Calcium in Drug Action

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PREFACE

Anyone surveying physiological and pharmacological journals can readily see that the biological actions of calcium ion are of increasingly widespread current interest. The scope of investigated actions and reactions in which a role for calcium ion is of some importance is so numerous as to convey the impression that calcium ion is everywhere and interacts with everything. This being so, the challenge in contemporary research is to focus on investigation of those actions of calcium ion which, in some manner, influence either significant physiological parameters or the manner in which pharmacological agents act.

This multi-authored book originated from a more limited Symposium on "Importance of Calcium as a Primary Locus of Drug Action" co-chaired by myself and Dr. Frank R. Goodman at the April, 1977 FASEB meeting in Chicago. This Symposium was organized in response to a perceived need for increased communication among workers in different areas of Ca^{2+}-related research. In the process of selecting the maximum of six areas for presentation within the format provided, it soon became apparent that this would result in only a limited sampling of current research efforts. Expansion of the number of areas to fourteen within a book format appeared to be the most logical mechanism to provide a more coherent interdisciplinary approach to consideration of various aspects of the roles of Ca^{2+} in drug action. This is not to imply that all relevant Ca^{2+}-related areas are surveyed within this volume. Rather, the fourteen chapters represent a sampling of the current status of our knowledge of Ca^{2+} as an essential component of the basic mechanisms by which various types of drugs exert their actions.

The specific goal of this volume is to foster an interdisciplinary approach to consideration of calcium in drug action. By this, I mean that Ca^{2+}-related research is usually system-oriented and included in sessions devoted to aspects of, for example, smooth muscle or secretory mechanisms. In contrast, there is no clearly delineated constituency for Ca^{2+} as a unitary field of research. However, there is a recognized need to compare and contrast the differing techniques and approaches employed to investigate the roles of Ca^{2+} as a basis for and a modifier of drug action. Hopefully, presentation of the varied cellular and subcellular actions of Ca^{2+} in different systems within the context of a single volume will encourage more widespread application of relevant techniques and approaches as well as increased communication within this general research area.

The organization of this book is intended to facilitate use by all investigators and students interested in any aspect of the cellular and subcellular basis of the roles of calcium in drug action. The fourteen areas considered have been arranged into three distinct sections emphasizing approaches to qualitative and quantitative analysis of Ca^{2+} distribution and movements (Chapters 1-4), subcellular sites and interactions of Ca^{2+} and drugs (Chapters 5-8), and the varied roles of Ca^{2+} in drug action in specific biological systems (Chapters 9-14). This separation is one based primarily on degree of emphasis and orientation. In all chapters, the objective is a definitive understanding of the cellular and molecular roles of Ca^{2+} in drug action.

As editor, I wish to convey my deepest appreciation to all of the contributors to this volume. The manner in which this volume was prepared necessitated a particularly high degree of coordination with respect to deadlines, preciseness of manuscript preparation and copy editing. The exceptional level of responsiveness and capability on the part of all of the authors made my job as editor a far less burdensome task than is often the case. It has been a rewarding experience to work with the contributors to this volume. Also, I want to thank Davida, Debbie and Bill Weiss for their patience during the many hours I spent preparing the final version of this volume and for their assistance in preparation of the subject index. Finally, the most important of all acknowledgements is due to Roma L. Chapin who did a superlative job in preparing the final copy of this entire volume.

George B. Weiss

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SECTION I

ANALYSIS AND ALTERATION OF CALCIUM ION DISTRIBUTION AND MOVEMENTS

The chapters in this first section focus especially on how movement of Ca^{2+} occurs across the cell membrane, how this movement is facilitated or regulated, and how drug-induced alterations in Ca^{2+} movements can produce changes in cellular responsiveness. The primary concern, in each case, is the cellular basis of the role of Ca^{2+} in coupling excitation to contraction. Though the techniques employed are varied and the approaches appear quite dissimilar, the actual conclusions derived are not basically different.

