Calcium Regulation by Calcium Antagonists

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FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

PREFACE

ALTHOUGH THIS BOOK WAS CONCEIVED as a result of a Symposium entitled "Calcium Regulation and Drug Design," the topics presented at the Symposium have undergone revision, expansion, and updating, and additional chapters from invited authors have been incorporated into the present volume. This book, therefore, represents a collage of various topics involving the roles of calcium and calcium antagonists in health and disease. Unlike other excellent recent books on calcium antagonists, the present volume does not focus exclusively on the cardiovascular system, but covers other systems as well. The emphasis is on basic science, although clinical correlates are included wherever applicable. Numerous authorities in the field have contributed their expertise in their respective areas to the compilation of this book.

The opening chapter by Dr. Nayler attempts to create order from chaos by presenting a rational classification of membrane calcium channel blockers based upon their pharmacological effects on the cardiovascular system. Dr. Triggle then provides an overview of structure–activity studies with the calcium channel blockers, and emphasizes the chemical and pharmacological heterogeneity of this class of compounds. The latter fact provides a strong impetus for the adoption of the pharmacological classification of calcium channel blockers proposed by Dr. Nayler. A comprehensive review of the cardiovascular electrophysiological and hemodynamic effects of the calcium channel blockers is presented by Dr. Muir. Dr. Daniel and his colleagues provide insight into the role of calcium in the regulation of vascular and nonvascular smooth muscle contractility, and outline the methodological approaches for studying calcium fluxes, sequestration, and mobilization in smooth muscle.

While the first four chapters focus on the membrane calcium channels, the next two explore the intracellular compartment. Dr. Brostrom and his colleagues review present-day knowledge about the intracellular calcium receptor, calmodulin, while the Editors contribute an update on the basic and applied pharmacology of a novel class of intracellular calcium antagonists, the methylenedioxyindenes.

The roles of calcium and calcium antagonists in the central nervous system are dealt with in the next two chapters. From Dr. Leong Way's laboratory comes an overview of the mechanism of interaction between calcium and opioid alkaloids and peptides, while Dr. Ferrendelli reviews

the evidence for an inhibitory effect of certain antiepileptic drugs on neuronal calcium conductance. Dr. Le Breton and his colleagues review the relationship of cyclic nucleotides and calcium in platelet function, while novel information on the salutary effects of the calcium antagonist nifedipine in atherosclerosis is presented from Dr. Henry's laboratory. Dr. Borowitz and coworkers review the role of calcium and the modulating influence of calcium antagonists on secretory systems.

We wish to acknowledge with gratitude the cooperation of the participants in the Symposium and the contributors to this volume with whom we had the distinct privilege of collaborating.

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CONTENTS

Calcium Antagonists: Classification and Properties

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Calcium antagonists (slow channel blockers, slow Ca^{2+} antagonists) are a heterogeneous group of substances with widely differing tissue specificities, potency and properties. Some of them exhibit other properties in addition to that of Ca^{2+} antagonism. In general these drugs can be considered as a subgroup of a much larger group of compounds which impede the entry of Ca^{2+} irrespective of the route of entry. The 'slow channel blockers' can be subdivided on the basis of their differing tissue specificities. The purpose of this article is to explore the possibility that a classification which is based on differing tissue specificities may reflect differing modes of action.

Verapamil, nifedipine and diltiazem (Figure 1) belong to a relatively newly recognized group of drugs known collectively as "calcium antagonists", (1) "slow channel inhibitors", (2) or "calcium entry blockers" (3,4). Other substances which are now thought to belong to this group include niludipine, nimodipine, prenylamine, fendiline, caroverine, cinnarizine and perhexeline. The possibility of using these and other closely related substances in the management of a variety of cardiovascular disorders - including infarction, (5,6,7) arrhythmias, (8,9) angina, (10,11) hypertension (12) and hypertrophic obstructive cardiomyopathies (13) is now being considered. However the "calcium antagonists" that are currently available for such use differ from one another not only in terms of their chemistry, bio-availability and stability, (1,14) but also in potency, (1,14) tissue specificity and possibly in their precise mode of action (15,16,17). Because no attempt has yet been made to subclassify these drugs, the purpose of this article is to explore the problem of providing a suitable classification. To do this it is necessary to briefly discuss the physiological significance of the

