NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

ISBN 0-315-55475-4

THE UNIVERSITY OF ALBERTA

SCREENING AND PHARMACOLOGICAL EVALUATION OF NOVEL 1,4-DIHYDROPYRIDINE Ca^{2+} CHANNEL MODULATORS

by

WANDIKAYI C. A. MATOWE (C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PHARMACEUTICAL SCIENCES (PHARMACOLOGY)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA

FALL 1989

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Wandikayi C. A. Matowe

TITLE OF THESIS: Screening and pharmacological evaluation of novel 1,4-dihydropyridine Ca^{2+} channel modulators

DEGREE FOR WHICH THESIS WAS PRESENTED: DOCTOR OF PHILOSOPHY

YEAR THIS DEGREE GRANTED: FALL 1989

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) .....

PERMANENT ADDRESS:

P.O. Box 334
MUTARE
ZIMBABWE

DATE: Sept. 22nd 19.89

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled SCREENING AND PHARMACOLOGICAL EVALUATION OF NOVEL 1,4-DIHYDROPYRIDINE CALCIUM CHANNEL MODULATORS

submitted by Wandikayi C. A. Matowe

in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

in PHARMACEUTICAL SCIENCES (PHARMACOLOGY).

.....
Supervisor

.....

.....

.....

.....
External examiner

Date: September 22, 1989

To my late mother, Mwakanyadzeni, my father, Mwari Haatongerwi, my sister, Chakawoneni, and to my wife, Patricia-Lynn.

ABSTRACT

1,4-Dihydropyridine (1,4-DHP) Ca^{2+} channel modulators comprise an important and growing class of structurally similar compounds, whose applications range from pharmacological probes of Ca^{2+} channel function, to therapeutically useful drugs for the treatment of cardiovascular disorders.

The research presented set out to screen for Ca^{2+} channel modulatory activity, and to characterize the structure activity relationships and pharmacological profiles of novel analogues of the prototype 1,4-DHP compounds, nifedipine (inhibitor) and Bay K 8644 (activator).

Isolated guinea pig ileal longitudinal smooth muscle (GPILSM) and left atrium were used to screen for *in vitro* Ca^{2+} channel modulator effects of several analogues of the above-mentioned prototype Ca^{2+} channel modulators. GPILSM was used to screen for inhibition of contraction of smooth muscle, and the left atrium to screen for Ca^{2+} channel activator effects on cardiac tissue. Standard pharmacological protocols were employed. The results of these experiments were then used to characterize the structure activity relationships of the novel compounds, and to derive rank orders of their relative potencies within a related group or subgroup in which modifications had been made at positions C3, C4 or C5 of the 1,4-DHP ring of the parent compound, or in the 1,4-DHP ring itself. Compounds with the best activity or effects differing from those of the parent compounds were further tested both *in vitro*

and *in vivo* in order to identify their tissue specificities, characterize their pharmacological profiles, and to elucidate their mechanism of action.

New structure activity relationships established included the following: a). For C-4 pyridyl substituted nifedipine analogues, the potency order was 2-pyridyl > 3-pyridyl > 4-pyridyl. b). Analogues possessing asymmetric C-3/C-5 esters tended to be more potent than those [illegible] metric esters. c). Bulky symmetric C-3/C-5 ester analogues with a C-4 3'-(1'-methoxycarbonyl-4'-methyl-1',4'-dihydro pyridyl) group produced compounds which selectively blocked depolarisation-induced contraction (DIC) of GPILSM. d). Analogues possessing C-3/C-5 isopropyl/phenylethyl esters were at least as active as nifedipine on GPILSM, and had much greater specificity for smooth muscle than cardiac tissue, some exhibiting partial agonist effects on the heart at low concentrations. e). Para position methylation, methoxylation or halogenation of the phenyl ring of these esters produced activity greater than that of nifedipine on GPILSM. f). For C-4 pyridyl substituted Bay K 8644 analogues, the relative potency order on cardiac tissue was 4-pyridyl > 3-pyridyl > 2-pyridyl. g). C-4 2-pyridyl Bay K 8644 analogues inhibited contraction of GPILSM, being the first compounds to possess differential acitivity on smooth and cardiac tissues, but C-4 3- and 4-pyridyl analogues of Bay K 8644 were activators.

Development of these novel compounds has provided potential therapeutic agents and valuable pharmacological tools for use in investigating Ca^{2+} channels of different tissues.

Acknowledgements

I wish to express deep gratitude to Dr. M. W. Wolowyk, for his supervision, guidance and encouragement throughout the course of this work. I would also like to thank Dr. E. E. Knaus and his post-doctoral researchers Dr. M. Ramesh and Dr. M. Akula for providing the novel compounds on which this work was carried out.

I also wish to thank my friends and colleagues, for their helpful suggestions, and especially Teresa Tsuruda for editing 'typos' and and for 'cheering me up with cups of coffee, particularly on Monday mornings when I felt like giving-up!'.

Finally, I wish to thank my wife Patricia, for her patience, support, encouragement and understanding, during the time I have been doing this program.

Table of Contents

List of Tables

List of Figures

List of abbreviations

AR:	Activity ratio
C-3:	position C-3 of 1,4-DHP ring
C-4:	position C-4 of 1,4-DHP ring
C-5:	position C-5 of 1,4-DHP ring
c-butyl:	cyclobutyl
c-hexyl:	cyclohexyl
c-pentyl	cyclopentyl
DIC	depolarisation-induced contraction
DHP:	dihydropyridine (dihydropyridyl)
GPILSM:	guinea pig ileal longitudinal smooth muscle
HPSS:	HEPES physiological saline solution
i-butyl:	isobutyl
i-propyl:	isopropyl
IC_{50}:	concentration of a compound causing half the maximal response elicited by an agonist
MeO-Et:	methoxyethyl
N-2:	depicting a 2-pyridyl structure
N-3:	depicting a 4-pyridyl structure
N-4:	depicting a 4-pyridyl structure
n:	no. of tests
N3-PhE:	3'-(1'-phenoxycarbonyl-1',4'-DHP) functional group
N4-PhE:	4'-(1'-phenoxycarbonyl-1',4'-DHP) functional group
N3-MeE:	3'-(1'-methoxycarbonyl-1',4'-DHP) functional group
N4-MeE:	4'-(1'-methoxycarbonyl-1',4'-DHP) functional group
N3-tBuE:	3'-(1'-butoxycarbonyl-1',4'-DHP) functional group

N4-tBuE:	4'-(1'-butoxycarbonyl-1',4'-DHP) functional group
RP:	Relative Potency
SAR	structure-activity relationships
SEM:	standard error of the mean
SI:	Selectivity Index
2-pyr:	2-pyridyl
3-pyr:	3-pyridyl
4-pyr:	4-pyridyl
t-butyl:	tertiary butyl
pIC_{50}:	log IC_{50}
VSC:	voltage-sensitive Ca^{2+} channel

1. INTRODUCTION

The advent of compounds now generally called calcium antagonists, has generated a great deal of on-going excitement and activity in basic and clinical science research. The large number of such compounds currently under investigation is a reflection of the anticipated potential applications of these agents, both as tools for elucidating the role of Ca^{2+} in cell activity and homeostasis, as well as therapeutic agents for the treatment of calcium-dependent pathological processes. Although many compounds possess the ability to antagonise the action of Ca^{2+} in the cell, the term calcium antagonist is now universally applied to only those compounds that exert their primary pharmacological effects through the inhibition of Ca^{2+} entry into the cell (Vanhoutte, 1987).

The efforts of Fleckenstein and his co-workers (1983a), probably had the greatest impact on influencing research on Ca^{2+} antagonists and their applications. Much of their research focused on identifying the physiologic function of Ca^{2+} and Ca^{2+} withdrawal in myocardial and visceral smooth muscle, and the development of therapeutic agents useful for treating pathologic conditions in these tissues. Fleckenstein is credited with developing the pharmacodynamic concept of calcium antagonism, and compounds possessing this property are in current therapeutic use as antihypertensive, antiarrhythmic, and cardioprotective agents.

Concurrent with Fleckenstein and co-workers' investigations, the pioneering studies of T. Godfraind and his co-workers (Godfraind and Kaba, 1969; Godfraind, 1982; Godfraind, 1983; Godfraind and Miller, 1983), particularly on elucidating the mechanisms of action of Ca^{2+} antagonists on vascular smooth muscle, also deserves credit for leading the field in Ca^{2+} channel modulator studies.

The discovery of compounds, within the dihydropyridine class of calcium antagonists, capable of promoting Ca^{2+} entry into cells, instead of inhibiting it (calcium channel agonists), has broadened the scope of applications to which compounds capable of modulating the movements of Ca^{2+} across cell membranes can be used. Collectively calcium channel antagonists and calcium channel agonists are called calcium channel modulators.

Investigations of the pharmacological properties of calcium channel modulators have led to a greater understanding of the structural basis of transmembrane Ca^{2+} movements, and of the physiological control processes involved in Ca^{2+} homeostasis in normal and under pathologic conditions. In the following discussion, a brief background of the physiology of Ca^{2+} in cardiovascular function and disease, and the mechanisms of action of calcium channel modulator drugs is reviewed.

1.1 Historical aspects of Ca^{2+} and cell function.

The role of Ca^{2+} in cell physiology and homeostasis has been extensively studied by biologists since the late nineteenth century. Ringer's classical studies (1882; 1883a; 1883b; 1886) on physiological salt solutions, formed the basis for future studies of the relationship between cell function and the external electrolyte environment (Bianchi, 1968). Ringer showed that the frog heart failed to maintain spontaneous beating and contractility in response to electrical stimulation, when $CaCl_2$ was excluded from the perfusing solution. Furthermore, he established that a bathing solution containing NaCl, $CaCl_2$ and KCl enabled cell function to be studied ex vivo, and thus initiated the use of isolated tissues, and even cells for studying physiological systems outside the whole animal for extended periods of time.

Chambers and Reznikoff (1926), using the unicellular organism amoeba, demonstrated that the cell membrane acted as a primary barrier to the movement of Ca^{2+} into the cell, and established the physiological role of low intracellular Ca^{2+}. Thus they established that there existed a differential distribution of Ca^{2+} across the cell membrane. Subsequently, Pollack (1928), using the same organism, discovered that a relationship existed between intracellular Ca^{2+} movements and cell function.

The discovery by Fatt and Katz (1953), that crustacean muscle fibres produced feeble, frequently non-propagating action potentials in normal external solution, but did so when external sodium was

replaced with either choline, tetraethylammonium chloride, tetrabutylammonium chloride, or even isosmolar sucrose, led them to postulate that such action potentials were produced through increased membrane permeability to Ca^{2+} or Mg^{2+}. This was the first evidence suggesting the existence of calcium channels in the cell membrane. Subsequently, Fatt and Ginsborg (1958), confirmed Ca^{2+} as the charge carrier, and demonstrated that Ba^{2+} and Sr^{2+} were equally capable of producing these action potentials.

Although other investigators had made the observation that contraction ensued when frog sartorius muscle was immersed in isotonic $CaCl_2$ (Graham and Sichel, 1939), Heilbrun was the first person to realise the crucial role of Ca^{2+} in mediating intracellular events, and to hold clear ideas about the source of the Ca^{2+} and the mechanisms of its release from this source (Heilbrun, 194C; Ebashi, 1987; Putney, 1987). Heilbrun's claim that Ca^{2+} was the physiological trigger for muscle contraction earned him the title of 'pioneer of the Ca concept'.

The development of methods such as voltage clamp and patch clamp techniques, the use of fluorescent dyes to measure Ca^{2+} within cells, electron probe microanalysis and immunolocalisation of components associated with Ca^{2+} homeostasis, have since confirmed the existence of specific pathways for Ca^{2+} movement into and out of cells (fig 1.1) (Hagiwara, 1983; Tsien, 1983; Garfield and Somlyo, 1985; Van Breemen et al., 1986a; Baker et al., 1987; Meldolesi et al., 1988). These methods have elucidated the complex inter-relationships between Ca^{2+} sources and the modes of Ca^{2+} mobilisation and subsequent resequestration into storage sites (Chiu

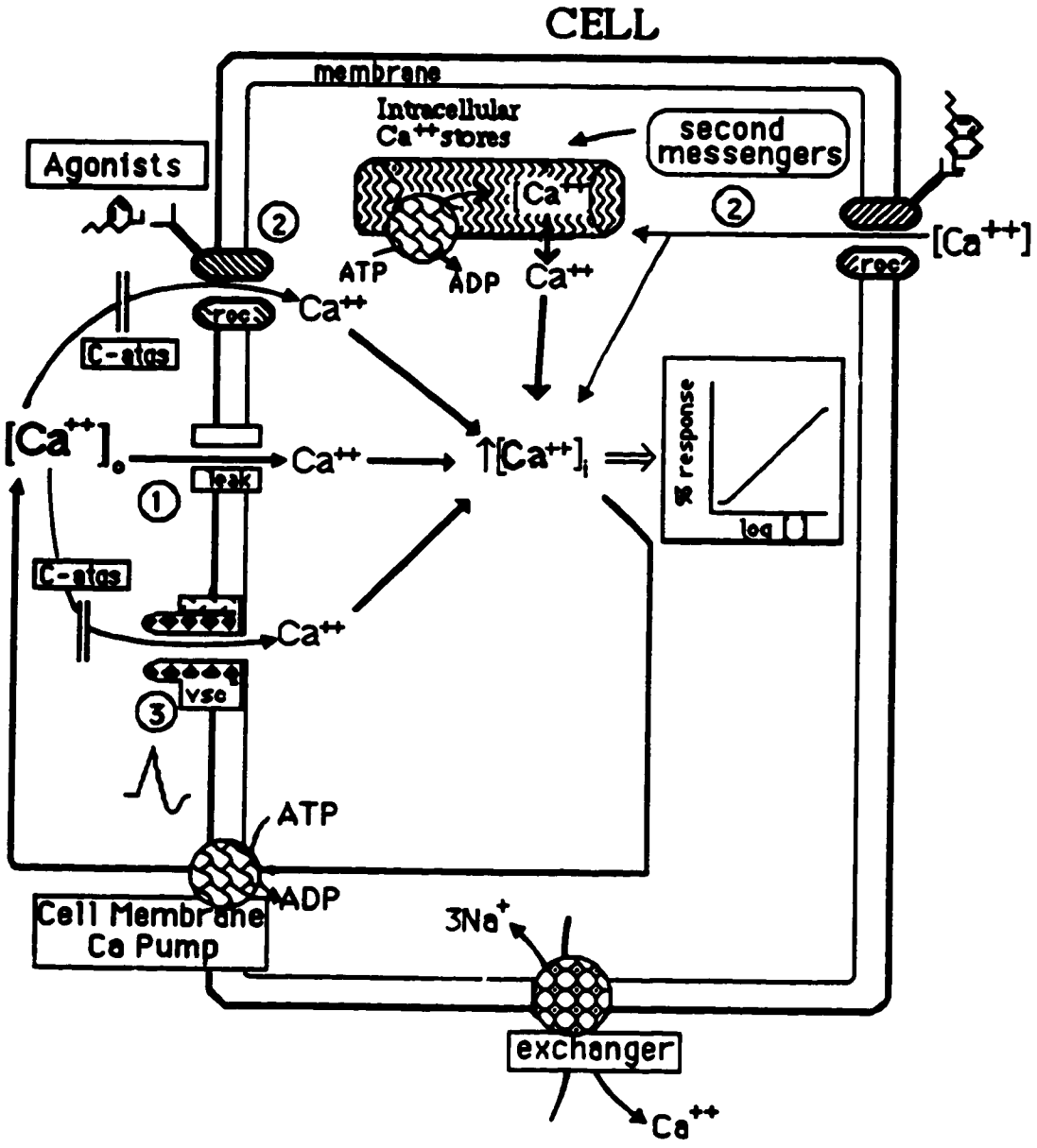


Figure 1.1 Schematic diagram of transmembrane Ca^{2+} movements: Influx occurs through 'leak', receptor-operated (ROC), and voltage-sensitive Ca^{2+} (VSC) channels. Efflux occurs through the Mg^{2+}-dependent Ca^{2+} pump (Ca^{2+} ATPase) and Na^{+}/Ca^{2+} exchange. (C-atgs = Ca^{2+} antagonists).

et al., 1986; Williamson, 1986; Putney, 1987; Somlyo et al., 1988). Calcium channel modulator drugs have contributed significantly in these studies, and the development of more potent and more selective drugs, with fewer side effects and a longer duration of action, remains a focal point for many medicinal chemists, pharmacologists and clinicians.

