′,5′-AMP produced
Synapse	Electrical	Transmitter release	+	+
Neuromuscular junction	Electrical	Transmitter release	+	+
Anterior pituitary	GHRF	Growth hormone release	+	+
Anterior pituitary	LHRF	LH release	+	+
Anterior pituitary	TRF	TSH release	+	+
Posterior pituitary	Electrical	Vasopressin release	+	?
Salivary gland	Epinephrine	Amylase release	+	+
Beta cell, pancreas	Glucose	Insulin release	+	+
Adrenal cortex	ACTH	Steroid release	+	+
Liver	Glucagon	Glucose synthesis and release	?	+
Thyroid	TSH	Thyroxine release	+	+
Corpus luteum	LH	Progesterone release	+	+
Stomach	Histamine	HCl secretion	+	+
Heart	Epinephrine	Glycogenolysis	+	+
Toad bladder	Vasopressin	$Na^+ + H_2O$ transport	?	+
Kidney tubule	PTH	Gluconeogenesis	+	+
Melanocyte	MSH	Melanin dispersion	+	+
Slime mould	?	Aggregation	+	+
Sea urchin egg	Sperm	Fertilisation	+	+
Adipocyte	Epinephrine	Lipolysis	+	+
Fly salivary gland	5-Hydroxy-tyramine	Secretion	+	+
Red blood cell	Epinephrine	Change in filterability	+	+
Thymocyte	PTH	Increased mitotic rate	+	?

The first important question concerning this second messenger hypothesis is the matter of whether or not adenyl cyclase activation is the sole effect resulting from the interaction of the first messenger with its receptor site on the cell surface. In many systems at least one additional effect has been noted (Rasmussen, 1970; Borle, 1968; Prince, Berridge, and Rasmussen, 1972). The first messenger stimulates the uptake of calcium into the cell (Figure 3). However, this might not invalidate the model depicted in Figure 2, if this uptake of calcium resulted from an effect of cyclic AMP within the cell rather than from a direct effect of first messenger on the cell membrane. This is not the case. Concentrations of exogenous cyclic AMP, sufficient to activate the particular cell, do not cause an increase in the entry of calcium into the cell. On the other hand, in most of these systems, the first messenger still activates adenyl cyclase in the absence of external calcium (Rasmussen and Nagata, 1970; Namm et al, 1968; Prince et al, 1972). The simplest hypothesis to

account for these facts is one in which first messenger–receptor interaction leads to both an increase in the calcium permeability of the plasma membrane, and an activation of the membrane-bound enzyme, adenyl cyclase (Figure 4).

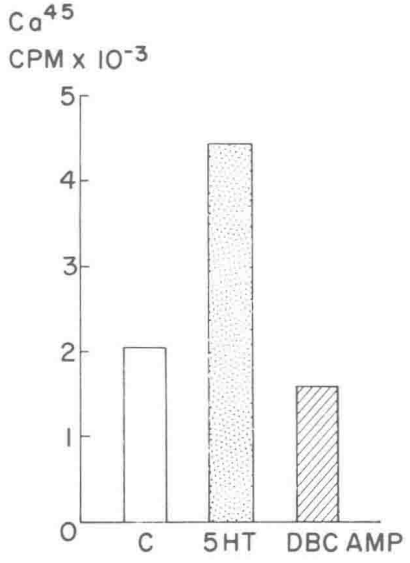

Figure 3. The effect of 5-hydroxytryptamine, 10^{-8}M, and dibutyryl cyclic AMP, 10^{-2}M, upon the uptake of calcium-45 by the isolated fly salivary gland (Prince et al, 1972), compared with controls.

In spite of the demonstrated effect of first messenger upon calcium entry, it is possible to question the importance of this effect in terms of cell activation, because exogenous cyclic AMP will, if added in high enough concentration, usually mimic many of the physiological effects of first messenger without altering calcium entry. However, before reaching such a conclusion it is important to note several other facts. Firstly, in many, but not all, of these cellular systems activation of the cell to perform its specific function

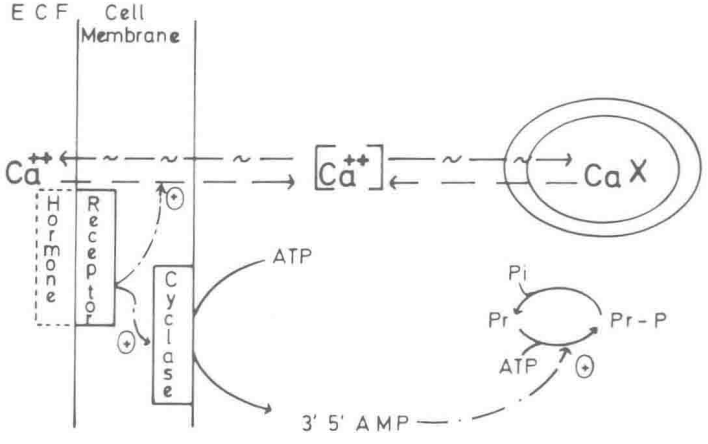

Figure 4. A model of cell activation in which hormone–receptor interaction has at least two simultaneous effects upon cell membrane function: an activation of membrane-bound adenyl cyclase; and an increase in calcium permeability.