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THE ROLE of CALCIUM in BIOLOGICAL SYSTEMS

Volume III

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FOREWORD

Calcium must certainly be the major bioelement of the times. Only a generation ago Ca^{2+} was known to physiologists and biochemists as a component of bone mineral and as a blood plasma constituent required in heart function and blood coagulation, but little more. Only a few, such as Baird Hastings and Walter Heilbrunn, saw more clearly into the future of Ca^{2+}, a future that was a long time coming. Then came the discovery of the role of Ca^{2+} in the contraction-relaxation cycle of skeletal muscle and the recognition that the free Ca^{2+} concentration of the resting sarcoplasm must normally be orders of magnitude lower than that in the blood plasma. Thus it was found that skeletal muscle must possess extremely efficient energy-dependent Ca^{2+} pumps. The discovery that mitochondria can accumulate Ca^{2+}, by my colleagues Vasington and Murphy, was at first regarded by many as an anomaly of in vitro conditions, since Ca^{2+} had earlier been found to uncouple oxidative phosphorylation. How could oxidative phosphorylation and Ca^{2+} transport be compatible? What possible role can mitochondria play in cellular Ca^{2+} distribution? And why does calcium phosphate form insoluble but noncrystalline granules in the mitochondrial matrix?

Answers to these and other questions came slowly at first, but in the 1970s a crescendo of Ca^{2+} research developed. Today we know dozens if not hundreds of different cellular and extracellular processes that are regulated by changes in the level of cytosolic or extracellular Ca^{2+}, in which at least three different membrane systems of the cell take part. Indeed, Ca^{2+} is now emerging as a most important and ubiquitous intracellular messenger, perhaps even broader in function than cyclic AMP, the original second messenger. What is even more remarkable is that cytosolic Ca^{2+} levels can regulate several different activities simultaneously in a single cell, raising fundamental questions regarding spatial and temporal regulatory fluctuations in cytosolic Ca^{2+} concentrations. Also remarkable are the biochemical mechanisms that keep calcium and phosphate, which occur in extracellular fluids and urine in supersaturating concentrations, from precipitating and turning us into stone. Central to all these questions is the chemistry of Ca^{2+}, its special features that endow it alone, of all the common cations, to participate in such a panoply of biological activities.

The papers in this volume address many aspects of these problems in the biochemistry and physiology of calcium and provide an important guide to recent progress.

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PREFACE

The purpose of this review is to summarize and correlate the recent advances in several fields of scientific research related to the involvement of calcium in the structure development and function of biological systems.

Considering the general interest in calcium, this publication which is a comprehensive collection of contributions on the biochemical properties of the ion, is aimed to be of interest to workers in many fields of biology and medicine whose investigations might be related, directly or indirectly to the role of this ion in biological systems. In addition to the benefit of presenting a concise review of the state-of-the-art on each subject, it will provide a useful reference source of the work done in a wide range of scientific disciplines such as biochemistry, analytical chemistry, cell biology, physiology, nutrition, pathology, pharmacology, toxicology, etc.

The text consists of six major divisions. The first deals with the chemistry of calcium and gives both the theoretical and practical basis to interpret the role of this element in the function of normal and pathological biological systems, as described by the other subsequent divisions.

It is not the aim of this publication to provide an exhaustive compilation of all the subjects concerning the biochemistry of calcium, but to give within the limits of the present work the most important and actual highlights related to this bioelement. In most instances the given information has been made as concise as possible to make feasible the coverage of all the different subjects, but without sacrificing the updated bibliographic references which constitute a quick access to the ultimate source of knowledge. To the contributors and publisher who have made possible this publication we are very much indebted.

Leopold J. Anghileri
Anne Marie Tuffet-Anghileri

THE EDITOR

Leopold J. Anghileri, Dr. Chem. is Senior Scientist and Research Group Leader at the Laboratory of Biophysics, Service of Nuclear Medicine and Diagnostic by Ultrasons and Thermography, Medicine Faculty B, University of Nancy, France. Dr. Anghileri graduated in 1951 from Buenos Aires University where he obtained his Dr. Chem. degree in 1957. Dr. Anghileri became a member of the American Chemical Society in 1961. Until 1964 he was Investigator at the Argentine Atomic Energy Commission. From 1964 to 1968 he was a fellow at the Johns Hopkins Medical Institutions, Division of Nuclear Medicine and Radiation Health, Institut de Radium, Laboratoire Curie, Paris, and Institut für Nuklearmedizin, Deutsches Krebsforschungzentrum, Heidelberg, West Germany. During the period 1970 to 1975 he was Investigator at the Tumorforschung, Ruhr Universität, Essen, West Germany.