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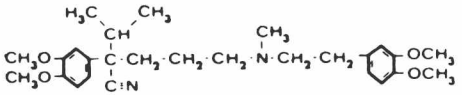
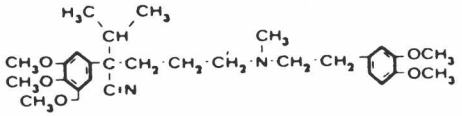
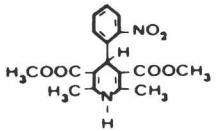
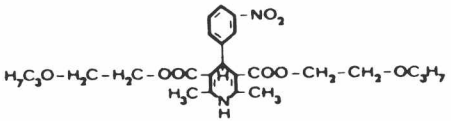
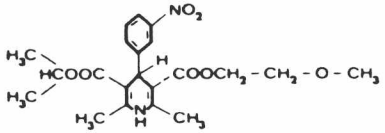
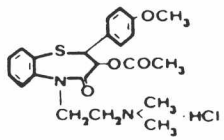
Verapamil (mol.wt. 454.59)	
Compound **D-600** (gallopamil, mol.wt. 485.59)	
Nifedipine (mol.wt., 346.34)	
Niludipine (mol.wt. 490.55)	
Nimodipine (mol.wt. 418.45)	
Diltiazem (mol.wt. 414.52)	

Figure 1. Structural formulas of some Ca^{2+} antagonists and their derivatives.

"slow Ca^{2+} current" (18) and the associated ion-selective channels.

The Slow Ca^{2+} Current

The slow Ca^{2+} current in heart muscle.

In normal heart muscle excitation involves the activation of two distinct inward currents (19,20). The first of these currents is carried by Na^+ and is recorded as the fast upstroke of the action potential. The Na ions move across the cell membrane through voltage-activated "channels" that are highly, but not totally, selective for Na^+. The second inward current is carried mainly (21,22), but not exclusively (23), by Ca^{2+}. It is activated slowly, contributes to the plateau phase of the action potential and is known as "the slow Ca^{2+} current". The Ca ions involved pass across the cell membrane through channels that are highly selective for Ca^{2+}. Like their Na^+ counterparts, the Ca^{2+}-selective channels are voltage activated but their threshold of activation (about-55mV) is higher than that of the Na^+ channels (-35mV). It is possible to envisage these "channels" as being pore-like structures in the plasmalemma, each pore having its own set of "activation" and "inactivation" gates. In this analogy voltage activation can be likened to the opening of "gates" which are closed during the resting state (24). Taking this hypothesis one step further it can be argued that the normal opening and closing of these "gates" involves voltage-dependent changes in the configurational state of the membrane. This same argument applies irrespective of whether the ion-selective "channels" are "pore-like" structures or a particular combination or organization of the membrane proteolipids that facilitates the inward movement of certain ions. Within this framework drugs which interact with the cell membrane may alter the configurational state of the membrane, thereby altering the ion selectivity or responsiveness of the "gated" channels and proteolipid complexes.

The slow Ca^{2+} current and excitation-contraction coupling.

The slow Ca^{2+} current accounts for the entry into the cytosol of between 5 and 10 μmoles Ca^{2+}/kg heart weight/beat (25). This is approximately one tenth of the Ca^{2+} needed to activate contraction (26). Probably the Ca ions that enter as the main charge carriers for the slow current serve as a trigger for the mobilization of Ca^{2+} from the intracellular stores (27,28). However, since the magnitude of the mechanical response varies according to the extracellular concentration of Ca^{2+} (29) it is probably the current-carrying Ca ions of the slow inward current which determine the amount of Ca^{2+} mobilized for interaction with the myofilaments. In other words, the Ca^{2+}-induced "trigger" release of Ca^{2+} from the intracellular storage sites simply provides an

internal amplification factor. Skeletal muscle differs from cardiac muscle in that it mobilizes all the Ca^{2+} it requires for excitation-contraction coupling directly from its own intracellular stores.

The slow Ca^{2+} current in smooth muscle, nodal and conducting tissues.

The occurrence of "Ca^{2+}-selective", voltage activated "channels" is not limited to the myocardium. They occur in most smooth muscle cells - including those in the coronary, cerebral and peripheral vasculature (30). The normal activity of pacemaker, nodal and conducting tissues (31) is also largely dependent upon them. Because of their widespread distribution it follows that substances which affect the functioning of these channels will have a profound effect on the circulation. By contrast, skeletal muscle is relatively unaffected (32).

Specificity of slow channels for Ca^{2+}.

Although Ca^{2+} is the main current carrying ion for the slow inward current part of the current is carried by Na^{+} (23). Certain divalent cations, including Ba^{2+} and Sr^{2+}, can substitute for Ca^{2+} as the main charge carrier (33). Others, including Ni^{2+}, Co^{2+}, and Mn^{2+} are inhibitory (34).