In the first chapter, evidence is summarized for the entry of Ca^{2+} current through a specific Ca^{2+} channel and the manner in which inorganic (divalent and trivalent ions) and organic (verapamil and related compounds) Ca^{2+} antagonists alter Ca^{2+} binding and entry. By use of structural comparisons of Ca^{2+} antagonists and of Ca^{2+} ionophore actions as well, the possibility is raised that comparative data obtained with these agents might help to elucidate the molecular nature of the Ca^{2+} entry processes. In the second chapter, the sarcolemmal origin of that Ca^{2+} important in excitation-contraction coupling in heart cells is delineated. Altered permeabilities of cultured heart cell membranes are employed to examine mechanisms of Ca^{2+} entry as well as effects of stimulatory and inhibitory agents on superficial Ca^{2+} binding. The third chapter is concerned with the binding of Ca^{2+} at two types of sites and the quantitative relationships between these two sites and corresponding Ca^{2+} uptake and washout components. The fourth chapter discusses mechanisms by which Ca^{2+} is transported across cell membranes from depots and/or transport

sites. Both the third and fourth chapters consider drug actions in terms of alterations induced in Ca^{2+} binding and uptake parameters.

All four of these initial chapters are concerned with the quantitative aspects of Ca^{2+} distribution and movements in smooth or cardiac muscle systems. The variety of techniques successfully employed include electron microscopic, histochemical, isotopic and electrophysiological ones. The level and precision of these approaches are increasingly cellular in nature and provide varied evidence of the primary involvement of Ca^{2+} as an essential modulator of actions of various drugs.

CHAPTER 1

CALCIUM, CALCIUM TRANSLOCATION, AND SPECIFIC CALCIUM ANTAGONISTS

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INTRODUCTION

It is now almost one hundred years since Sidney Ringer (1882) described the importance of Ca^{2+} in the maintenance of frog heart contractility. Subsequent to this observation, it has been increasingly recognized that Ca^{2+} plays a critical and central role in a multitude of biological events at both the intra- and extracellular levels (Duncan, 1976; Kretsinger, 1976a; Table 1). However, Ca^{2+} distribution across the cell membrane is far from equilibrium, since if the resting membrane potential (~-60mV) were equal to the Ca^{2+} equilibrium potential, then the intracellular Ca^{2+} activity should be some 100-fold greater than the extracellular activity. This is quite clearly not so and although accurate measurements of free ionized intracellular Ca^{2+} concentrations have not been made in many systems the concensus of evidence firmly indicates that $[Ca^{2+}_{int}] < 10^{-7}M$ (Baker, 1972; 1976; Blaustein, 1974; Reuter, 1973). Such a low intracellular Ca^{2+} concentration accords with the binding constants of Ca^{2+} for those intracellular proteins whose activity is known to be modulated by Ca^{2+} (pK_D values ~ 6-7; Kretsinger, 1976a,b) and indicates the "trigger" function of an increased intracellular Ca^{2+} concentration (Heilbrunn, 1956).

The very large driving force for Ca^{2+} entry indicates that there must exist specific mechanisms for the removal of intracellular Ca^{2+}. A schematic representation of the several processes that are involved in the regulation of intracellular Ca^{2+} is given in Figure 1. Subsequent to entry Ca^{2+} may be removed through complexation with cytoplasmic constituents (including the internal membrane surface), or by sequestration into the intracellular

TABLE I. PARTIAL LISTING OF CALCIUM-DEPENDENT EVENTS

Excitation-contraction coupling	*Stimulus-secretion coupling*
Ciliary motility	*Blood clotting cascade*
Modulation of activities of intracellular enzymes (phosphorylase b kinase; adenylate and guanylate cyclases; phosphodiesterase activator protein, etc.)	
Lymphocyte transformation	*Egg cell activation*
Cell aggregation and adhesion	*Membrane stabilization and fusion*
Regulation of membrane excitability (electrical and chemical)	*Microtubule assembly*

structures, mitochondria (Lehninger, 1974; Carafoli and Crompton, 1976) and sarcoplasmic reticulum (MacLennan and Holland, 1975; Carafoli *et al.*, 1975). Although there can be no doubt as to the importance of Ca^{2+} uptake by the active transport processes mediated by mitochondria and sarcoplasmic reticulum the cell must, in order to maintain its total Ca^{2+} sensibly constant, ultimately transport Ca^{2+} to the external medium. Two major processes for such removal of intracellular Ca^{2+} have been described: in one process the extrusion of Ca^{2+} is directly coupled to the hydrolysis of ATP and in the second process the extrusion of Ca^{2+} is coupled to an influx of Na^{+}.