1.2 Calcium and cellular mechanisms - current understanding

1.2.1 Ca^{2+} - intracellular messenger.

Ca^{2+}, is an important intracellular signal for a number of physiological systems in animals. Ca^{2+} mediates a variety of actions such as smooth muscle contraction, cardiac contractility, pacemaker activity, neuroendocrine, endocrine and exocrine secretion (Rasmussen and Barrett, 1984; Abdel-Latif, 1986; Godfraind et al. 1986). Intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, is a major determinant of cell activity in many cell types. $[Ca^{2+}]_i$ rises in response to a number of extracellular stimuli such as membrane depolarisation, neurotransmitter and hormone binding to receptors in the cell membrane. Stimuli lead to opening of calcium channels in the cell membrane directly, or they may generate intracellular messengers such as diacylglycerol (DAG) and inositol trisphosphate (IP_3), which lead to calcium channel opening or release of intracellularly stored Ca^{2+}. The open channels then allow Ca^{2+} to move down its electro-chemical gradient into the cell. Some second messengers such as IP_3, cause the release of stored Ca^{2+}

from sarcoplasmic reticulum storage sites (Rasmussen, 1986; Berridge, 1987). The resulting elevation of $[Ca^{2+}]_i$ initiates a cascade of reactions (phosphorylation - dephosphorylation), culminating in a physiological response. Termination of the signal comes about in part when the level of $[Ca^{2+}]_i$ returns to its resting levels. A variety of mechanisms including calcium resequestration into sarcoplasmic reticulum and extrusion to the extracellular space, are involved.

Ca^{2+}, therefore, acts as a final common messenger for a number of diverse signals and is precisely controlled to ensure proper interpretation of the signal in the cell. Failure of the regulatory processes leads to pathological conditions involving altered Ca^{2+} homeostasis.

At rest, the intracellular concentration of free Ca^{2+} is approximately 10^{-8} M, vs an extracellular concentration of about 10^{-5} to 10^{-4} M, a 10,000 fold difference (Rasmussen and Barrett, 1984; Baker, 1986). The resting state is thus a dynamic equilibrium in which an electro-chemical gradient tending to drive calcium into the cell is held in check by mechanisms which extrude cell Ca^{2+} (i.e. Ca^{2+}-ATPase and Na^{+}/Ca^{2+} exchange) and is carefully controlled in order to maintain the Ca^{2+} gradient.

In many cells, the intracellular levels of calcium, along with those of other second messengers, fluctuate periodically (Berridge, 1987; Berridge and Galione, 1988). Berridge hypothesised that these fluctuating concentrations reflect the spatial and temporal aspects of cellular signalling, which in turn mirror the spatial distribution of the calcium (or other second messenger), and the

temporal variability of its levels, resulting in oscillations of the calcium transients. Calcium mobilising agonist concentration could then encode messages to the cell in two ways. Some cells could use frequency-modulated signals in the form of calcium oscillations, to elicit appropriate responses. Such signals would be easier to control than amplitude-modulated signals, especially with regard to activity close to threshold levels, or for minimising noise in the signals (Berridge and Galione, 1988). As well, cells could use the size of the stimulus (amplitude- modulation) to encode responses. In many cell types, the response, which is controlled both in intensity (magnitude of the response), and in duration, is mediated by both types of signals (Berridge, 1987).

1.2.2 Ca^{2+} mobilisation and signal termination.

Ca^{2+} mobilisation in cell activation differs depending on the cell type, the response required of the cell, the sources of activator Ca^{2+} available to the cell, the way the mobilisation process is triggered, and the mobilisation process itself. Smooth muscle and cardiac tissues are dependent to a greater or lesser extent on extracellular $[Ca^{2+}]_o$ (Sperelakis, 1983; Van Breemen et al., 1986a; Hess, 1988). This dependence on $[Ca^{2+}]_o$ renders the tissues sensitive to the action of calcium channel modulators because these drugs exert their effects primarily on calcium channels of the plasma membrane. Conversely, tissues that are more dependent on $[Ca^{2+}]_i$ are less sensitive to the action of calcium channel modulators.

In cardiac and vascular smooth muscle cells, Ca^{2+} and/or cyclic nucleotides (cAMP and cGMP) control the degree of phosphorylation of key regulatory proteins and enzymes that mediate the cell/tissue response (Fleckenstein, 1983a; Loutzenhiser et al., 1985; Silver, 1988; Walsh et al., 1988; Weishaar et al., 1988). However, Ca^{2+} mobilisation and signal termination processes differ somewhat in the two tissues. The differences arise from differences in both effector mechanisms (contractile-element components and their proportions and interactions in the tissue) and regulatory processes (proportions and types of phosphorylation/dephosphorylation reactions and the enzymes (isozymes) responsible) in a tissue-type (Rasmussen et al., 1987; Silver, 1988).

1.2.2.1 Smooth muscle cells.

In vascular smooth muscle, rapid changes in $[Ca^{2+}]_i$ result from influx of extracellular Ca^{2+} and release of bound Ca^{2+} in the sarcoplasmic reticulum, followed by extrusion to the extracellular space and resequestration back into the sarcoplasmic reticulum (Sperelakis, 1983; Rasmussen et al., 1987). The total cycle lasts from several seconds up to several minutes (cf. the cardiac cycle which is of much shorter duration - see below).

Ca^{2+} exerts its effects by stimulation of protein kinase activity through two regulatory cofactors: a). calmodulin - a calcium binding protein which activates myosin light chain kinase, and b). phosphatidylserine plus diacylglycerol - which activate protein kinase C (Johns et al., 1987; Rasmussen et al., 1987;

Silver, 1988; Stoclet et al., 1988). Phosphorylation of the myosin light chain is necessary for rapid tension generation (phasic contraction), and is also involved in sustaining the contractile response (tonic contraction) (Abdel-Latif, 1986; Rasmussen, 1986a; Rasmussen, 1986b). Other protein kinases can also be activated by Ca^{2+} directly, or through an interaction with the calcium-calmodulin complex, as occurs with protein kinase C. Efflux of Ca^{2+} requires energy and results from the action of Ca^{2+}/Mg^{2+} ATPases (Ca^{2+} pumps located in the sarcolemma and in the sarcoplasmic reticulum membranes), which actively pump Ca^{2+} out of the cell and also into the sarcoplasmic reticulum. A reversible, electrogenic Na^{+}/Ca^{2+} exchange mechanism also contributes to returning $[Ca^{2+}]_i$ to its resting level (Blaustein and Nelson, 1982).

1.2.2.2 Cardiac cells.

In cardiac muscle, $[Ca^{2+}]_i$ fluctuates on a beat-to-beat basis. The cycle lasts from several milliseconds to over a second depending on the heart rate and the animal species. The actual mobilisation sequence has not been fully elucidated and certain aspects remain speculative (Seed and Walker, 1988), however both Ca^{2+} influx from the extracellular space as a result of cell membrane depolarisation, and release from sarcoplasmic reticulum, are involved. Ca^{2+} released from the sarcoplasmic reticulum may play a greater role in mediating contraction on a beat-to-beat basis. Wendt-Gallitelli and Jacob (1982), using electron probe

micro-analysis, demonstrated that when isolated cardiac muscle was left unstimulated for a prolonged period, Ca^{2+} stores were depleted. The first contraction following this quiescent period was smaller, and was not considered to be due to Ca^{2+} released from the sarcoplasmic reticulum, but from Ca^{2+} entering the cell during the cardiac action potential. When stimulation continued, sarcoplasmic reticulum storage sites were gradually refilled, and contraction increased with each beat in a "positive staircase".

Increase in $[Ca^{2+}]_i$ was shown to lead to phosphorylation of a 22-27 kDa polymeric protein, phospholamban, located in cardiac sarcoplasmic reticulum (Seed and Walker, 1988; Stoclet et al., 1988). The phosphorylations mediated by cAMP and calmodulin-dependent protein kinases occurred at three different sites of the protein, producing additive increases in the Ca^{2+} pumping activity of cardiac sarcoplasmic reticulum vesicles, and thus were thought to augment faster cycling of Ca^{2+} fluxes.

1.3 Ca^{2+} and cardiovascular disease.

In pathological conditions involving smooth muscle contractility, impairment of transmembrane Ca^{2+} movements result in the tissues' inability to maintain the Ca^{2+} gradient at rest, or to return the concentration to resting levels after cell activation. The consequence would be increased intracellular Ca^{2+}, resulting in increased tone and/or spasm.

Systemic hypertension is characterised by increased peripheral vascular reactivity and tone, resulting in increased resistance to

blood flow and therefore high blood pressure. Abnormalities in ion homeostasis have been postulated to be the primary pathogenic factors in hypertension (Bohr and Webb, 1986). McCarron (1985) and MacGregor (1985) presented opposing but equally convincing arguments about the roles of Ca^{2+} and sodium in altered vascular reactivity and increased peripheral resistance. Van Breemen et al., (1985) demonstrated greater uptake of Ca^{2+} in vascular smooth muscles from the spontaneously hypertensive rats (SHR) strain - a laboratory model for hypertension, than in normotensive Wistar-Kyoto (WKY) rats. Their experiments also demonstrated that in SHR rats there is an elevated influx of Ca^{2+} through 'leak' channels in the unstimulated muscle and through voltage sensitive channels in the depolarised muscle.

The strongest arguments for the role of altered Ca^{2+} regulation as the central pathophysiological mechanism underlying cardiovascular disease have been proposed by Hermsmeyer (1987), and Hall (1988). Hall reported that differences in dietary calcium intake separated normotensives from hypertensives better than did the dietary intake of sodium or potassium. Hermsmeyer stated that the action of calcium antagonists, not only to decrease total peripheral resistance, but to normalise blood pressure, and the fact that hypertension is not always associated with increased intracellular Na^{+}, makes a strong case for the role of Ca^{2+} as the central element in hypertension.

Primary disturbances of calcium homeostasis are found in pathologic conditions of the heart and other organs or tissues where $[Ca^{2+}]_o$ is required to mediate intracellular events. The

disastrous sequelae of uncontrolled hypertension on the heart and kidney (Cheung et al., 1986; Frohlich, 1989; Kannel, 1989) are also associated with aetiologies involving Ca^{2+}. Calcium antagonist intervention is useful in preventing and/or controlling some of these conditions (Dormandy, 1988; Flameng, 1988; Henry, 1988).

1.4 Calcium channels and transmembrane Ca^{2+} movements

Permeability of the plasma membrane of excitable cells to Ca^{2+} has been studied for almost four decades, yet it is only within the last few years that we have begun to understand and appreciate the complexity of pathways of transmembrane Ca^{2+} movements and intracellular Ca^{2+} mobilisation. Calcium channels are membrane-spanning macromolecular complexes that form a pore, and are capable of transferring millions of permeant ions (Ca^{2+}, Ba^{2+}, Sr^{2+}) per second (Tsien, 1987; Vaghy et al., 1988). They differ from pumps, exchange mechanisms or ionophores in that they are gated, and on opening allow Ca^{2+} and other permeant ions to pass through the membrane from regions of high concentration to regions of low concentration.

The existence of at least four different types of calcium channels has been proposed to explain transmembrane calcium movements in smooth muscle cells (Loutzenhiser et al., 1985; Schramm and Towart, 1985; Van Breemen et al., 1986). The channels have been designated as 'Leak', 'Stretch-sensitive', 'Receptor-operated' and 'Voltage-sensitive' calcium channels.

1.4.1 Leak channels.

Leak channels were assumed to exist based on the tendency of Ca^{2+} to diffuse passively into cells down its electro-chemical gradient when there is no activation. All measurements of Ca^{2+} influx using radiolabelled Ca^{2+} reveal a passive, basal influx which is generally unaffected by drugs, but is blocked by inorganic cations such as La^{3+} and Mn^{2+} (Cauvin et al., 1983). The role of this intrinsic leak mechanism in smooth muscle cell activation is not fully understood, but without counter-balancing mechanisms, it is of sufficient magnitude to elicit a contractile response.

1.4.2 Stretch-sensitive channels.

The existence of the stretch-sensitive calcium channel (Loutzenhiser et al., 1985), was hypothesised on the basis that mechanical effects promote Ca^{2+} influx (Bülbring et al., 1968), but there is no experimental proof that this entry is through defined channels. This influx could actually be through cell membranes which have temporarily lost their integrity.

1.4.3 Receptor-operated channels.

In many cells, activation of certain membrane receptors by neurotransmitters and hormones causes a rise in intracellular Ca^{2+} without causing membrane depolarisation (pharmaco-mechanical coupling) (Bolton, 1979; Meisheri et al.,

1981; Haeusler, 1985; Johns, 1987: Putney, 1987). This phenomenon suggested the existence of receptor-operated channels (ROCs). Hallam and Rink (1989), have recently reviewed the evidence for the existence of these Ca^{2+} entry processes. The most convincing evidence is summarised below:

1. Rasmussen and Barrett (1984), Rasmussen et al., (1987) and Putney (1987) have established that in smooth muscle calcium-mobilising receptors induce a biphasic response which is identifiable through a rapid phasic contraction due to inositol trisphosphate (IP_3)-induced release of Ca^{2+} from sarcoplasmic reticulum, and a more slowly generating but sustained tonic response attributable to influx of extracellular Ca^{2+}.

2. Functioning single channels with a selectivity of Ba^{2+}:Na^{+} of 30:1 (Hymel et al, 1988; Zschauer, 1988), whose conductance was similar to that of voltage-gated Ca^{2+} channels, under similar experimental conditions, were demonstrated to exist in reconstituted vesicles enriched in plasma membrane from platelets.

3. In kinetic studies of $[Ca^{2+}]_i$ transients in fura-2-loaded platelets using stopped-flow fluorimetry, ADP evoked an increase in $[Ca^{2+}]_i$ within 20 ms in the presence of $[Ca^{2+}]_o$, whereas in Ca^{2+}-free medium the rise in $[Ca^{2+}]_i$, which results from release of intracellularly stored Ca^{2+}, was delayed by about 200 ms (Sage and Rink, 1987).

Since second messengers can also cause release of Ca^{2+} from intracellular storage sites, there is a need to clarify the terminology pertaining to Ca^{2+} channels activated by cell membrane receptor activation and those activated by second messengers within the cell. Meldolesi and Pozzan (1987) defined ROCs as molecules or molecular complexes in which receptor and channel functions reside in the same entity. They then gave the name second messenger operated channels, (SMOCs), to channels activated by second messengers subsequent to membrane receptor activation.

1.4.4 Voltage-operated channels.

Voltage-operated Ca^{2+} channels (VOCs) have been identified in a wide variety of tissues (Hurwitz et al., 1980; Reuter, 1986; Bolton et al., 1987; Spedding, 1987; Tsien, 1987). Three distinct types, L-, N- and T-types, have been well-characterised, using single channel recording techniques. They can also be distinguished from each other by their different sensitivities to pharmacological agents.

T-type channels have a low threshold for opening which occurs by small depolarisation from very negative potentials, and pass a rapidly inactivating transient Ca^{2+} current. They have a small conductance and are insensitive to modulation by dihydropyridines and are not affected by omega conotoxin, a toxin isolated from the marine snail Conus geographicus, which blocks neuronal N- and L-type channels (Tsien, 1987).