The Identification of Ca^{2+}-antagonists

Techniques which are currently being used to identify substances that alter slow channel transport involve measurements of the height and duration of the action potential, monitoring the rate of uptake of radioactively labelled Ca^{2+}, and electrophysiological techniques that involve suppression of the fast Na^{+} current. Use of these techniques is based on the tacit assumption that the drugs we are dealing with act only on the slow channels. Later in this chapter we will summarize the data which suggests that such an assumption may no longer be justified. For the moment, however, it is pertinent to work within this framework.

(a) Height and duration of the action potential.

To illustrate the use of changes in the configuration of the action potential to establish the presence or absence of "Ca^{2+} antagonism" we will concentrate on the cardiac action potential. The currents which contribute to the height and duration of the cardiac action potential are complex (20). In addition to the inward currents already referred to there are at least two and possibly three outward (35) K^{+} currents. Accordingly, unless a substance is specific for only the inward Ca^{2+} current studies

which depend upon the monitoring of changes in the height and duration of the action potential are not particularly helpful in identifying either inhibitors or activators of slow channel transport. This difficulty is further compounded by the fact that the subsarcolemmal concentration of Ca^{2+} influences the outward K^{+} currents, (35) and hence the shape and duration of the action potential.

(b) Isotopic Techniques

The use of radioactively labelled Ca^{2+} to monitor changes in the magnitude and duration of the slow Ca^{2+} current is also difficult, partly because of the small amounts of Ca^{2+} that are involved. When considered on a beat to beat basis the Ca^{2+} that enters heart muscle cells by way of the slow Ca^{2+} current represents less than 2 percent of the total tissue Ca^{2+} and if, as seems probable, this small component is rapidly recycled to the exterior its accurate detection during the time course of the action potential presents substantial technical difficulties. There is, however, another and more serious objection to the use of labelled Ca^{2+} for this purpose. This difficulty centres around the fact that Ca^{2+} can enter the myocardium and other excitable cells through several different routes - including in exchange for Na^{+}, by passive diffusion, and (Figure 2) in exchange for K^{+}. Consequently even if the uptake of radioactively labelled Ca^{2+} is reduced there can be no certainty that the particular route of entry that is being affected is "the slow channels".

(c) Electrophysiological techniques

These involve the use of techniques to suppress the fast inward Na^{+} current. Either a voltage can be applied so that the transmembrane potential difference is clamped above the level at which the Na^{+} current is activated, (36) or tetrodotoxin (37) - which specifically inhibits the Na^{+} current, can be added, or the membrane can be depolarized by raising the external K^{+} (38,39). Under each of these conditions the slow inward current can be activated by adding aminophylline or isoproterenol, (39) and by electrical stimulation.

Substances That Alter Slow Channel Transport

Theoretically, substances or interventions that alter slow channel transport may do so in a variety of ways:-

(a) they may alter the amount of Ca^{2+} which is available to act as the charge carrier;

(b) they may, by interacting with the cell surface, evoke a configurational change which either facilitates or impedes the approach of Ca^{2+} to the channels; alternatively -

(c) the configurational change in the membrane may induce a

change in the Ca^{2+}-carrying capacity of each channel; or - (d) the number of channels that are operative at any given time may be affected. This could be achieved by altering the threshold of activation, changing the kinetics of channel activation and/or recovery, by facilitating the formation of "de novo" channels or by activating "sleeping" channels.

Activators of slow channel transport.

The catecholamines, including norepinephrine, epinephrine and isoproterenol augment the slow Ca^{2+} current (39,40). They do this by increasing the number of channels that are activated at a given voltage, without affecting the rate of channel activation or deactivation (40). Cyclic AMP has the same effect (41). This ability of the catecholamines to recruit new channels may indicate the existence of a heterogenous population of voltage-activated channels, one population being under cyclic AMP control. Alternatively there may be several different states of activation for each channel. Irrespective of which, if either, of these alternatives is correct, it follows that the amount of slow channel activity that is available at any one moment is influenced by the circulating level of catecholamine. The recent discovery of 'calciductin', a protein that appears to be associated with the slow channels and which can be phosphorylated by a cyclic AMP-dependent pathway points towards the possibility of heterogeneity within the channels. There are other reasons for believing that the channels themselves must be heterogenous. Thus, for example, the drugs we are discussing have no inhibitory effect on the Ca^{2+}-dependent excitation-induced release of norepinephrine from the sympathetic nerve terminals.

Inhibitors of slow channel transport.