Active Ca^{2+} transport across the cell membrane mediated by a Mg^{2+}, Ca^{2+}-activated ATPase has been best described for the red blood cell (Schatzmann, 1975), but it likely operates in a number of other systems including L cells (Lamb and Lindsay, 1971), brain (Nakamura and Schwartz, 1971) and smooth muscle (Hurwitz *et al.*, 1973), and in general this transport system shows a considerable resemblance to that of sarcoplasmic reticulum. A second system for moving Ca^{2+} across cell membranes is Na^{+} - Ca^{2+} coupled transport. The most detailed knowledge of this system derives from studies with squid axons (Baker, 1972; Blaustein and Russell, 1975; Baker and McNaughton, 1976), but the system is widely distributed (Blaustein, 1974) and appears to be of major importance in the regulation of intracellular Ca^{2+} levels. Ca^{2+} extrusion by this process depends upon the presence of external Na^{+} and is generally described as insensitive to inhibitors of Na^{+}, K^{+} - ATPase or metabolic poisons and only indirectly coupled to ATP-utilizing processes. The energy for the uphill transport of Ca^{2+} is presumed to be derived from the coupling of Ca^{2+} extrusion to the movement of Na^{+} down its electrochemical gradient, the nature of

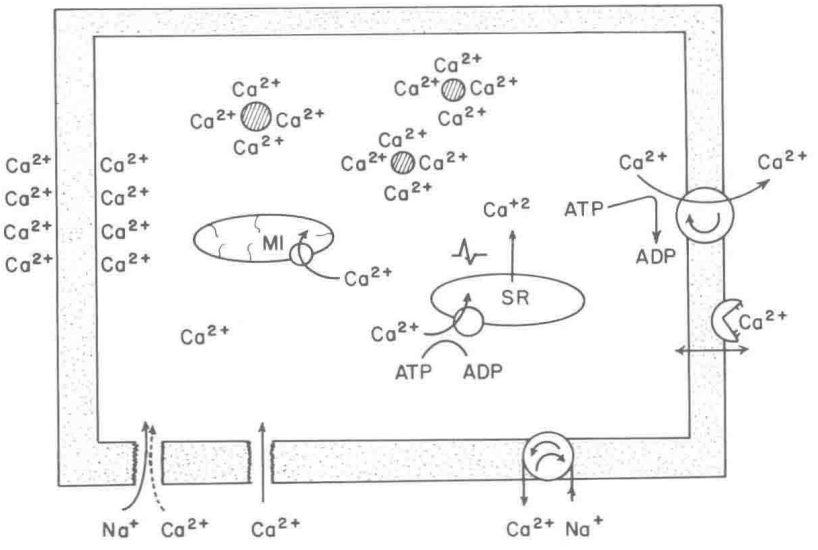


Fig. 1. A schematic representation of cellular calcium regulation. Intracellular calcium may be bound to intracellular proteins (●), to the internal surface of the cell membrane or sequestrated in mitochondria or sarcoplasmic reticulum. Calcium pumping across the plasma membrane is represented by a Ca^{2+} - ATPase and a Na^{+} - Ca^{2+} exchange mechanism. Specific entry mechanisms for calcium include the "Na^{+}" and the "Ca^{2+}" channels. Additionally, ionophoric species may bypass specific calcium entry and exit routes.

the coupling determining the steepness of the Ca^{2+} gradient achieved. The most recent studies in squid axon (Baker and McNaughton, 1976) do suggest a role for ATP in maintaining Na^{+}-dependent Ca^{2+} efflux, but it is not established whether ATP hydrolysis occurs or whether ATP acts in allosteric fashion to control the affinities of Ca^{2+} and Na^{+} binding sites. It is likely that the process is electrogenic with 3 Na^{+} entering for each Ca^{2+} leaving, a stoichiometry that can generate the physiological Ca^{2+} gradient. Ca^{2+} entry can be observed if the Na^{+} gradient is reversed, this process presumably representing operation of the carrier system in reverse.