N-type channels are found in neurons. They are activated by strong depolarisation from negative holding potentials, possess conductance and opening time intermediate between T- and L-type channels, and are also insensitive to modulation by dihydropyridines.

L-type calcium channels are the widely distributed, classical slow-opening channels (Meldolesi and Pozzan, 1987; Tsien, 1987). They have a high conductance and prolonged open time, and appear to be involved primarily in the regulation of $[Ca^{2+}]_i$. Inactivation is slow and appears to be controlled by a dual mechanism, a time- and voltage- dependent mechanism, and a $[Ca^{2+}]_i$-dependent mechanism. They are regulable by hormones and neurotransmitters and are subject to modulation by dihydropyridine Ca^{2+} modulators.

In heart muscle, cAMP levels have been found to increase the probability of single channels to remain open with no change in their kinetics or unitary conductance. This cAMP-dependent modulation accounts for the positive inotropic effects of a-adrenergic agonists, and the negative inotropic effects of muscarinic agents. The effects are mediated through a_1 and M_2 receptors respectively, which exert opposite effects on adenylate cyclase.

Evidence from biophysical, biochemical and pharmacological studies has now shown that different subtypes of the L-type channel may exist (Meisheri et al., 1981; Glossman et al., 1984; Bolton et al., 1988). Tsien et al., (1987) listed three major differences found between L-type Ca^{2+} channels in chick dorsal root ganglion

(DRG) cells and those found in cardiac cells. 1. Omega conotoxin blocked L-type channels in chick DRG cells with high affinity, but exerted no detectable effect on cardiac L-type channels. 2. Cardiac L-type channels demonstrated both a Ca^{2+} entry-dependent and voltage-dependent mechanism for inactivation, but the Ca^{2+} entry-dependent inactivation appeared to be less important in DRG L-type channels. 3. Elevated cAMP levels greatly enhanced cardiac L-type channel activity but diffusible cAMP derivatives failed to increase DRG L-type channel activity.

Differences in the sensitivities of the L-type Ca^{2+} channels in different tissues to different ligands, may therefore reflect a difference in the channel structure, indicating a different form (iso-species or subtype) of the channel in different tissues.

1.4.5 Calcium channel structure

The richest source of L-type calcium channels is skeletal muscle, however the kinetics of the channel in this tissue appear to preclude its involvement to any significant extent in skeletal muscle contraction (Beaty et al., 1987; Fosset and Lazdunski, 1987; Vaghy et al., 1988). Specific binding sites for calcium antagonists have been identified on this channel in T-tubule membranes from rat, frog and guinea pig (Fosset et al., 1982; Goll et al., 1983; Galizzi et al., 1984).

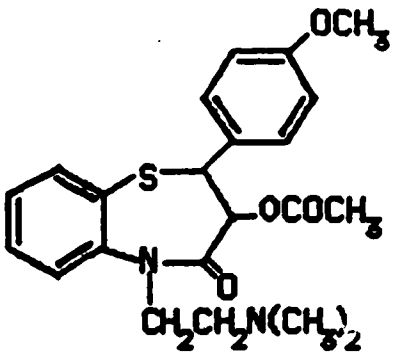
Based on molecular weights determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, Vaghy et al., (1988), have suggested that the channel

comprises five subunits, corresponding to protein subunits of molecular weights 165 kDa, 140 kDA, 50 kDa, 33 kDa and 30 kDa. The 165 kDa subunit was shown to have the binding sites for 1,4-dihydropyridine and phenylalkylamine drugs. Although there is no conclusive evidence yet, photoaffinity labelling of the membrane-bound diltiazem receptor seems to indicate that this subunit possesses the diltiazem binding site as well.

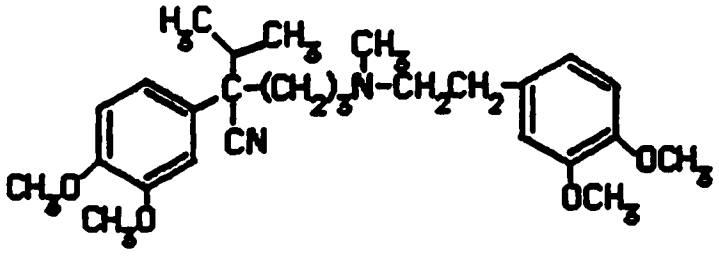
The 50 kDa subunit can be phosphorylated by protein kinases, and hence may be a site of regulation for the channel. The 165 kDa protein has several membrane-spanning regions and an amino acid homology of 55% with a rat brain Na^+ channel. The function of the other proteins remains unidentified although the 140 kDa subunit appears to have two or three membrane-spanning regions and may thus form part of the channel itself.

1.5 Calcium channel modulators

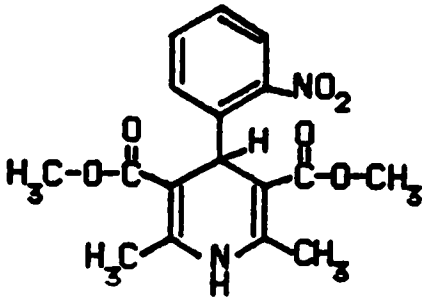
A number of structurally diverse compounds that modulate the transmembrane movement of Ca^{2+} are now available (Triggle and Swamy, 1980; Fleckenstein, 1984). The best known of these compounds (fig. 1.2) are verapamil, diltiazem and nifedipine which are calcium channel antagonists, and Bay K 8644 which is structurally very similar to nifedipine but has diametrically opposite effects (Schramm et al. 1983; Loutzenhiser et al. 1984; Rogg et al. 1985) and is the prototype calcium channel activator. Of the three prototype calcium antagonists, nifedipine is the most potent calcium antagonist.



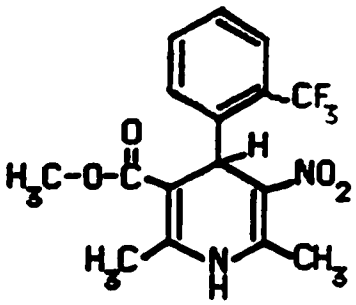
Diltiazem



Verapamil



Nifedipine



(R)-(+)-Bay K 8644

Figure 1.2 Prototype Ca^{2+} antagonists specific for the slow Ca^{2+} channel.

The predominant pharmacological effects of the calcium channel inhibitors or antagonists are:- vasodilation of peripheral, cerebral and coronary blood vessels, negative inotropic effects and inhibition of sinoatrial and atrioventricular nodal excitation (Vanhoutte 1987). They have therefore become a clinically important group of drugs for the treatment of cardiovascular disorders including hypertension, ischaemic heart disease, coronary artery spasm, Raynaud's disease, hypertrophic cardiomyopathy and some supraventricular tachyarrhythmias.

Specific receptor sites for the calcium antagonists have been identified on L-type calcium channels of different tissues (Triggle and Swamy, 1983; Glossman et al., 1984; Spedding and Berg, 1984; Spedding, 1985; Laragh et al., 1986; Schwartz et al., 1988; Vaghy et al., 1988). Biochemical and <u>in vitro</u> studies indicate that there is some degree of interaction between the different binding sites (fig. 1.3) of these calcium antagonists (Godfraind et al., 1986). Spedding (1983) reported additive effects of the inhibitory activities of diltiazem and nimodipine on guinea pig taenia coli. Furthermore, binding of diltiazem and nimodipine to their respective binding sites was reported to be mutually synergistic (Yousif and Triggle, 1985).

Although calcium antagonists share a common mechanism of action, they differ significantly in their pharmacological and clinical effects (Cauvin et al., 1983; Saida and Van Breemen, 1983; Hof, 1984; van Zwieten, 1985). Verapamil greatly decreases sino-atrial and atrio-ventricular nodal conduction and is thus useful for the treatment of supraventricular arrhythmias. It is also more likely

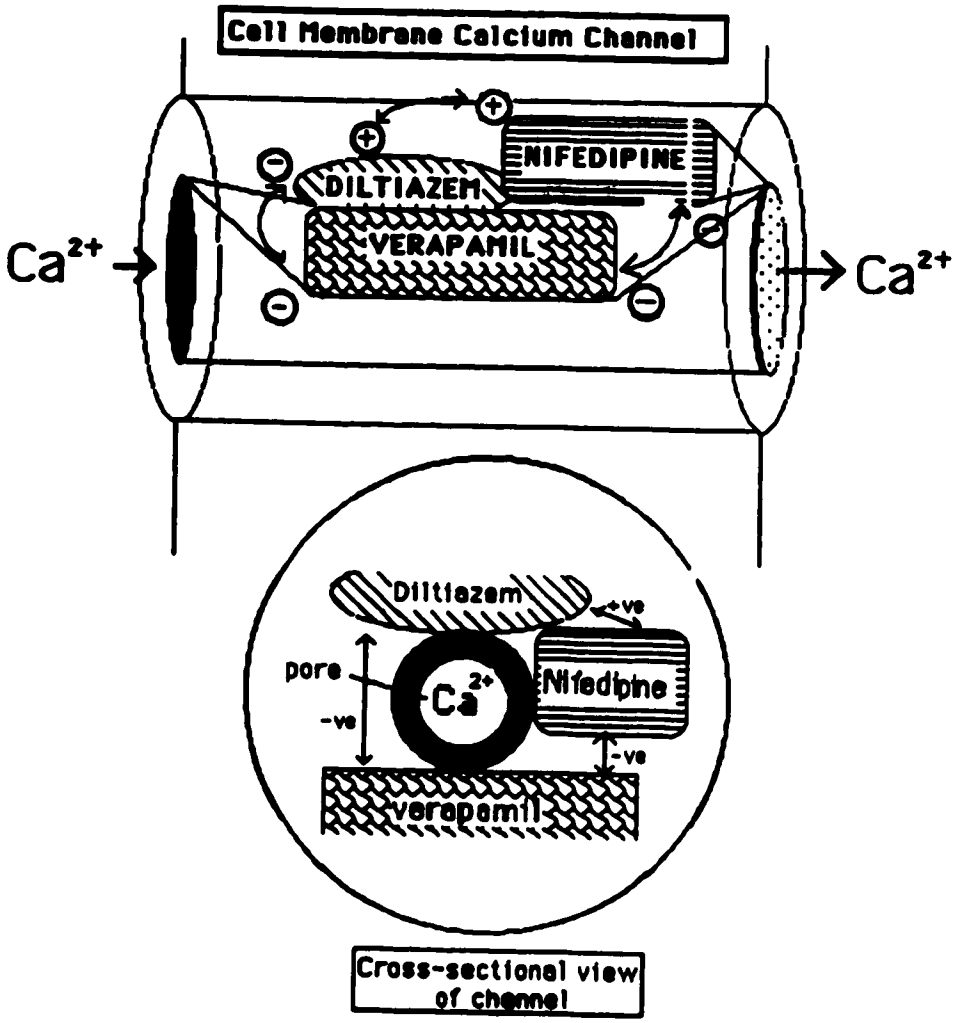


Figure 1.3 Interaction of the binding sites of Ca^{2+} antagonists

to interact with other ion channels and/or receptors (Scriabine, 1987). In the heart, it produces a greater degree of negative inotropic effect than diltiazem or nifedipine and its congeners.

The pharmacology of nifedipine was first described by Fleckenstein in 1971. It is a potent vasodilator that lowers arterial pressure and prevents the development of hypertension in experimental animals (Kazda et al., 1983). In humans it reduces peripheral vascular resistance with little or no sodium and water retention or increase in heart rate, and is indicated for angina because of its coronary artery relaxation effects and reduction of myocardial oxygen demand.

Diltiazem is an effective vasodilator used primarily for spastic coronary artery diseases. It is also effective in treating supraventricular tachycardias, including sinus tachycardia and accelerated AV junctional tachycardia (Schneeweiss and Schettler, 1988).

1.5.1 Classification of calcium antagonists.

Several attempts have been made to classify calcium antagonists. Classification has been difficult because the agents comprise such a diverse group both chemically and pharmacologically. Six major classes (table 1) have been proposed for agents that inhibit transmembrane calcium flux through slow Ca^{2+} channels (Vanhoutte, 1987). The first three classes which are selective for slow calcium channels are: 1) benzothiazepines, exemplified by diltiazem, 2) dihydropyridines, exemplified by nifedipine and 3) phenylalkyamines represented by verapamil. The other three classes are nonselective for calcium channels and were characterised as: 4) flunarizine-like, 5) prenylamine-like and 6) others including perhexiline and caroverine. Several papers dealing with the prototype calcium antagonists have been published. I shall now only discuss the dihydropyridine class since they comprise the subject matter of this thesis.

1.5.2 1,4-dihydropyridine calcium antagonists.

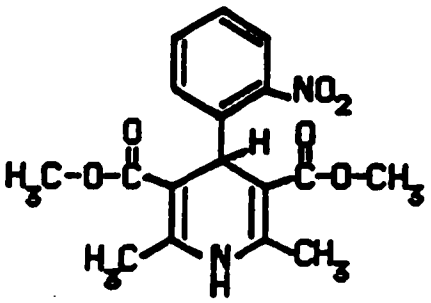
Although nifedipine is considered the prototype 1,4-dihydropyridine (1,4-DHP) calcium antagonist, it shares this distinction with a few other compounds. Niludipine, ryosidine and SKF 24260 (figure 1.4), although not marketed, are other first generation 1,4-DHP compounds that had comparable pharmacological profiles to that of nifedipine (Scriabine, 1987). A major limitation in the therapeutic use of these calcium antagonists was

Table 1.1

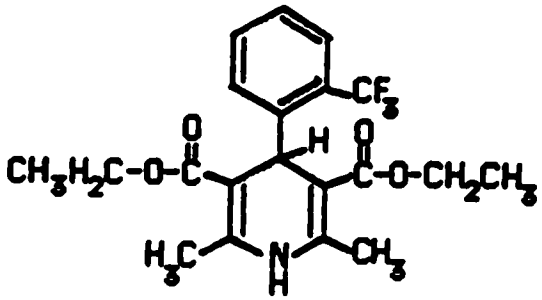
Current classification of Ca^{2+} antagonists.

	SELECTIVE FOR VSCs			NONSELECTIVE FOR VSCs		
EFFECT	I	II	III	IV	V	VI
SI of myocardial VSCs	+	+	+	-	-	-
Inhibit Na^+ fast channels	-	-	-	0	+	+
Inhibit cardiac inotropism	+	+	-	-	0	0
Damping of SA and AV node activity	+	+	+	0	+	+
Inhibit VSCs of VSM.	+	+	+	+	+	+
Inhibit myogenic VSM activity	+	+	+	-	0	0
Inhibit ROCs of VSM.	+	+	+	+	+	+
Antihypertensive	+	+	+	-	0	0
Antagonise effects of Bay K 8644	+	+	+	-	-	-

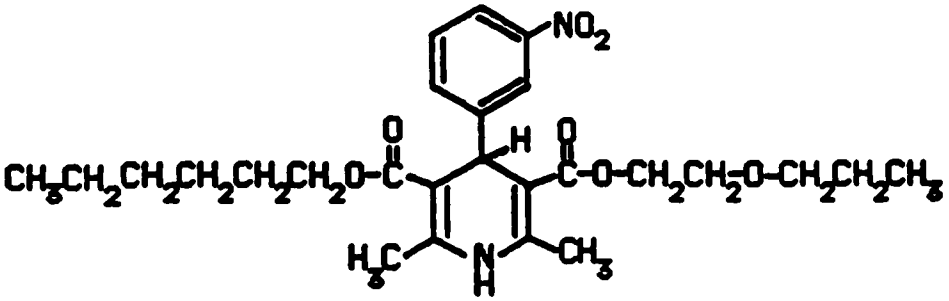
I - phenylalkylamines; II - 1,4-DHPs; III - Benzothiazepines; IV - Flunarizine-like; V - Prenylamine-like; VI - Others (perhexiline, caroverine). SI = selective inhibition; VSM = vascular smooth muscle;



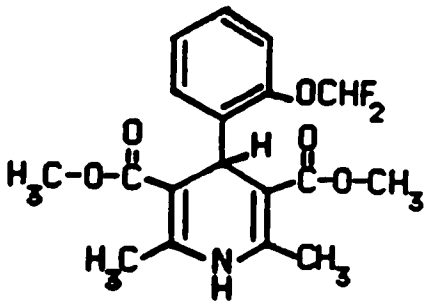
nifedipine



SKF 24260



Niludipine



Ryosidine

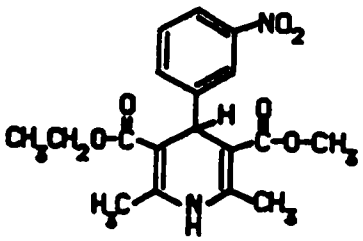
Figure 1.4 First generation 1,4-DHP Ca^{2+} antagonists.

related to their lack of selectivity between tissues (i.e. cardiac vs smooth muscle) and/or specificity within tissues (i.e. preferential activity in certain vascular beds).