Many substances inhibit slow channel transport. In addition to the divalent cations already cited (Mn^{2+}, Co^{2+} and Ni^{2+}), protons, La^{3+}, the metabolic inhibitors cyanide and dinitrophenol, are effective inhibitors (24). Other inhibitory agents include acetylcholine, (42) papaverine, (43) pentobarbital, lidoflazine, (44) and adenosine (45) as well as verapamil, nifedipine and diltiazem (1). Precisely how many of these substances interfere with slow channel transport is unknown, although in the case of the metabolic inhibitors we can probably account for their effect in terms of the energy requirements (24) needed for maintaining the configurational state of the cell membrane compatible with the maintenance of normal slow channel ultrastructure.

Classification of Slow Channel Blockers (or Antagonists)

Fleckenstein (1) originally classed some of the substances listed in Table I as "calcium antagonists" on the basis of two requirements:

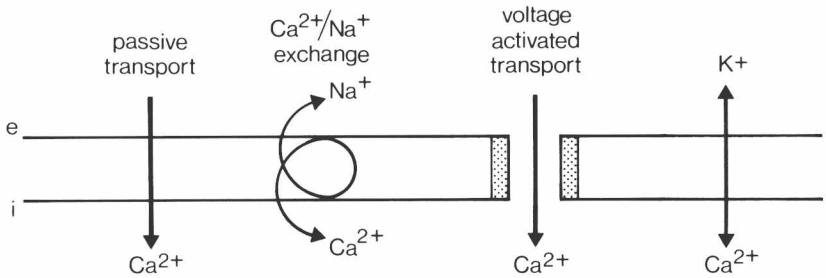


Figure 2. Schematic representation of possible routes of Ca^{2+} entry into a myocardial cell.

Table I: Substances Classed as Ca^{2+} Antagonists

	References
Verapamil	1
methoxyverapamil (D-600)	1
prenylamine	1
nifedipine	1
lidoflazine	44
nimodipine	52,53
diltiazem	1
bepridil	68
caroverine	70
niludipine	53
fendiline	1

(a) the predominant characteristic of these substances is their ability to inhibit the slow Ca^{2+} current; and
(b) this inhibition could be overcome by adding Ca^{2+}.

These criteria still apply. However, the continued unqualified use of the term "calcium antagonist" requires reappraisal because of its lack of specificity with respect to the site and precise mode of drug action. Thus "calcium antagonism" can be expressed at a variety of sites, including the cell membrane, the myofibrils, the sarcoplasmic reticulum and the mitochondria. When used in therapeutic concentrations, however, the drugs we are discussing express their "calcium antagonistic" properties at only one site - the cell membrane. Even at the cell membrane there are other ways in which substances can interfere with transmembrane Ca^{2+} movements - apart from the entry of Ca^{2+} through the voltage-activated "channels". Possibly, therefore, there is some merit in considering drugs of the type shown in Table I as being a subgroup of a much larger group of drugs which, for want of a better term, may be called "Ca^{2+}-entry blockers" or "Ca^{2+}-entry antagonists" (34). This group of drugs - "the Ca^{2+} entry blockers" or "Ca^{2+}-entry antagonists" (Figure 3) would include any drug which impedes the inward movement of Ca^{2+}, irrespective of the route of entry. The known routes of Ca^{2+} entry into cardiac and smooth muscle cells include by passive diffusion, in exchange for Na^{+}, in exchange for K^{+}, and through the voltage-activated, ion selective channels we have been discussing. As far as cardiac and smooth muscle cells are concerned, therefore it is possible that four different sub groups of Ca^{2+}-entry blockers (or antagonists) will ultimately become available. However, the drugs which are currently available are specific only for the subgroup that involves the influx of Ca^{2+} through the voltage activated, ion selective channels. Since these channels are slowly activated relative to the channels that selectively facilitate the rapid influx of Na^{+} during the fast upstroke phase of the action potential it may be more appropriate to refer to these substances as "inhibitors of slow channel transport". An alternative term - "Ca^{2+} channel blocker" has already appeared in the literature but may be inappropriate because the slow channels are not totally selective for Ca^{2+}. They also admit some Na^{+} and the resultant "slow" Na^{+}-dependent current is blocked by some of the currently available antagonists, including verapamil and methoxy verapamil (23). Presumably as new Ca^{2+} entry blocking drugs are developed substances that specifically inhibit the influx of Ca^{2+} through routes of entry other than the slow channels will become available. For example, substances that specifically inhibit the entry of Ca^{2+} in exchange for Na^{+} or K^{+} may be developed. Such substances could be of clinical importance because these other routes of Ca^{2+} entry may be involved (43) in the massive influx of Ca^{2+} that occurs (46) when flow is re-introduced to a previously ischaemic zone - as may occur, for