Dr. Anghileri has presented invited papers at international meetings. He has published more than 210 research papers. His current major research interest includes hyperthermic treatment of cancer and the calcium-magnesium membrane relationship in tumors.

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The late **Anne Marie Tuffet-Anghileri, Dr. Sci.** was Investigator at the Centre National de la Recherche Scientifique, Paris. Dr. Tuffet-Anghileri graduated in 1969 from Paris University. She worked in carcinogenesis at the Laboratoire Curie, Paris and in Molecular Biology (DNA-metal ions interactions) at the Paris University.

To my wife and dedicated co-worker Anne Tuffet-Anghileri (1937-1981) whose life was sacrificed for and by the Science.

L. J. Anghileri

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Robert Neher

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I. INTRODUCTION

When adrenocortical or gonadal tissue is stimulated by physiological concentrations of the trophic hormones corticotropin (ACTH) and lutropin (LH), respectively, a rapid neosynthesis of steroid hormones occurs. In this process, as in many similar processes of cell communication,[1] extracellular Ca^{2+} appears to be as important for the stimulatory response as the hormone itself. In discussing this particular role of Ca^{2+}, we shall refer mainly to adrenocortical tissue where most of the experimental data were obtained. These will be complemented by data obtained in gonadal tissue, considering that effects of Ca^{2+} may differ due to the use of different species or tissue preparations, such as perfused glands, tissue slices, isolated or cultured cells, or cell-free homogenates. The most reliable and abundant data are derived from work with isolated adrenal cells of the zona fasciculata-reticularis. This review will not deal with the effects of Ca^{2+} on the release of the respective trophic hormones.

II. THE GENERAL ROLE OF EXTRACELLULAR Ca^{2+}

In 1953, Birmingham et al.[2] were the first to report a requirement for Ca^{2+} in the action of ACTH on steroidogenesis in rat adrenal tissue in vitro. The Ca^{2+}-dependence of steroidogenesis in rat Leydig cells stimulated by LH was reported by Janszen et al.[3] in 1976.

Before discussing in detail the role of extracellular Ca^{2+} in the acute action of a trophic steroidogenic hormone or an equivalent agonist, it seems appropriate to review briefly the present knowledge about the mechanism of action as exemplified by the acute action of ACTH in isolated adrenocortical cells of the zona fasciculata-reticularis (Figure 1).[4-13]

According to this simplified scheme, ACTH binds to a plasma membrane receptor to be coupled to the membrane bound adenylate cyclase complex (AC) which, in the presence of its GTP-binding subunit and Mg^{2+}, converts ATP to cyclic AMP (pool 1). The intracellular level of cyclic AMP is determined by its rate of formation, its rate of degradation by phosphodiesterase (PDE) to 5′-AMP, and by its distribution into several intracellular pools and the extracellular space. The degree of steroidogenesis appears to be determined by the level of cyclic AMP in pool 3 where it is bound to the regulatory subunit (R_2) of a cyclic AMP-dependent protein kinase (R_2C_2), thus, activating the enzyme by dissociation of the free catalytic subunits C_2. The activated protein kinase phosphorylates specifically one or more preformed and relatively labile protein substrates. These P-proteins, by an unknown mechanism involving other labile proteins and possibly microfilaments and microtubuli, might promote the translocation of precursor cholesterol across the inner mitochondrial membrane to its matrix site. This process is considered to be the rate-limiting step in the stimulation of steroidogenesis. Once cholesterol has reached the cytochrome P_{450} subunit of the mixed function oxidase in the same compartment it binds immediately to it and, in the presence of NADPH and molecular oxygen, is split into pregnenolone and a C_6-fragment. Pregnenolone is subsequently converted in several steps in various compartments to corticosteroids which leave the cell. When adrenocortical cells are stimulated by cholera toxin, by extracellular cyclic AMP, or a metastable Ca^{2+} complex, the same mechanism of action exists involving the activation of a protein kinase, although the extent to which Ca^{2+} is required varies greatly as discussed below. Adrenocortical cells of the zona glomerulosa which are specialized to produce aldosterone can be stimulated, in addition to the previously mentioned agonists, by angiotensin and high K^+. When stimulated by these latter two agonists, the left part of the mechanism as shown in Figure 1 has to be replaced at least in part by another, yet unknown, mechanism since cyclic