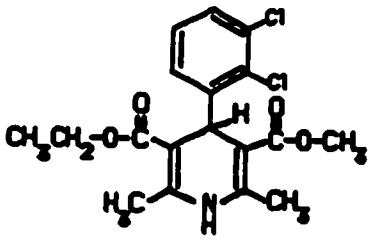
Attempts to improve this pharmacological profile has led to the development of a number of second generation compounds. Some of the second generation 1,4-DHPs (fig. 1.5) have demonstrated clinical advantages over the first generation compounds. Desirable improvements include a longer duration of action, greater differential of activity between cardiac and smooth muscles, greater tissue specificity and greater selectivity for different vascular beds. Examples of second generation 1,4-DHP compounds that are now in clinical use are nimodipine and nicardipine which are highly selective for cerebral and coronary vasculature respectively.

The approach used to develop second generation 1,4-DHPs has been one commonly used by medicinal chemists and pharmacologists to develop new therapeutic agents or to improve the pharmacological or therapeutic profile of a drug or class of drugs. Certain functional groups of a 'lead' molecule were altered to produce derivatives; a systematic study of the structure-activity relationships (SARs) of a number of the derivatives is made l ding to identification of features which are responsible for altering or improving the activity of the parent compound.

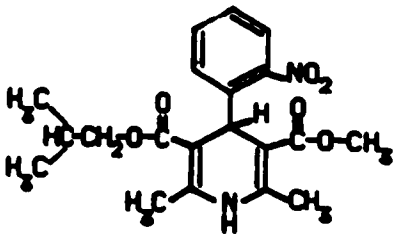
Using the prototype 1,4-DHP nifedipine as the 'lead' molecule, several studies have identified the structure-activity relationships of 1,4-DHP calcium antagonists (Bossert et al.,1979; Triggle and Janis, 1985; Dagnino et al., 1986; Wolowyk et al., 1987; Ramesh et



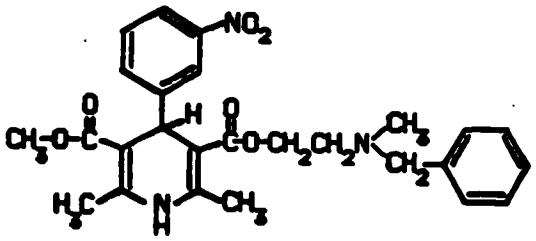
Nitrendipine



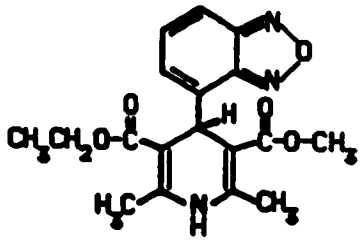
Felodipine



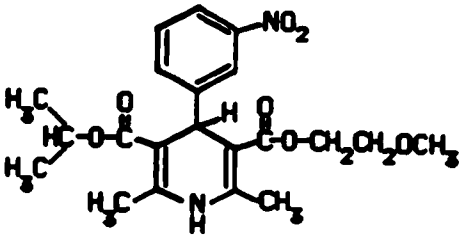
Nisoldipine



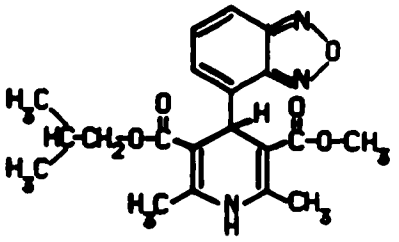
Nicardipine



Darodipine



Nimodipine



Isradipine

Figure 1.5 Some second generation 1,4-DHP Ca^{2+} antagonists.

al., 1987). (The SARs are discussed in detail in the discussion section).

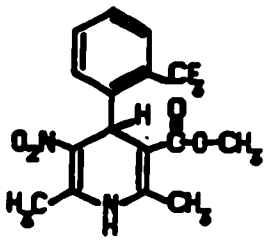
1.5.3 1,4-DHP calcium channel agonists

Bay K 8644 is the prototype compound of closely related 1,4-DHP compounds now generally known as calcium channel agonists or activators (fig. 1.6), (Schramm et al., 1983a; Schramm et al., 1983b; Spedding and Berg, 1984). It was shown to be a potent vasoconstrictor and positive inotropic agent. While nifedipine and related 1,4-DHP calcium antagonists exert their antagonist effects by binding to a specific dihydropyridine binding site of the calcium channel, thereby 'closing the channel' (Triggle and Swamy 1980), Bay K 8644 and the Ca^{2+} channel activators bind to the same site but promote or facilitate the opening of the channel.

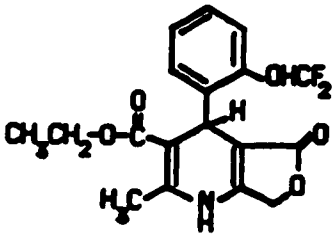
Bay K 8644 exists as two enantiomeric forms, with the calcium channel agonist properties residing in the (-)-isomer, and the (+)-isomer possessing the antagonist properties. The racemic mixture displays agonist activity at low concentrations and antagonist actions at high concentrations.

Another 1,4-DHP, 202-791, shows the same dualistic activities with agonist activity residing in the (+)-isomer and antagonist activity in the (-)-isomer (Vaghy et al. 1987). Similarly to Bay K 8644, the racemate has agonist activity at low concentrations and antagonist actions at high concentrations.

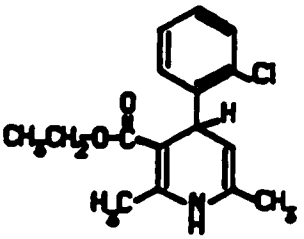
Most 1,4-DHPs have enantiomeric forms since they tend to have a chiral carbon at position 4 of the dihydropyridine ring (figs. 1.4,



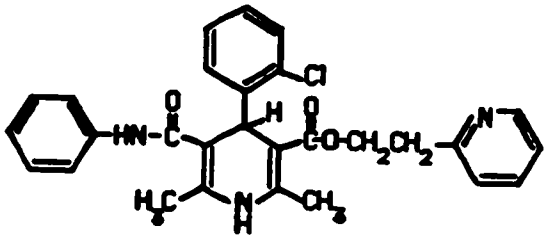
(S)-(-)-Bay K 8644



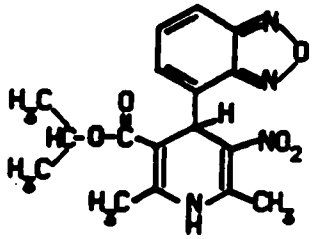
CGP 28 392



H 160/51



YC - 170



(R)-(+)-202 791

Figure 1.6 1,4-DHP calcium channel agonists.

1.5 and 1.6), however, the great majority do not display this dualism of action. Differences in the inhibitory effects however have been reported for the enantiomers of the antagonist compounds.

Bay K 8644 is a competitive inhibitor of nifedipine and other 1,4-DHP calcium antagonists on cardiac and smooth muscles. The 1,4-DHP nucleus is therefore unique in that calcium activator and inhibitory effects exist in the same chemical moeity. Based on this observed dualism of action of some 1,4-DHPs and the close structural similarities of these compounds, it is reasonable to assume that dihydropyridine compounds may be agonists, partial agonists or antagonists at the voltage sensitive calcium channel.

1.5.3.1 Uses for calcium channel activators

While 1,4-DHP calcium channel antagonists are now used clinically and structural modification continues to provide improved therapeutic agents, calcium channel agonists have yet to be developed to the point of clinical application. Bay K 8644 increases cardiac contractility in the same dose ranges that cause constriction of blood vessels (10^{-10}-10^{-6}M), the latter effect increasing peripheral resistance and hence increasing myocardial work. This has precluded its use use as a cardiotonic agent.

Potential uses for Bay K 8644 have been reported. In a study on endotoxin-induced shock in rats, Ives et al. (1987) reported a remarkable reversal of the condition by Bay K 8644, at concentrations that had no effect in normal animals. This reversal was marked by dramatic increases in blood pressure and cardiac

function and much improved haemodynamics. This is one example of possible therapeutic applications for calcium channel agonists.

Pillai and Roth (1987) reported that Bay K 8644 counteracted the morphine-induced hyperthermic response in the rat, and restored Ca^{2+}/Mg^{2+} ATPase activity in the hypothalamus of these animals. Paradoxically, this also occurs with calcium channel blockers. Johnson et al., (1988) found that the (+)-isomer of Bay K 8644 inhibited platelet activation by competitive antagonism of thromboxane A_2 in micromolar concentrations. 1,4-DHP antagonists also exerted the same effect. These results suggest that DHPs may possess other effects which are distinct and separate fr[illegible]d possibly unrelated to their calcium channel agonist/ant[illegible]st effects.

It is very probable that as more work is done with 1,4-DHPs, new pharmacological effects will be discovered and these may redirect the focus of possible applications or indicate other uses for these compounds as pharmacological or biochemical probes.

II. PROPOSED RESEARCH.

2.1 Preamble

In the last decade, calcium channel research and calcium channel pharmacology have developed into an area of intense pharmacological activity. The diversity of chemical compounds possessing the ability to inhibit transmembrane calcium fluxes has contributed to this activity. Despite advances made torward unravelling the structure of the calcium channel itself, detailed information regarding its regulation and physiological function is still unknown.

Among the Ca^{2+} channel modulators available, the 1,4-DHPs have captured special interest due to their potency and tissue selectivity as calcium antagonists, particularly in blood vessels. The discovery of calcium channel activator properties in some of the compounds has added to the challenge of defining structural requirements of pharmacophores at the calcium channel.

Second generation 1,4-DHP calcium antagonists with differential activity between tissues, and great selectivity within tissues, have been developed. While some of these compounds are already in clinical use or are undergoing clinical trials as antihypertensive agents, more are still in the early stages of development, and may display even better pharmacotherapeutic profiles than the compounds currently available.

1,4-DHP calcium channel agonists could be useful as cardiotonic agents, but currently available compounds are not used therapeutically because they tend to constrict blood vessels in the same dose-ranges that increase cardiac contractility. Attempts to develop compounds possessing calcium channel activator effects on cardiac muscle, yet devoid of these effects on smooth muscle are being made (Franckowiak et al., 1988; Holland et al., 1988). Separation of the calcium activator and inhibitory effects of the dihydropyridine nucleus is expected to lead to the production of such compounds.

Using the 1,4-DHP compounds nifedipine and Bay K 8644 as 'lead' molecules, Dr. E.E. Knaus and two of his research associates, Dr. Manian Ramesh and Dr. Murthy Akula, have made several substitutions of different functional groups on the dihydropyridine nucleus (fig 2.1). The subject matter of this thesis is therefore threefold: 1) to make a systematic pharmacological characterisation of the structure-activity relationships (SARs) of these novel 1,4-DHPs, 2) to attempt to elucidate the interaction of 1,4-DHPs with their receptor(s) on the calcium channel, through the observed resultant changes in pharmacological responses, and 3) to identify compounds with potential as therapeutic agents.

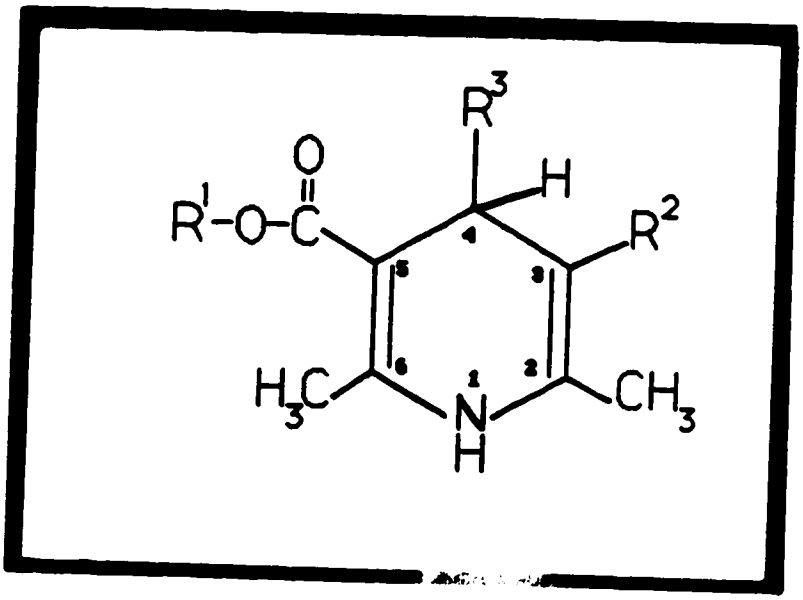


Figure 2.1 1,4-dihydropyridine nucleus showing positions (R^1, R^2 and R^3) where substitutions were made to produce novel compounds. Numbers in the ring indicate the numbering of the atoms in the 1,4-DHP nucleus.

2.2 Objectives

Specific objectives of this thesis were therefore to use isolated tissues and intact animals to:

1. quantitate the *in vitro* agonist/antagonist pharmacological profiles of new 1,4-DHP calcium channel modulators on smooth muscle using standard pharmacological screening tests.
2. quantitate the pharmacological effects of the new 1,4-DHPs on cardiac contractility in the isolated guinea pig left atrium.
3. establish some structure-activity relationships (SARs) of the new compounds on the above-mentioned tissues and to identify structural features that impart differential activity and tissue-selectivity on these tissues.
4. attempt to elucidate the exact nature of the interaction of the dihydropyridine calcium channel modulators with the dihydropyridine binding site of the voltage-sensitive calcium channel.
5. establish the *in vivo* haemodynamic effects of some of the more interesting novel compounds in order to identify potential new drugs, and to identify relevant pharmacologic effects not seen *in vitro*.

III. MATERIALS AND METHODS

3.1 Choice of methods.

The screening methods used were chosen because they were fast and efficient, and could confirm or negate the existence of Ca^{2+} channel modulator effects in a compound economically. These methods were considered to be sufficiently definitive to provide conclusive evidence for the mode and site of action of a compound. Since it is rare that any one 'test' can fulfil these requirements, it was necessary to carry out a number of tests such that when the results were combined, the information obtained would provide the answers to the questions raised.

Although it was desirable to do as many tests as possible in order to meet all the requirements for pharmacological characterisation of calcium channel activators, the number of compounds that had to be tested, and the time and resources available imposed limits as to what could be achieved. To achieve the desired objectives with maximal economy, a number of tissues were examined for suitability and the following tissues were chosen as best fitting the requirements: a) guinea pig ileal longitudinal smooth muscle (GPILSM); b) guinea pig left atrium; and c) rabbit bladder. These tissues were found most suitable for the following reasons:

1. They are easily isolated.
2. They are available in fairly large quantities (GPILSM).
3. They retain viability and produce large and consistent and reproducible responses for fairly long periods after isolation.
4. They respond to and recover from stimulation rapidly and therefore save time in repetitive experiments.
5. They are dependent on $[Ca^{2+}]_o$ for contraction, therefore provide a good system for testing L-type Ca^{2+} channel modulators.