THE Ca^{2+} ENTRY PROCESS

Given the existence of the several mechanisms that operate to maintain a low intracellular free Ca^{2+} concentration it is clear that there must also exist mechanisms that serve to increase $[\ Ca^{2+}_{int}\ ]$ and couple membrane excitation to intracellular Ca^{2+}-modulated events. This Ca^{2+} may be derived from intracellular

stores or from extracellular sources (Figure 1). Although initial emphasis was placed by Hodgkin and Huxley (1952) on Na^+ and K^+ as the current carrying species during squid axon excitation, there is now substantial evidence for this, and many other tissues, that Ca^{2+} entry through "specific calcium channels" also contributes to the total membrane current (Baker, 1972; Reuter, 1973; Hagiwara, 1975; Triggle and Triggle, 1976). Thus, early work with crustacean muscles (Fatt and Katz, 1953; Fatt and Ginsborg, 1958; Hagiwara, 1975) showed that long lasting action potentials could be observed in the presence of tetraethylammonium (TEA - a K^+ channel antagonist), that neither Na^+ nor Mg^{2+} was essential to the maintenance of excitation, that Ca^{2+} removal abolished action potentials, that the amplitudes and durations of the action potentials increased with increased [Ca^{2+}_{ext}] and that Ca^{2+} could be replaced by Sr^{2+} or Ba^{2+}.

Although the inward current of the squid axon action potential contains only a very small Ca^{2+} component (0.001 % of the Na^+ entry), this preparation has facilitated the characterization of the Ca^{2+} current (Baker, 1972; Baker and Glitsch, 1975). The photoprotein aequorin emits light in the presence of ionized Ca^{2+} (Blinks *et al.*, 1976) and its injection into squid axons has permitted the differentiation of two quite distinct components of Ca^{2+} entry. An early phase of Ca^{2+} entry associated with short depolarizing pulses parallels the rise in Na^+ permeability and is abolished by tetrodotoxin whereas the later phase of Ca^{2+} entry associated with longer depolarizing pulses is insensitive to tetrodotoxin or TEA (Figure 2). Apparently, the initial phase of Ca^{2+} entry uses the fast sodium channels which are approximately 100 times more permeable to Na^+ than to Ca^{2+}. The delayed Ca^{2+} entry is through channels apparently quite distinct from those used by Na^+ (TTX - sensitive) or K^+ (TEA - sensitive) and with an ion selectivity, $Ca^{2+}:Na^+:K^+$, 1:0.01:0.01, quite distinct from that of the sodium channel (Reuter and Scholz, 1977). The slow calcium channels do, however, have properties consistent with a Hodgkin-Huxley system since they show voltage- and time-dependent activation and inactivation; these properties are, however, markedly different from those of the early sodium channel since the calcium current is activated at a more positive membrane potential (and indeed can be seen when the Na^+ current has been inactivated by a depolarizing pulse), has a more positive equilibrium potential and is inactivated much less rapidly (Bassingthwaighte and Reuter, 1972; Reuter, 1973; Kohlhardt, 1975).

Further distinction between the fast sodium and slow calcium currents is provided by observations that these currents are associated with kinetically distinct gating currents associated with particle displacement in the opening and closing of ion channels (Armstrong, 1975; Goldman, 1976; Adams and Gage, 1976) and, of

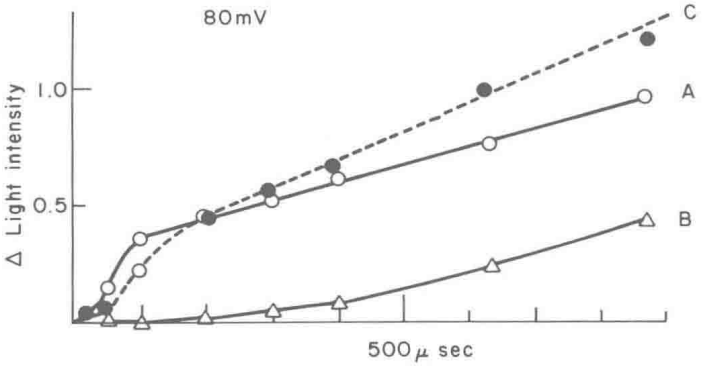
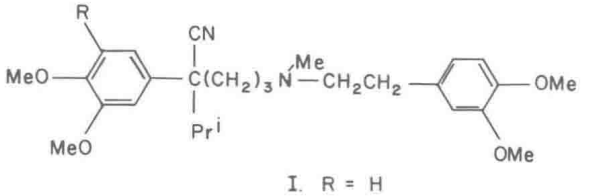