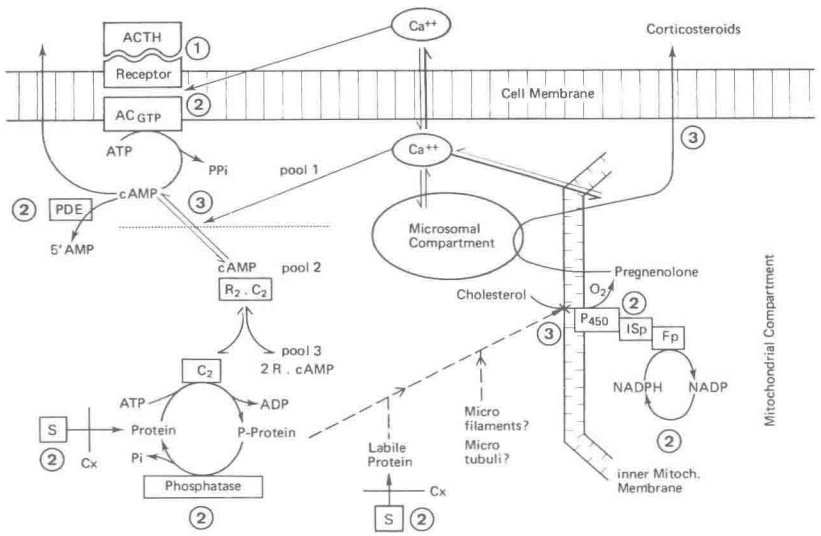

FIGURE 1. Mechanism of acute stimulation by ACTH of adrenocortical steroidogenesis. Effects of Ca^{2+} on ① hormone binding; ② enzyme regulation; ③ translocation of products. ACTH = adrenocorticotropin; cAMP = cyclic 3′5′-adenosinemonophosphate, cyclic AMP; AC = adenylate cyclase; PDE = phosphodiesterase; R_2C_2 = protein kinase holoenzyme; S = protein synthesis; Cx = cycloheximide; P_{450} = cytochrome P_{450}; ISp = iron-sulfur protein (adrenodoxin); Fp = flavoprotein (adrenodoxin reductase).

AMP does not seem to be involved.[14,15] Nevertheless, the action of these two regulators of aldosterone production is known[14-16] to be as Ca^{2+}-dependent as the steroidogenic response to ACTH.

Since 1972, various groups studying ACTH-induced steroidogenesis[14,17-22] reported that at low or physiological levels of ACTH (10^{-13} to 10^{-10} *M*), the Ca^{2+} requirement is absolute, but at higher levels to ACTH the Ca^{2+} requirement diminishes. The ED_{50} of ACTH for steroid formation increases for several orders of magnitude with decreasing extracellular Ca^{2+}, whereas the intrinsic activity or maximal capacity of steroid production decreases only about half, unless an excess of the Ca^{2+} chelator EGTA eliminates virtually all Ca^{2+} and abolishes any steroid formation. Interesting enough, Ways et al.[23] found that the ACTH analog $ACTH_{6-39}$ acted as a weak agonist at high Ca^{2+} concentration and as an ACTH antagonist at low Ca^{2+} concentration.

In contrast to the ACTH-induced steroidogenesis, cyclic AMP or cyclic GMP-induced steroidogenesis proved to be much less Ca^{2+} dependent.[3,17-21,24,25] This is also apparent from the fact that cyclic AMP-induced steroidogenesis is less sensitive to Ca^{2+}-antagonists, such as verapamil, ruthenium red, or La^{3+} than ACTH-induced steroidogenesis.[26-28] The stimulatory effect of cyclic nucleotides can be observed even in the presence of excessive EGTA. These findings indicate that the requirement for Ca^{2+} in ACTH action, while involved in more than one step, is greater for events preceding the formation of cyclic AMP than for those that follow. It can be concluded that Ca^{2+} has an important activating effect on the ACTH-receptor-adenylate cyclase complex (cf. Section V).