Rabbit bladder was used for studies on the calcium agonist effect of some compounds because it displayed responses intermediate between those of GPILSM and rabbit aorta, and was dependent primarily on extracellular Ca^{2+} for contraction (Fovaeus et al., 1987). It had the same advantages as GPILSM and did not require extra K^+ in the buffer (Schramm et al., 1983), in order to respond to calcium channel activators.

Two animal models were chosen for <u>in vivo</u> testing, the spontaneously hypertensive rat (SHR) and the rabbit. The spontaneously hypertensive rat (SHR) was chosen for three reasons:

a). Rats are relatively cheap and easy to handle (compared to larger animals).

b). SHR is a good model for testing antihypertensive drugs as it develops hypertension at age 7-15 weeks without exception (Yamori, 1984).

c). Precedent - this model has been used extensively for studying various aspects of hypertension.

The rabbit was used because it was also fairly cheap and has been used extensively for these types of studies. It was also chosen because confirmation of pharmacological findings in guinea pig tissues and in the rat would strengthen the premise that the compounds would work in the same manner in humans.

3.2. Novel compounds, standard drugs and physiological solutions

All novel compounds tested and Bay K 8644 were synthesized in Dr. E. E. Knaus's medicinal chemistry laboratories at the University of Alberta. Standard drugs used were carbachol (Aldrich Chemical Co. Inc., Milwaukee, Wis.); (-) arterenol, atropine, isoproterenol and nifedipine (all from Sigma). All other chemicals were purchased from Fisher, Canlab, Aldrich or Sigma.

For most <u>in vitro</u> experiments HEPES buffered physiological saline solution (HPSS) of the following composition in mM/L was used: NaCl 137.0, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.6, D-glucose 11.9, HEPES 9.0, and adjusted to pH 7.4 with normal NaOH. In experiments using KCl, isotonic KCl was prepared by equimolar substitution of NaCl in the HPSS. Ca^{2+}-free HPSS was prepared by omitting Ca^{2+} from the HPSS and adding 2 mM EGTA (Yamamoto et al., 1984). In experiments on isolated bladder, the HPSS was modified to the following composition: NaCl 140.0, KCl 5.0, $MgCl_2$ 1.0, $CaCl_2$ 1.5, D-glucose 10.0, HEPES 5.0, and adjusted to pH 7.4 with normal

NaOH (Yamamoto et al., 1984).

3.3 IN VITRO EXPERIMENTS

3.3.1 Guinea pig ileal longitudinal smooth muscle - GPILSM:

Male Hartley strain guinea pigs (Charles River Canada Inc., St. Constant, Quebec) weighing 350-700g were sacrificed by stunning and exsanguination. The method used to isolate the desired organ was that of H. P. Rang (1964). The animal was laid on its back and a longitudinal midline incision was made in the abdominal wall, exposing the abdominal viscera. The caecum was lifted and shifted to the right side of the animal, exposing the ileo-caecal junction. The ileum was then cut a few centimetres above this junction and freed of mesenteric attachments for about twenty centimetres rostrally. A second cut was made at the end of the cleared section to isolate the section. Taking care not to stress the tissue, the section was stretched over a glass rod of appropriate diameter and immersed in HPSS that had been aerated with 100% oxygen.

Two incisions were made on either side of the line of mesenteric attachment with a blunt scalpel blade, taking care not to cut all the way through the ileum. A cotton-tipped applicator (Q tip) was then used to separate the longitudinal smooth muscle which is the outer layer of the intestinal smooth muscle, by tangentially

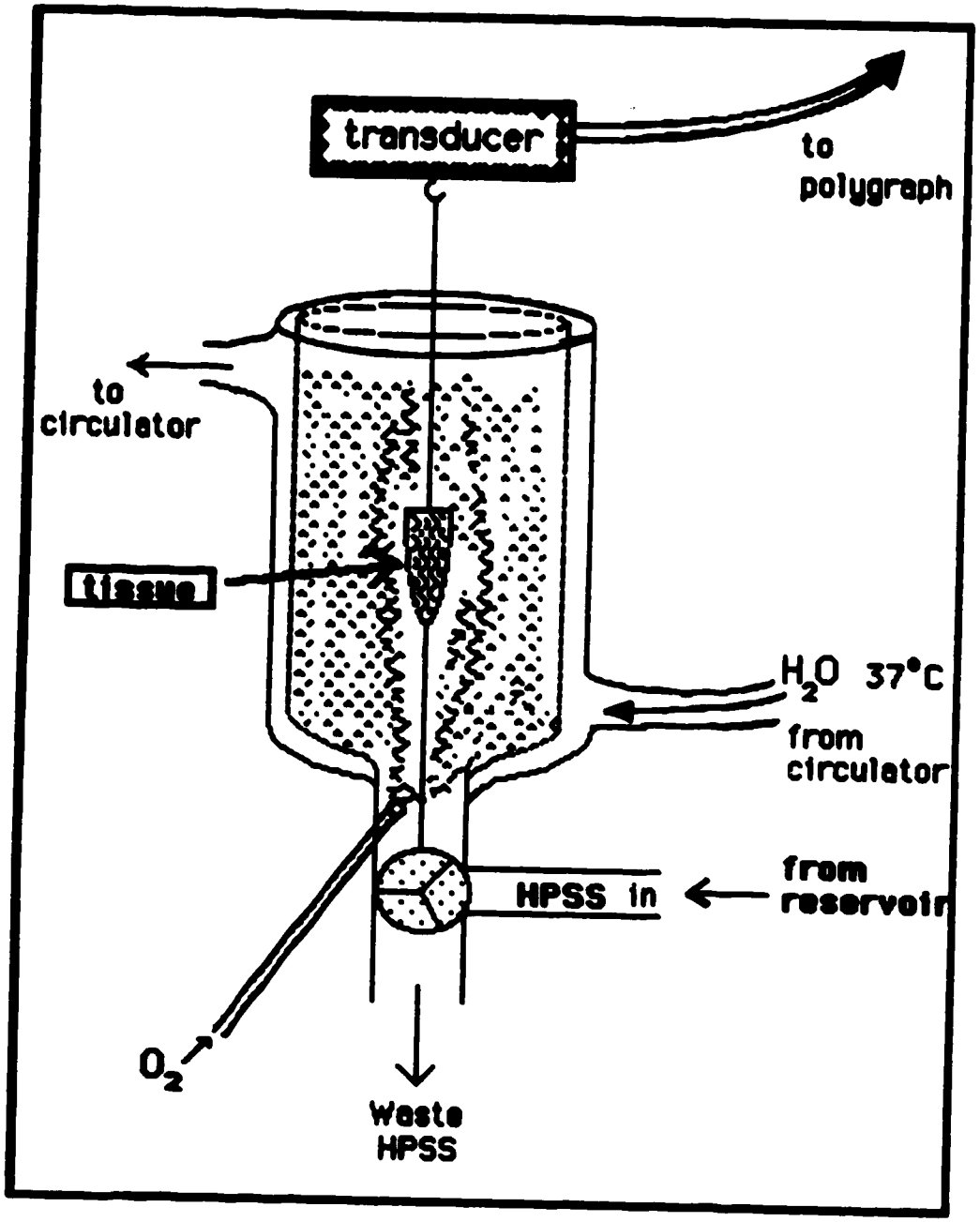


Figure 3.1 Isolated tissue set-up for smooth muscle and right atrium. The left atrium was attached to a stimulating electrode and suspended similarly as the other tissues.

brushing it away from the underlying tissue; this was isolated as a sheet along the length of the section. 1.5 to 2 cm sections of this tissue were then prepared by tying each end with a piece of thread and using the threads to suspend the tissue in a tissue bath of 10 ml capacity (figure 3.1). One thread was fixed to the bottom of the bath and served to anchor the tissue, the other end was used to attach the tissue to a force-displacement transducer (Grass FT03). The transducer was coupled to a polygraph machine (Grass Model 7D) which recorded isometric tension responses for the experiments.

The bath was filled with HPSS maintained at 37^{o}C by a water circulator (Haake Model E52 or Braun Thermomix II) and aerated with 100 % oxygen. An initial tension of about 500 mg was applied to each tissue. Tissues were left to equilibrate for about 45 minutes with changes of HPSS every fifteen minutes before the desired experiments were carried out. The resting tension of the tissues invariably declined during the equilibration period and was readjusted to 250 mg at the beginning of the experiment. For all isolated tissue experiments, chart speed was set at an appropriate speed (usually 2.5 - 5.0 cm/min), during the equilibration period in order to observe tension changes occurring in the tissues.

3.3.2 Atrial tissues

A guinea-pig was sacrificed as described above and the thoracic cavity opened to expose the thoracic organs. Working as quickly possible the heart was excised and immediately immersed in HPSS at 37^{o}C. Extraneous tissues attached to the atria were dissected

away. The atria were then separated into left and right atria. A thread was attached to each end of an atrium. The spontaneously beating right atrium was suspended in a tissue bath, in much the same way as GPILSM and allowed to equilibrate in HPSS.

The procedure for the left atrium differed from that of the right atrium in that the bottom thread was used to anchor the left atrium to a platinum stimulating electrode (25 gauge platinum wires embedded 1.0 mm apart in plastic). The tissue was electrically-driven at a constant rate (square pulses of 1.5 msec duration at a frequency of 1.5 hertz) through a Grass stimulus isolation unit (SIU5) attached to a Grass S44 stimulator.

Both atria were allowed to equilibrate under a resting tension of about 250 mg for one hour with HPSS changes every 15 minutes. Optimal length-tension relationships were determined for each tissue prior to beginning each experiment (Li-Kwong-Ken, 1986). The right atrium was used to study chronotropic effects and the left atrium for studying inotropic effects.

3.3.3 Rabbit bladder

Male rabbits (New Zealand albino) weighing 1.5 to 3.0 kg were sacrificed by cervical dislocation and exsanguination. The lower abdominal wall was opened by a midline incision to expose the lower abdominal contents. The bladder was isolated according to the methods described by Creed (1971) and Fovaeus et al., (1987). The bladder which appears as a pale yellow fluid-containing organ, was taken out, emptied of residual urine and rinsed thoroughly in HPSS.

An incision was made longitudinally to open up the organ. A transverse cut was made above the level of the openings of the ureters to isolate the lower trigone which was discarded. The top section of the bladder was also cut away leaving a roughly rectangular piece of tissue. Vesical mucosa and perivesical connective tissue and fat were removed. Strips about 1 cm in length and 0.1-0.2 cm in diameter were made along the grain of the muscle. Thread was attached to each end of a tissue strip and these were treated in the same manner as GPILSM. Initial resting tension for this tissue was set between 0.75 and 1.0 g, and was readjusted to 0.5 g at the beginning of the experiment.

3.4 IN VIVO BLOOD PRESSURE MEASUREMENTS

3.4.1 Spontaneously hypertensive rats (SHR strain)

Conscious male SHR rats (colony derived from rats obtained from Charles River Canada Inc., St. Constant, Quebec and maintained and bred by the Animal Care Unit, University of Alberta, Edmonton, Alberta) were restrained in cages and warmed on a heated plate to 28 - 32 ^{o}C. Indirect systolic blood pressure and heart rate of the animals were measured using the tail-cuff method (Yen et al., 1978; Borg and Viberg, 1980; Matsuda et al., 1987), modified to accommodate a semi-automatic pressure generating system. Pulse and pressure were recorded through a pneumatic pressure transducer (Gould P23 ID) attached to the Grass polygraph.

Control blood pressure measurements were made after the animals were warmed sufficiently to produce a discernible pulse in the transducer. At least three measurements were taken and the average of these was used as control. Drug was then administered to the animal by intra-peritoneal injection (i.p.) and measurements were taken at appropriate intervals. Animals were kept restrained for the duration of the experiment (but not longer than three hours).

3.4.2 Anaesthetised rabbit

Male rabbits (New Zealand albino) weighing 1.5 to 3.0 kg were anaesthetised with pentobarbital (Abbott Laboratories - 30 mg/kg i.v. through a marginal ear vein or 40 mg/kg i.p.) or Rompun cocktail (zylazine - 8 mg/kg (Chemagro Ltd), ketamine - 40 mg/kg (M.T.C. Pharmaceuticals), acepromazine - 0.5 mg/kg (Ayerst Laboratories)). Lidocaine (Xylocaine 1% (Astra Pharmaceutical Canada Ltd.)) was injected into the neck area and the trachea exposed and intubated. Supplemental pentobarbital (8-15 mg/kg) was injected as needed during the course of the experiment.

The animal was then artificially respirated using a piston-type respirator (Ugo Basile 6025 Cat/Rabbit Ventilator) at a rate of 25 - 40 breaths per minute, with a tidal volume of 10 ml/kg.

The jugular vein (left or right) was cannulated and catheterised (18G Quik-cath intravascular Over-The-Needle Teflon catheter with Luer plug - (Travenol Laboratories)). This line was then used for injection of normal saline and drugs. The carotid artery (opposite side to catheterised vein) was also cannulated and catheterised

and was used for measuring arterial pulse pressure. This was done through a pressure transducer (Gould P23 ID) attached to a Grass model 7D polygraph. Heparin (50 units/kg in normal saline) was then infused into the animal through the venous line.

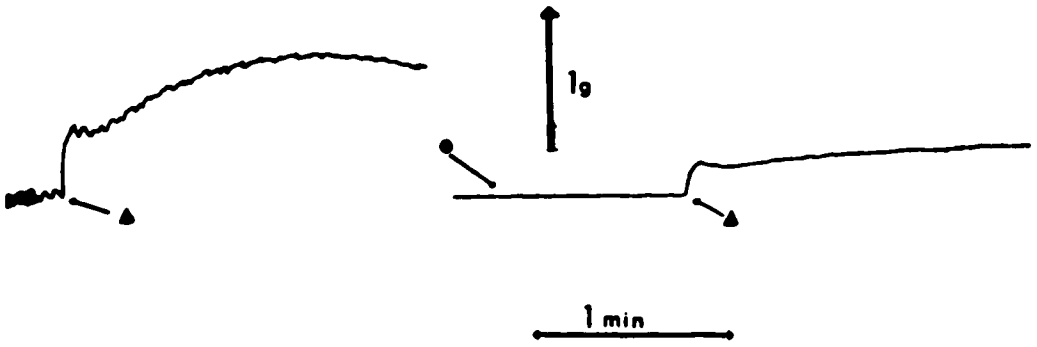
The animal was allowed to equilibrate for up to an hour, depending on stability of blood pressure and ECG, before experiments were started.