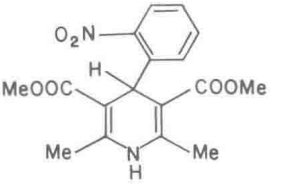
Fig. 2. The two phases of calcium entry in squid axon (measured by aequorin light response) showing the relation between the voltage-clamp pulse (80 mV depolarization) duration (abscissa) and the increased light intensity per pulse. A, before TTX; B, in presence of TTX; C, after removal of TTX. (Reproduced with permission from Baker, Progr. Biophys. Mol. Biol., 24, 177. Copyright Pergamon Press).

particular importance, pharmacological differentiation of the Na^+, K^+ and Ca^{2+} channels is possible with selective antagonists. Tetrodotoxin and tetraethylammonium are well known for their actions on Na^+ and K^+ channels respectively (Hille, 1970) and the inorganic ions Mg^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, La^{3+} and the organic agents verapamil (I), D-600 (II) and Nifedipine (BAY-1040; III) have gained prominence as Ca^{2+} channel antagonists (Fleckenstein, 1971, 1972; Reuter, 1973). There is an obvious analogy between this differentiation of ion channels and the differentiation of pharmacological receptors through specific antagonist action.



I. R = H

II. R = OMe



III

There is now considerable evidence that a calcium entry process similar to that seen in the squid axon occurs in a number of excitable tissues and that a calcium channel is utilized that is distinct from that carrying the early sodium current. The basis for the differentiation of such a process rests on the following properties:

a. Membrane currents and potential changes can be seen

in Na^+-free solution but are very rapidly abolished in the absence of both Na^+ and Ca^{2+}. Potential changes measured in Na^+-free solution are basically identical to those seen in Na^+-containing media in the presence of TTX or in preparations where the Na^+ channel has been inactivated by prior depolarization.

b. The calcium current is insensitive to TTX and TEA but is sensitive to antagonism by Mg^{2+}, Mn^{2+}, Co^{2+}, La^{3+}, verapamil, D-600 and Nifedipine.

c. Sr^{2+} and Ba^{2+} can substitute for Ca^{2+}.

d. The threshold, voltage- and time-dependent activation and inactivation parameters and gating currents are quite distinct from those determined for the early sodium current.

As judged by the application of the criteria listed above calcium channels mediating calcium translocation have been described in a wide variety of preparations, from protozoan to mammalian (Table II), although it must be noted that in many instances complete ionic, electrophysiological and pharmacological characterization is not available. Analysis of the calcium channel activity has not yet reached the stage achieved for the sodium channel where it has been possible to determine single channel conductances and to measure channel density, the latter being of the order of 50-500 TTX binding sites/μM^2 (Ritchie *et al.*, 1976). However, calcium current density in cardiac muscle is at least a hundred-fold less than the sodium current density; whether this reflects a corresponding reduction in channel density and/or flux rate through the channel is not known.

CALCIUM CHANNEL ANTAGONISTS

The di- and trivalent cations, Mn^{2+}, Ni^{2+}, Co^{2+}, La^{3+} and the organic molecules verapamil, D-600 and Nifedipine are defined as calcium channel antagonists and their actions serve as one important component of the definition of calcium channels. It must be emphasized, however, that neither the sites nor the mechanisms of action of these antagonists have been precisely defined.

It is plausible that the calcium channel organization is basically similar to that suggested for the sodium channel by Hille (1975). An important component of Hille's model is the channel cation coordination site which constitutes a rate limiting selectivity filter. The energetics of cation interaction at