Choleratoxin is a potent pharmacological stimulator of cyclic AMP and steroid synthesis in steroidogenic tissues.[14,20,29] This stimulus appears to be less dependent on extracellular Ca^{2+} than ACTH. It is assumed that the activation of adenylate cyclase by the toxin does not involve the coupling of the hormone receptor to the catalytic subunit, but is caused by the inhibition of GTP hydrolysis.[30-32]

A few years ago, Neher and Milani[26,33-35] found that acute steroidogenesis in isolated adrenocortical cells can be triggered, in the absence of ACTH, choleratoxin, or extracellular cyclic nucleotides, by extracellular Ca^{2+} alone when this cation is presented as a metastable complex under specific conditions (cells primed for Ca^{2+}). Recently, Shima[15] and Yanagibashi[27] also observed a similar stimulatory effect of Ca^{2+} in the absence of ACTH in rat glomerulosa cells and bovine fasciculata cells, respectively. This Ca^{2+} trigger proved to be a valuable tool for the elucidation of the role of Ca^{2+} in the mechanism of action of ACTH-induced steroidogenesis.

In the following sections, the various possible sites of action of Ca^{2+} will be discussed in detail (cf. sites ①, ②, and ③ in Figure 1). It may be pointed out that most of these effects are highly specific for Ca^{2+} with the exception of Sr^{2+} which is able to substitute for Ca^{2+} at equal or slightly higher concentrations.[34,36-39]

III. CELLULAR Ca^{2+} UPTAKE OR EXCHANGE

The absolute requirement for extracellular Ca^{2+} in steroidogenesis induced by low ACTH concentrations or by the metastable Ca^{2+} trigger suggests that some Ca^{2+} has to be bound or taken up by the cell. In fact, Leier and Jungmann[40] described a stimulus-dependent net accumulation of $^{45}Ca^{2+}$ in whole adrenal glands in vivo and in vitro which was not only an increased rate of Ca^{2+} exchange. Unfortunately, it is not clear how these changes are related to the actual steroidogenetic process of the zona fasciculata cells, since Leslie and Borowitz[41] reported some evidence for a Ca^{2+} pump in plasma membranes of adrenal medulla but not adrenal cortex. Some $^{45}Ca^{2+}$ accumulation was found only in adrenocortical Golgi apparatus fraction. In contrast, Laychock et al.[42] reported some slowly increased Ca^{2+} uptake by a bovine adrenocortical 27,000 × g particulate fraction after stimulation by high ACTH concentrations in vitro or by several nucleotides of which some were nonsteroidogenic. The physiological significance of this effect remains to be elucidated.

Experiments by Jaanus and Rubin[43] with perfused cat adrenal glands produced no evidence for a net Ca^{2+} uptake by ACTH, but the rate of Ca^{2+} efflux was found to be reduced. ACTH seemed to cause an intracellular translocation of Ca^{2+} rather than an uptake of extracellular Ca^{+2}. Similarly, in isolated rat adrenocortical cells, no ACTH-induced Ca^{2+} uptake or exchange could be detected,[35] which is in agreement with the finding of Matthews and Saffran[37,44] that ACTH-induced steroidogenesis in normal Ca^{2+}-containing Krebs Ringer solution was not accompanied by a change in transmembrane potential. Nevertheless, Yanagibashi et al.[27,45] reported a dose-dependent increase in Ca^{2+} uptake in rat adrenocortical cells stimulated by 10^{-10} *M* ACTH which could be inhibited by 10^{-5} *M* verapamil. Although Ca^{2+} ionophores such as A23187 promoted a marked $^{45}Ca^{2+}$-uptake, they were found to inhibit steroidogenesis in intact cells[28,34,46,47] in the presence or absence of $Ca.^{2+}$ It is likely that Ca^{2+} ionophores are unspecifically disturbing the accurately balanced Ca^{2+} distribution in various intracellular compartments,[48,49] thus, interfering with many enzymatic processes and inhibiting, e.g., protein synthesis.[46] However, under different conditions such as in adrenocortical slices, basal, and ACTH-induced steroidogenesis, but not cyclic AMP-induced steroidogenesis, was reported[50] to be increased by A23187 in the presence of Ca^{2+}. According to tissue preparation or to conditions, cyclic AMP formation is inhibited by A23187[35,46] or not.[47]

So far, the question of increased Ca^{2+} uptake or exchange in stimulated adrenal cells of zona fasciculata-reticularis remains controversial. This seems to be due to differing experimental procedures of cell preparation and particularly to analytical limitations in the study of Ca^{2+} metabolism in isolated cells. These cells are maintaining a Ca^{2+}-gradient between 10^{-7} to 10^{-2} *M* within the various intracellular compartments.[48,49]