3.5 Experimental protocols.

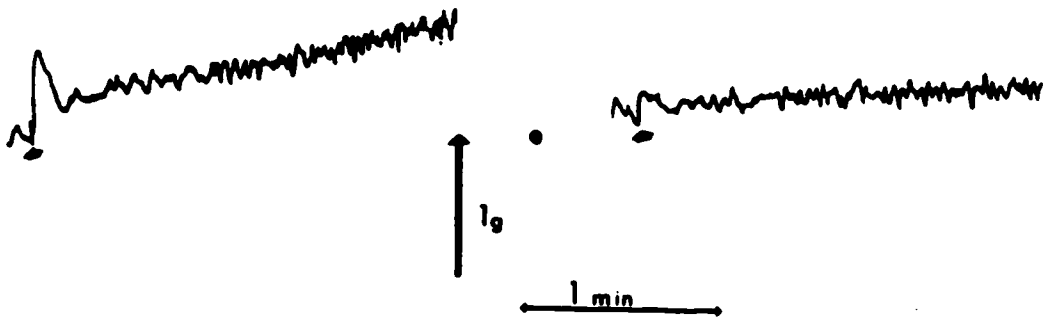
After an appropriate equilibration period, experiments were usually started by exposing the tissue or animal to a test dose of an agonist drug. This served to establish control responses against which the action of the compounds could compared. The following protocols were used

3.5.1 Evaluation for Ca^{2+} antagonism on GPILSM.

1. Increase chart speed to 25 cm/min, add 65 ul of 2.5 x 10^{-5} M carbachol (final conc. = 1.6 x 10^{-7} M), wait for response (fig. 3.2) to reach maximum of the tonic (slow onset and slow to peak contraction) response.
2. Reduce chart speed to control speed (2.5-5.0 mm/min), wash tissue once, wait 15 minutes, and repeat step 1.
3. Repeat steps 1 and 2 until consistent responses were obtained (usually two to three times).



a). Inhibition of GPILSM MRM response (carbachol 1.78×10^{-7} M) response by the C-4 2-pyridyl Bay K 8644 analogue, Reek 30 (1.13×10^{-5} M).



b). Inhibition of GPILSM contractile response to Reek 32 (C-4 3-pyridyl Bay K 8644 analogue - 1.9×10^{-5} M) by Ca^{2+} antagonist nifedipine (4.0×10^{-8} M).

Figure 3.2 Typical responses of GPILSM to agonists and antagonists.

4. After last wash in step 2, wait 5 minutes, add small dose of compound.
5. Wait 10 minutes, then repeat step 1.
6. Wash tissue twice, wait 15 minutes, and repeat steps 2 and 3.
7. After tissue response recovered to control level, repeat steps 4 and 5 with larger concentration of compound.
8. Repeat steps 3 to 7.

Tissues that did not produce at least half a gram of tension after the second agonist challenge were replaced with new tissues and the tissues equilibrated as before. The same protocol was used when 80 mM isotonic KCl was used, except that HPSS was removed from the tissue bath and was replaced with an equal volume of the KCl.

The response just preceding the antagonist dose was taken as control, and the effect of the antagonist dose was calculated as % decrease of the subsequent response to carbachol (or KCL), from the control.

3.5.2 Evaluation of novel compounds on G.P. left atrium.

After optimal length-tension relationship determination, a cumulative dose-response curve of the β-adrenergic agonist isoproterenol (INA) was done. Cumulative doses of the agonist were added in a geometrical fashion, in steps of one third or one half $\log_{10}$ using the modified method of Van Rossum (1963). Each

sequential dose was added after the previous dose had reached its peak until the tissue achieved maximum response. The tissue was then washed twice, allowed 15 minutes to re-equilibrate, then the test compound was added in the same manner as INA. The same protocol could also be used when the compound was an antagonist to determine an antagonist dose response curve. Figure 3.3 shows typical responses obtained on the left atrium to both agonists and antagonist compounds.

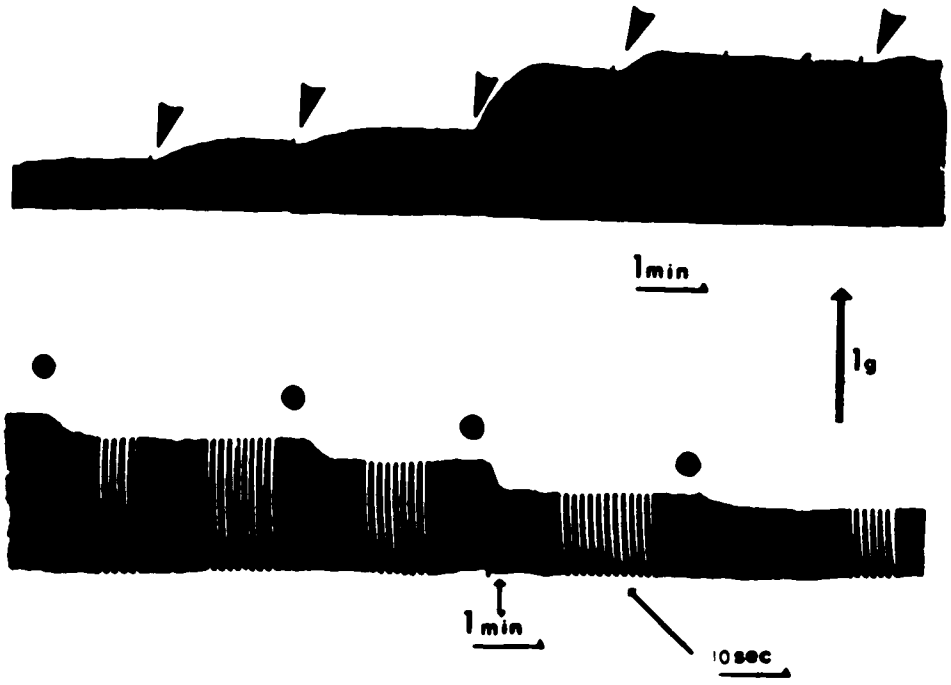


Figure 3.3 Typical responses of guinea pig left atrium to cumulative doses of agonists ▲ and antagonists ●.

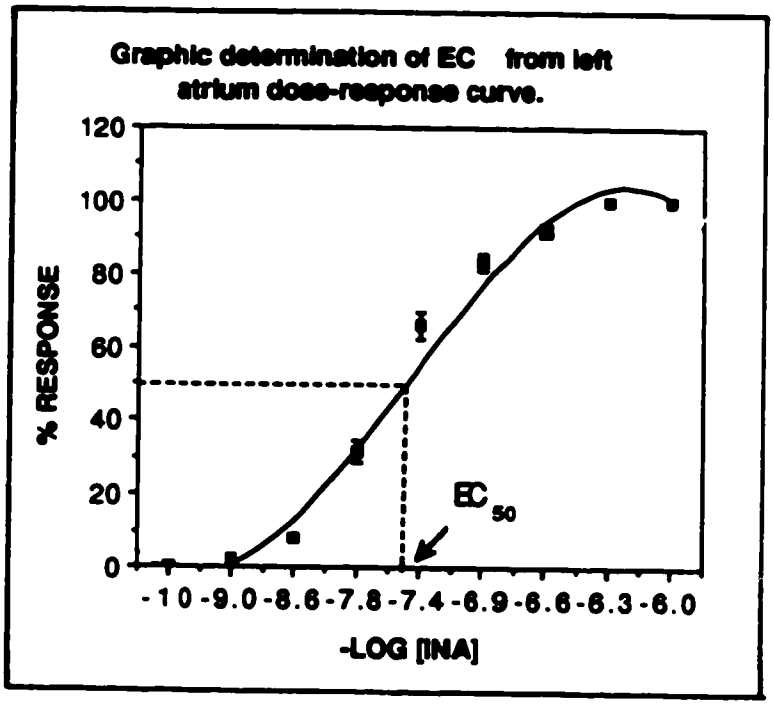


Figure 3.4 Graphic determination of EC_{50} from the dose-response curve of INA on guinea pig left atrium. When the determination was done using linear regression analysis values used were obtained from the linear portion of the curve.

3.6 Analysis of data.

For all in vitro tests, a minimum of three determinations of the IC_{50} or EC_{50} value for each compound was made. Results were reported as the arithmetic mean of IC_{50}s or EC_{50}s (+/- the standard error), determined graphically or by linear regression analysis (fig. 3.4).

Comparison of IC_{50} or EC_{50} values was performed automatically using Fisher's L.S.D. (least significant difference test) after analysis of variance (ANOVA) was done using the Statview 512+ Mcintosh computer program. This program automatically compared the EC_{50} or IC_{50} values with each other, once ANOVA determined that there was a significant difference in the group. The level for signicant differences was determined at the 5% level (i.e. $p < 0.05$.).

3.6.1 Criteria for comparing analogues.

The establishment of criteria for the determination of comparative parameters for pharmacological activity of drug molecules is a relatively discretionary process based upon the investigator's knowledge or experience with a particular drug or class of drugs. The basis for determining criteria may include the chemical class(es) of the drugs, the observed pharmacological or clinical effects, and the mechanism(s) of action of the drugs, if known.

Cavero and Spedding, (1984), proposed that experimental results should provide a quantitative estimation of a relative potency, the index for potency being the negative log of the concentration causing half the maximal effect, (pIC_{50}). The approach used in this thesis was to use a slightly different quantitative unit of relative potency by classifying the compounds into structurally related groups or subgroups, and dividing the IC_{50} value of the least potent compound in a group by the IC_{50} value of each

compound in the group or subgroup, the figure arrived at being then designated the relative potency (RP), of that compound respectively. The least potent compound within the group or subgroup therefore always had a value of one.

Comparison of the compounds was based on the structural modifications made on the structure of the lead compound, and the relative potencies of the compounds. The specific comparative criteria for the groups of analogues is presented in the relevant results sections.

IV. NIFEDIPINE ANALOGUES

4.1 Introduction.

The chemistry and pharmacology of dihydropyridine compounds have been intensively studied over the past two decades. The major focus of these studies has been to identify structural modifications made on the structure of nifedipine in order to improve the pharmacological profile of the 1,4-DHP class of Ca^{2+} antagonists. This has led to the identification of important structure-activity relationships (SARs) of the 1,4-DHPs useful in the development of new therapeutic agents for the treatment of cardiovascular disorders.

Loev and coworkers (1974) reported the first systematic pharmacological characterisation of a variety of 'Hantzsch-type' dihydropyridine compounds as hypotensive agents. Subsequently, Rodenkirchen and co-workers (1979), identified SARs of nifedipine analogues in isolated canine cardiac muscle, and described in detail the effects of altering different functional groups of the dihydropyridine nucleus on pharmacological activity. Several investigators have since added to the growing list of 1,4-DHP SARs as they discovered the influence of many different substituents on the 1,4-[illegible]clei.

4.2 Established SARs of 1,4-DHP Ca^{2+} antagonists.

Loev and coworkers (1974) determined the basic SARs of 1,4-DHP Ca^{2+} antagonists necessary for activity. Subsequent work by other investigators (Torwart et al., 1981; Fossheim et al., 1982; Janis and Triggle, 1983; Meyer et al., 1984; Arrowsmith et al., 1986; Dagnino et al., 1986; Fossheim, 1986; Li-Kwong-Ken, 1986; Dagnino et al., 1987; Triggle and Janis, 1987) has enlarged the list of SARs to include conformational and steric effects of certain substituents on the 1,4-DHP nucleus. Figure 4.1 shows some of these features and I have reviewed the SARs to provide a more current reflection of our understanding.

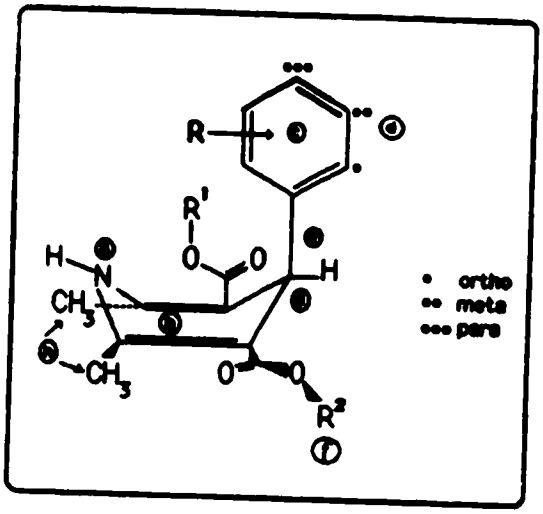


Figure 4.1 Structure and conformation of 1,4-DHPs showing features that influence Ca^{2+} antagonist activity.

A more current list of 1,4-DHP SARs follows below:

a. A 1,4-dihydropyridine ring in which the N-1 nitrogen is a secondary amine is critical; oxidation of the 1,4-DHP ring greatly reduced activity.

b. Increasing the planarity of the 1,4-DHP ring increased activity; shifting the double bonds in the ring (e.g. 1,2-DHP) reduced activity.

c. Increasing the size of the substituents at position C-4 of the dihydropyridine ring in the sequence hydrogen, methyl, alkyl, cycloalkyl, heterocyclic, phenyl, substituted phenyl increased activity in that order.

d. For substituted C-4 phenyl analogues of nifedipine, generally ortho substituents were the most active, followed by meta substituents. Para substituted analogues tended to be the least active.

e. The orientation of the C-4 phenyl ring substituent is perpendicular to the plane of the 1,4-DHP ring. Deviation from this orientation diminished activity.

f. Ester substituents at position C-3 and C-5 of the dihydropyridine ring conferred optimal activity. Absence of a substituent or presence of electron-withdrawing substituents diminished activity.

g. Chirality of the C-4 carbon due to asymmetric C-3 and C-5 ester substituents, increased calcium antagonist potency, and the resultant enantiomers usually displayed stereoselective effects.

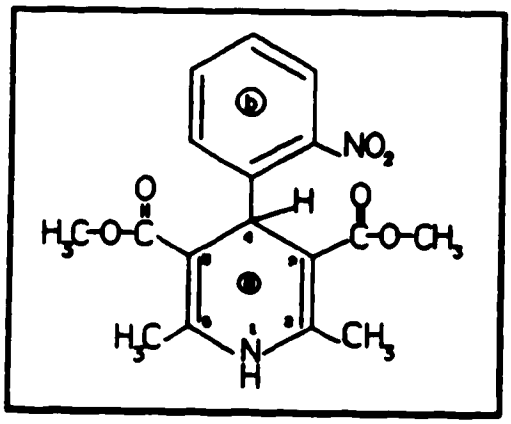
h. Substituents at position C-2 and position C-6 should be lower alkyl (methyl) for optimal activity; increasing the size of these groups diminished or abolished activity.

4.3 Objective of the evaluation of novel analogues of nifedipine.

Knowledge of the SARs of 1,4-DHP Ca^{2+} antagonists has led to the development of compounds with improved pharmaco-therapeutic profiles such as increased duration of action, greater potency, greater selectivity for certain target organs, and fewer side effects. The goal of this study was therefore to further characterise novel analogues of nifedipine, identify structural modifications that improved the pharmacological profile as described above. The results would also be compared to other 1,4-DHPs in order to find any relevant pharmacological differences.

4.4 Novel analogues screened for Ca^{2+} antagonist activity.

Figure 4.2 shows the structure of nifedipine, the prototype 1,4-DHP Ca^{2+} antagonist. The numbering of the ring atoms is provided for ease of reference to the structural changes. This numbering system will be used throughout the rest of the thesis.



nifedipine

Figure 4.2 Structure of the 'Lead' compound nifedipine showing numbering of the atoms of the 1,4-DHP ring (a), and the 2-nitrophenyl moiety (b).

The following structural modifications relative to nifedipine were made to produce the novel 1,4- and 1,2-DHPs which were screened for Ca^{2+} antagonist activity:

1. replacement of the 2-nitrophenyl substituent at the C-4 position with methyl, cyclohexyl, phenyl, substituted

phenyl, pyridyl, substituted pyridyl and 1-oxido-2-pyridyl groups.

2. replacement of the 1,4-dihydropyridine ring with a 1,2-dihydropyridyl ring.
3. replacement of one or both methyl groups of the esters at positions C-3 and C-5 of the 1,4-dihydropyridyl ring with different alkyl, cycloalkyl, aryl, substituted aryl and pyridyl groups.

4.5 Comparative criteria for Ca^{2+} antagonists.

Criteria for comparing the analogues relative to each other and to nifedipine, included the following:

a). the relative potency (RP).

b). the effect of symmetry/asymmetry of the C-3 and C-5 ester substituents (fig. 4.1).

c). the effect of substitutions made at the C-4 position, and where applicable, the effect of substitutions on a dihydropyridyl at the C-4 position.

d). the effect of varying the position of the nitrogen atom in the C-4 heterocycle, where applicable.

The Ca^{2+} antagonist activity of nifedipine, the lead compound, was not always close to that of the analogues. Consequently, the RP value of nifedipine varied from group to group, depending on the IC_{50} of the weakest compound in that group. This, however,

provided a reference point for each compound, and allowed each compound to be compared to nifedipine. Comparison of the effects of specific modifications on Ca^{2+} antagonist activity could therefore be made relative to nifedipine, and to other analogues in the group.

4.6 Results.

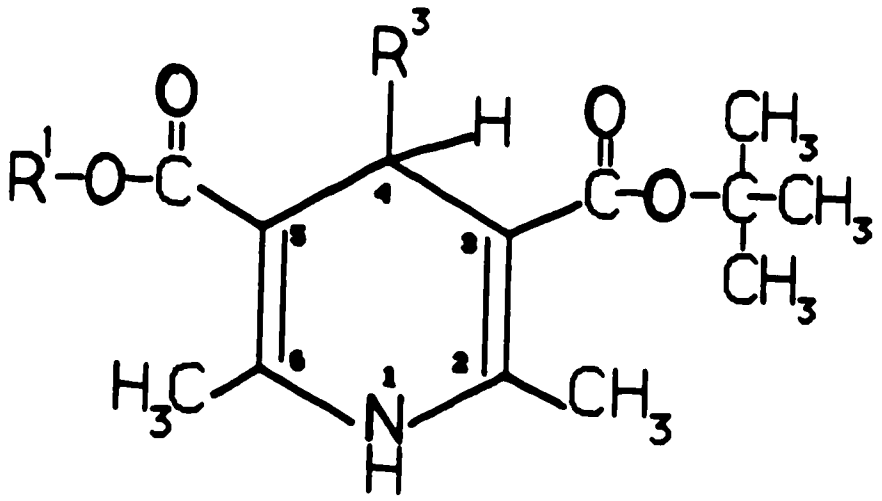
4.6.1 C-4 pyridyl 1,4-DHPs with symmetric C-3/C-5 t-butyl esters or asymmetric 3-t-butyl-5-methyl diesters.

The modifications made on the structure of nifedipine to produce the compounds in this group were: a). replacement of the 2-nitrophenyl ring system with a 2-, 3- or 4- pyridyl ring (position C-4); b). replacement of the C-3 and C-5 methyl esters with t-butyl esters to produce bulky symmetric esters and c). replacement of the C-3 methyl ester with a t-butyl group to produce asymmetric esters.

Table 4.1 shows the RP and IC_{50} values of novel analogues of this group. Relative to nifedipine, these compounds were not very potent. The most potent compound Reek 5, with a RP of 27.3, was about two orders of magnitude less potent than nifedipine. The least potent compound in the group, Reek 3 (RP = 1.0), was more than three orders of magnitude less potent than nifedipine, and more than an order of magnitude less potent than Reek 5.

Within the group, significant differences (Fisher's P.L.S.D., $p < 0.05$) were seen between Reek 1 and Reek 5, and also between Reek 4 and Reek 5. The rest of the compounds were not significantly different from each other. The C-4 2-pyridyl substituent with

Table 4.1



Compound	R^1	R^3	IC_{50}	+/-	SEM	n	RP
Reek 1	t-butyl	3-pyr	$3.73x10^{-6}$		0.17	4	8.5
Reek 2	t-butyl	4-pyr	$1.27x10^{-5}$		0.17	3	2.5
Reek 3	t-butyl	2-pyr	$3.17x10^{-5}$		0.54	3	1.0
Reek 4	methyl	3-pyr	$2.50x10^{-6}$		0.04	3	12.6
Reek 5	methyl	2-pyr	$1.16x10^{-6}$		0.10	3	27.3
Reek 6	methyl	4-pyr	$7.57x10^{-6}$		0.75	3	4.2
nifedipine.....			$1.43x10^{-8}$		0.38	18	2217

GPILSM Ca^{2+} antagonist activity of C-4 pyridyl-substituted analogues with symmetric C-3/C-5 t-butyl diesters or asymmetric C-3 t-butyl C-5 methyl diesters. IC_{50} is the concentration of compound producing 50% inhibition of the maximal response due to carbachol; n = no. of tests; SEM = standard error; RP = Relative Potency.

asymmetric esters gave the best activity (RP = 27.3), but when C-3 and C-5 substituents were changed to bulky symmetric (t-butyl) esters, potency decreased. This suggested that large symmetric ester groups may not be favourable for increasing potency. This observation was supported by the fact that a significant difference existed between symmetric and asymmetric analogues when the data was pooled for the two groups (fig. 4.3).

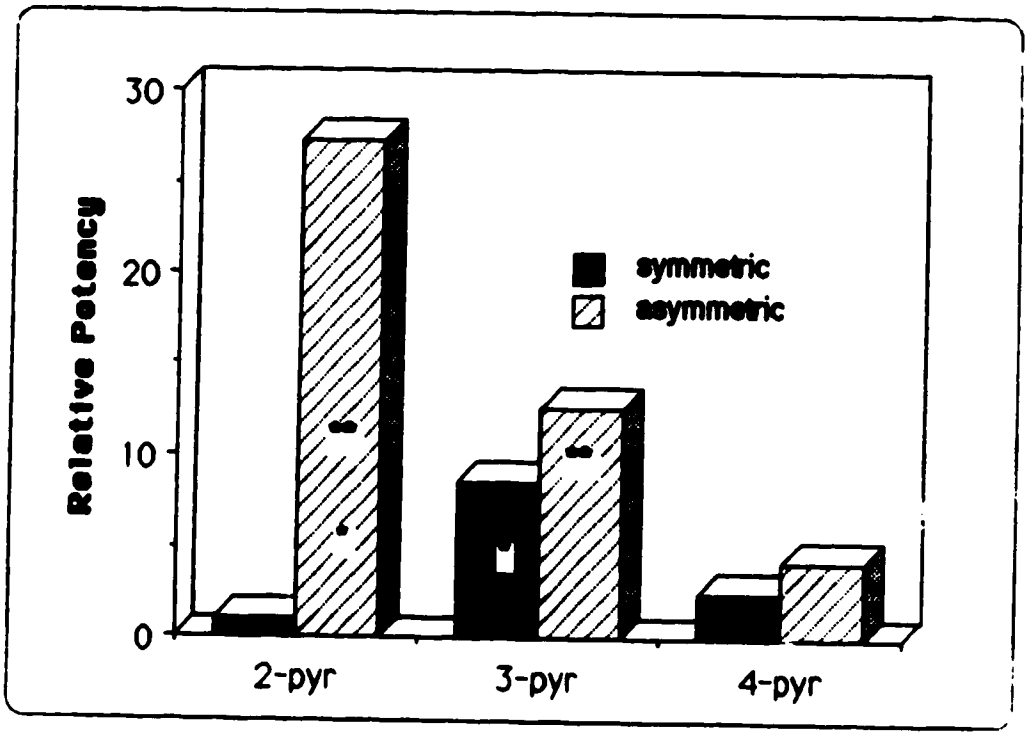


Figure 4.3 Relative potencies of symmetric and asymmetric analogues in Table 4.1. (significantly different at $p < 0.05$).

There was little difference in the activity of symmetric and asymmetric C-4 3-pyridyl analogues (Reek 1 and Reek 4), but the symmetric analogue was slightly more potent.

There was also little difference in the activity of symmetric and asymmetric C-4 4-pyridyl analogues (Reek 2 and Reek 6) with the

asymmetric analogue being more potent than the symmetric one.

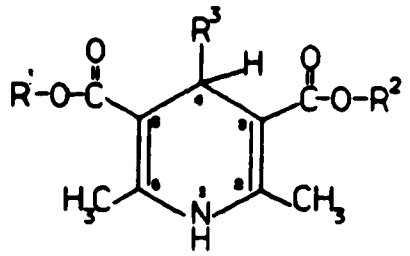
Comparison of the pyridyl substituents at C-4 showed that for the asymmetric esters, the RP order was 2-pyridyl > 3-pyridyl >4-pyridyl. The order was somewhat changed for the symmetric esters, where 3-pyridyl was greater than 4-pyridyl, which in turn was greater than 2-pyridyl.

4.6.2 C-4-pyridyl analogues with symmetric cycloalkyl diesters and asymmetric alkyl cycloalkyl diesters

While the C-4 substituent remained a 2-, 3- or 4-pyridyl group as in the previous section, compounds in this series were structurally different in that at least one of the C-3 or C-5 esters was a cyclic functional group. Other substitutions included changing the C-5 methyl ester of nifedipine to a different alkyl ester group.

Table 4.2 shows the substituents made at position C-5 (R^1), and the relative potencies and IC_{50} values within this group. Of the first six compounds, AM1 was significantly different from the rest of the compounds ($p < 0.05$). The only other significant difference was between AM3 and AM4. The relative potencies and differences between symmetric and asymmetric compounds (AM1-AM6) are shown graphically in figure 4.4. The activity of the group was fairly similar to that of the dialkyl diesters in Table 4.1 above. Collectively the compounds were about one to two orders of magnitude less potent than nifedipine, but overall they were more active than the compounds in Table 4.1. This improvement can be attributed to

Table 4.2



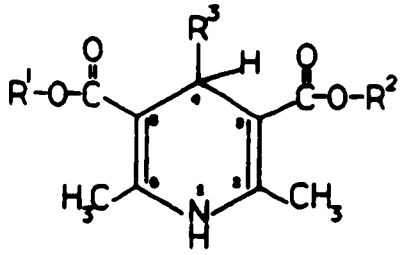
Compound	R^1	R^2	R^3	IC_{50}	+/- SEM	n	RP
a. AM1*	c-hexyl	c-hexyl	2-pyr	8.19×10^{-7}	1.25	6	3.1
AM2	c-hexyl	c-hexyl	3-pyr	4.12×10^{-7}	0.79	3	6.1
AM3**	c-hexyl	c-hexyl	4-pyr	4.50×10^{-7}	2.05	3	5.6
AM4**	methyl	c-hexyl	2-pyr	1.70×10^{-7}	0.34	3	14.8
AM5	methyl	c-hexyl	3-pyr	2.37×10^{-7}	0.83	4	10.6
AM6	methyl	c-hexyl	4-pyr	3.86×10^{-7}	0.36	4	6.5
b. A(2)1a*	t-butyl	c-hexyl	2-pyr	2.51×10^{-6}	0.91	3	1.0
A(2)1b	i-butyl	c-hexyl	2-pyr	6.08×10^{-7}	0.81	3	4.1
A(2)2a	ethyl	c-hexyl	2-pyr	2.71×10^{-7}	0.20	3	9.3
A(2)2b	i-propyl	c-hexyl	2-pyr	3.89×10^{-7}	0.21	3	6.5
A(2)3a	MeO-Et	c-hexyl	2-pyr	9.03×10^{-7}	1.10	3	2.8
nifedipine				1.43×10^{-8}	0.38	18	176

GPILSM Ca^{2+} antagonist activity of C-4 pyridyl-substituted 1,4-DHPs with symmetric cyclohexyl diesters and asymmetric alkyl cycloalkyl diesters. (c- = cyclo; i- = iso; MeO-Et = methoxyethyl).

* - significantly different from the rest.

** - significantly different from each other.

Table 4.2 cont'd



c.	A(2)48	i-propyl	c-butyl	3-pyr	5.29×10^{-7}	0.28	3	4.7
	A(2)49b	i-propyl	c-butyl	2-pyr	4.18×10^{-7}	0.19	4	6.0
	A(2)23a	i-propyl	c-pentyl	3-pyr	9.38×10^{-8}	2.43	3	26.2
	A(2)23b	i-propyl	c-pentyl	2-pyr	2.83×10^{-7}	0.99	4	8.9
nifedipine.....					1.43×10^{-8}	0.38	18	176

GPILSM Ca^{2+} antagonist activity of C-4 pyridyl-substituted 1,4-DHPs with symmetric cyclohexyl diesters and asymmetric alkyl cycloalkyl diesters. (c- = cyclo; i- = iso; MeO-Et = methoxyethyl).

* - significantly different from the rest.

** - significantly different from each other.

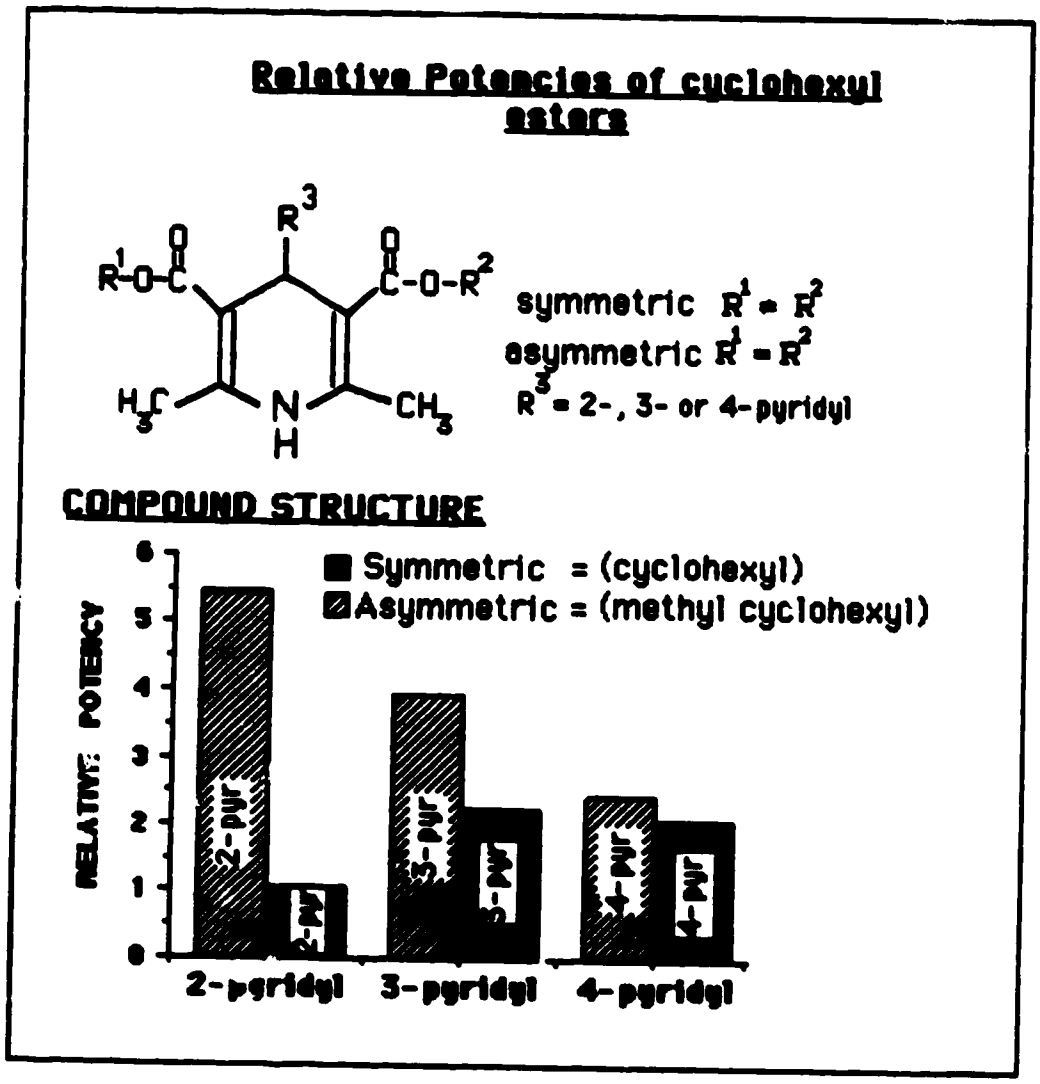


Figure 4.4 Relative potencies of C-4 pyridyl-substituted 1,4-DHPs with symmetric cyclohexyl diesters and asymmetric methyl cyclohexyl diesters. (On the same scale nifedipine was 65).

the cyclic substituent R^2, which increased activity by about two orders of magnitude.

Among compounds with one c-hexyl ester, asymmetry increased potency in the order methyl > ethyl > isopropyl > i-butyl > methoxyethyl > t-butyl. This trend supported the previously seen tendency for potency to increase linearly with a greater discrepancy in the size of the C-3 vs C-5 esters.

Among compounds where the R^1 substituent was isopropyl, the c-pentyl substituted compounds, A(2)23a and A(2)23b, had the best activity. The order of potency was c-pentyl > c-hexyl > c-butyl.

Although A(2)23a, a C-4 3-pyridyl compound had the best activity of the entire group, the general trend of 2-pyr > 3-pyr > 4-pyr appeared to be maintained for the group as a whole, when the esters differed considerably in size, however this order changed inconsistently when the ester substituents were closer in size For this series of analogues, potency appears to be dependent on the size of the C-3 and C-5 esters as well as the C-4 pyridyl group. This may indicate that these substituents all play a significant role in the pharmacophoric conformation of the molecule, which results from an interaction of these groups with their binding sites on the 1,4-DHP binding site.

4.6.3 C-4 N-methyl-tetrahydropyridyl 1,4-DHPs with symmetric and asymmetric dialkyl diesters

In this series of nifedipine analogues the major change made was at the C-4 substituent at which the 2-nitrophenyl ring of nifedipine

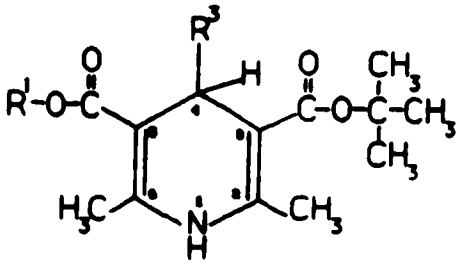
was replaced with a N-methyl-tetrahydropyridyl ring. C-3 and C-5 esters were maintained as symmetric t-butyl diesters or asymmetric C-3 methyl C-5 t-butyl diesters.

The relative potencies of this series of analogues are listed in table 4.3. The most potent compound in the series was Reek 7, with an RP of 27.1. This value was significantly different from those of the other analogues in the group, and was comparable to values obtained for the more active analogues in Table 4.1.

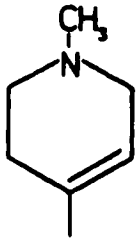
Collectively, symmetric diesters appeared to be more potent than asymmetric esters. This may be due to the inordinately more potent effect of Reek 7 relative to the rest of the compounds. The influence of the position of the nitrogen atom in the C-4 heterocycle was altered from that of the symmetric esters of the groups discussed in sections 4.6.1 and 4.6.2 above, the order now being N-3 > N-4 > N-2 (Reek 7 > Reek 10 > Reek 8). Potencies of the asymmetric ester compounds were very close to each other and not significantly different, leading to the conclusion that these analogues were very similar in their interaction with the Ca^{2+} channel and none influenced channel function in a manner distinctly different from the others.

Relative to modifications previously described, an overall decrease in activity was produced by the modifications made in this series of analogues. There was no advantage offered by these novel analogues in terms of novel pharmacology or improvement of the pharmacological profile. The overall effect of reducing the C-4 dihydropyridyl ring to a tetrahydropyridyl ring with an N-methyl substituent tended to decrease activity relative to nifedipine

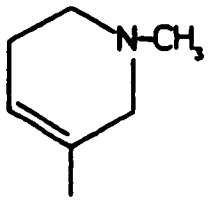
Table 4.3



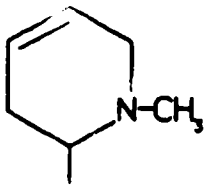
Compound Structure



N4-Me



N3-Me



N2-Me

Compound	R^1	R^2	R^3	IC_{50}	+/- SEM	n	RP
Reek 7	t-butyl	t-butyl	N3-Me	$1.45x10^{-6}$	0.14	4	27.1
Reek 8	t-butyl	t-butyl	N2-Me	$1.91x10^{-5}$	0.23	4	2.1
Reek 9	methyl	t-butyl	N4-Me	$3.55x10^{-5}$	0.46	3	1.1
Reek 10	t-butyl	t-butyl	N4-Me	$1.50x10^{-5}$	0.32	4	2.6
Reek 11	methyl	t-butyl	N2-Me	$3.93x10^{-5}$	0.35	3	1.0
Reek 12	methyl	t-butyl	N3-Me	$2.73x10^{-5}$	0.62	3	1.4
nifedipine.....				$1.43x10^{-8}$	0.38	18	2748

GPILSM Ca^{2+} antagonist activity of C-4 N-methyl-tetrahydropyridyl 1,4-DHPs with symmetric t-butyl and asymmetric methyl t-butyl diesters.

(RP = 2748).

4.6.4 C-4 (N-ester substituted) 1',6'-dihydropyridyl symmetric and asymmetric dialkyl diester analogues of nifedipine

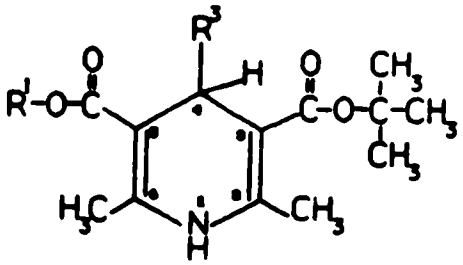
This series of analogues comprised nine compounds, with six analogues (Reek 13 - Reek 18) possessing symmetric C-3,C-5 t-butyl diesters, and the other three possessing asymmetric C-3 methyl, C-5 t-butyl diesters (Reek 19 - Reek 21). The C-4 position of all the compounds possessed an N-ester substituted 1',6'-dihydropyridyl ring with three different ester substituents on the nitrogen atom of the C-4 1',6'-dihydropyridyl ring (methyl, phenyl and t-butyl), and the attachment point of the C-4 substituent being either at the 3' or 4' position of the 1',6'-dihydropyridyl ring.

The range of IC_{50} values (RPs 1-12.3) for the compounds was quite narrow (Table 4.4) and their activities were not significantly different from each other. Collectively C-4 N4-ester substituted compounds had greater activity than N3-ester substituted compounds. Symmetry of the dialkyl diesters produced greater potency than asymmetry with the exception of Reek 19.

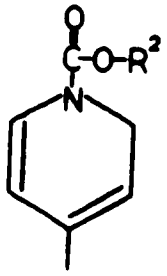
The effect of esterification of the N-methyl group could not be evaluated directly since an analogous group (table 4.3) had C-4 tetrahydropyridyl instead of a dihydropyridyl ring.

The trends in this group were generally less defined since the activities of the compounds were comparable. Overall, there was no improvement in activity resulting from these modifications.

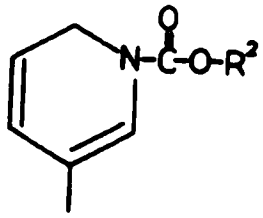
Table 4.4



Compound Structure



N4



N3

N4-MeE

N4-PhE

Compound	R^1	R^3	IC_{50}	+/- SEM	n	RP
Reek 13	t-butyl	N3-PhE	$2.55x10^{-5}$	0.27	4	1.3
Reek 14	t-butyl	N4-PhE	$3.23x10^{-6}$	0.18	3	10.6
Reek 15	t-butyl	N4-MeE	$9.33x10^{-6}$	0.27	3	3.7
Reek 16	t-butyl	N3-MeE	$4.85x10^{-6}$	0.07	3	7.0
Reek 17	t-butyl	N3-tBuE	$1.00x10^{-5}$	0.76	3	3.4
Reek 18	t-butyl	N4-tBuE	$2.77x10^{-6}$	0.38	3	12.3
Reek 19	methyl	N3-tBuE	$4.50x10^{-6}$	0.33	3	7.6
Reek 20	methyl	N3-MeE	$3.41x10^{-5}$	0.85	3	1.0
Reek 21	methyl	N3-PhE	$3.41x10^{-5}$	0.27	3	1.0
nifedipine.....			$1.43x10^{-8}$	0.38	18	2385

GPILSM Ca^{2+} antagonist activity of C-4 (N-ester)-substituted 1',6- dihydropyridyl 1,4-DHPs with symmetric t-butyl and asymmetric methyl t-butyl diesters. (E = ester).

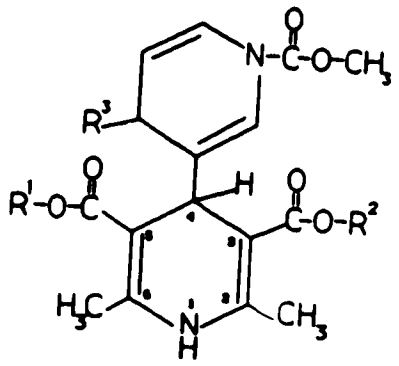
4.6.5 Ca^{2+} antagonist activity of 1,4-DHPs with symmetric methyl, ethyl, i-propyl, i-butyl, t-butyl and asymmetric methyl, i-butyl and i-propyl diesters containing a C-4 3'-(1'-methoxy carbonyl-4'-methyl-1',4'-dihydropyridyl) group.

The modifications made on the structure of nifedipine to produce this series of analogues are shown in Table 4.5 along with the basic structure of the compounds.

Activity of the analogues was two to three orders of magnitude less than that of nifedipine (RP = 455). For symmetric C-3/C-5 diesters where the R^3 substituent was a methyl group (Table 4.5a), activity increased with an increase in the size of the symmetric esters from methyl to isopropyl diesters, then declined with further increase in the size of the ester group. The order of potency for these diesters was i-propyl > t-butyl > i-butyl > ethyl > methyl. Asymmetry in which there was a large disparity in the size of the corresponding C-3/C-5 diesters increased activity (Reek 51), but when the disparity in size was reduced activity decreased (i.e. Reek 51 > Reek 58). Among compounds in Table 4.5a, Reek 47 was significantly different from the rest.

Compounds containing an R^3 n-butyl group (Table 4.5b), displayed a similar trend as above for symmetric diesters but greatest activity was now shifted to a smaller ester group (ethyl vs i-propyl). Again activity decreased when the diester substituents were decreased or increased in size from ethyl. The order of potency thus was ethyl > i-propyl > t-butyl $\geq$ i-butyl > methyl. Asymmetry also decreased activity but greater disparity in the size

Table 4.5



Compound	R^1	R^2	R^3	IC_{50}	+/ SEM	n	RP
a).							
*Reek 47	methyl	methyl	methyl	6.50×10^{-6}	2.27	3	1.0
Reek 48	ethyl	ethyl	methyl	2.93×10^{-6}	0.73	3	2.2
Reek 49	i-propyl	i-propyl	methyl	9.70×10^{-7}	2.90	3	6.7
Reek 50	t-butyl	t-butyl	methyl	1.33×10^{-6}	0.04	4	4.9
Reek 51	methyl	i-butyl	methyl	8.54×10^{-7}	1.43	4	7.6
Reek 52	i-butyl	i-butyl	methyl	1.40×10^{-6}	0.22	3	4.6
Reek 58	methyl	i-propyl	methyl	2.05×10^{-6}	0.56	3	3.2
nifedipine..	.....	.....		1.43×10^{-8}	0.38	18	455

GPILSM Ca^{2+} antagonist activity of 1,4-DHPs with symmetric methyl, ethyl, i-propyl, i-butyl, t-butyl and asymmetric methyl, i-butyl and i-propyl diesters containing a C-4 3'-(1'-methoxycarbonyl-4'-methyl- 1',4'-dihydropyridyl) group.

Table 4.5 cont'd

Compound	R^1	R^2	R^3	IC_{50}	+/- SEM	n	RP
b).							
Reek 53	methyl	methyl	n-butyl	1.39×10^{-6}	0.20	3	4.1
Reek 54	ethyl	ethyl	n-butyl	9.92×10^{-7}	0.94	3	5.7
Reek 55	i-propyl	i-propyl	n-butyl	1.21×10^{-6}	0.47	3	4.7
Reek 56	i-butyl	i-butyl	n-butyl	2.95×10^{-6}	1.70	3	1.9
Reek 57	t-butyl	t-butyl	n-butyl	3.28×10^{-6}	2.24	3	1.7
Reek 59	methyl	i-propyl	n-butyl	5.64×10^{-6}	1.31	3	1.0
Reek 60	methyl	i-butyl	n-butyl	4.42×10^{-6}	1.34	3	1.3
nifedipine.....				1.43×10^{-8}	0.38	18	394
c).							
Reek 61	methyl	methyl	phenyl	1.90×10^{-6}	0.21	3	4.3
Reek 62	ethyl	ethyl	phenyl	4.36×10^{-7}	0.25	3	18.9
Reek 63	t-butyl	t-butyl	phenyl	2.91×10^{-6}	0.26	3	2.8
Reek 64	i-butyl	i-butyl	phenyl	8.25×10^{-6}	1.23	4	1.0
Reek 65	i-propyl	i-propyl	phenyl	5.28×10^{-7}	1.45	3	15.6
nifedipine.....				1.43×10^{-8}	0.38	18	577

GPILSM Ca^{2+} antagonist activity of 1,4-DHPs with symmetric methyl, ethyl, i-propyl, i-butyl, t-butyl and asymmetric methyl, i-butyl and i-propyl diesters containing a C-4 3'-(1'-methoxycarbonyl-4'- substituted 1',4'-dihydropyridyl) group.

of the diesters increased activity as observed in table 4.5a. There were no significant differences in the activities of this sub-group of analogues.

For compounds containing an R^3 phenyl group (Table 4.5c), the order of potency was the same as for those where R^3 was a n-butyl group, potency increasing with an increase in the symmetric diesters from methyl to ethyl, then declining as the diesters became larger. There were no significant differences among analogues in this sub-group. As well, there were no compounds with asymmetric diesters available for comparison.

Some compounds in this series could be compared to compounds in table 4.4. When the R^3 substituent was a methyl group, activity was enhanced almost four-fold when the C-3/C-5 diesters were n-butyl groups (Reek 50 vs Reek 16). When the R^3 substituent was increased in size activity still increased but only one and a half times (Reek 57 vs Reek 16). When the R^3 group was changed to a phenyl group activity was comparable to that of the n-butyl substituted analogue (Reek 63 vs Reek 16).

This indicated that a small R^3 substituent enhanced activity but when the size of the R^3 group increased the effect was lost. This may therefore indicate that steric effects may be involved in allowing a small group to bind to the receptor site to induce a more favourable conformation, but when the group is too large the steric effects do not allow for favourable binding of the C-4 substituent.

4.6.5.1 Differential effects between muscarinic-receptor mediated (MRM) contraction and depolarisation-induced contraction (DIC) on GPLISM exhibited by some 1,4-DHPs with symmetric t-butyl and asymmetric methyl t-butyl diesters containing a C-4 3'-(1'-methoxycarbonyl-4'-substituted-1',4'-dihydro pyridyl) group.

It was discovered during testing of the compounds in this group that some preferentially inhibited the response to KCl-induced contractions (DIC) for longer periods than they did carbachol-induced contractions. The response due to carbachol (MRM) recovered to control or near control levels after two to three washes after the removal of the antagonist from the tissue bath. Some analogues inhibited the DIC response permanently and the tissue response did not recover at all (fig. 4.4).

The structural requirement to produce this effect was possession of large symmetric diesters at positions C-3 and C-5, and a small (R^3 = methyl) substituent in the C-4 pyridyl ring. Compounds possessing smaller symmetrical diesters were not able to inhibit recovery of the KCl response permanently, but recovery of the response was faster with smaller C-3/C-5 ester, the rate of recovery being methyl > ethyl > i-propyl. Compounds possessing t-butyl or i-butyl esters permanently inhibited the DIC response.

The ability to selectively inhibit DIC permanently was limited to compounds possessing a small R^3 substituent and large C-3/C-5 diesters (Table 4.5a), since compounds possessing C-3/C-5 diesters which were asymmetric (Reek 51), or possessed small symmetric esters

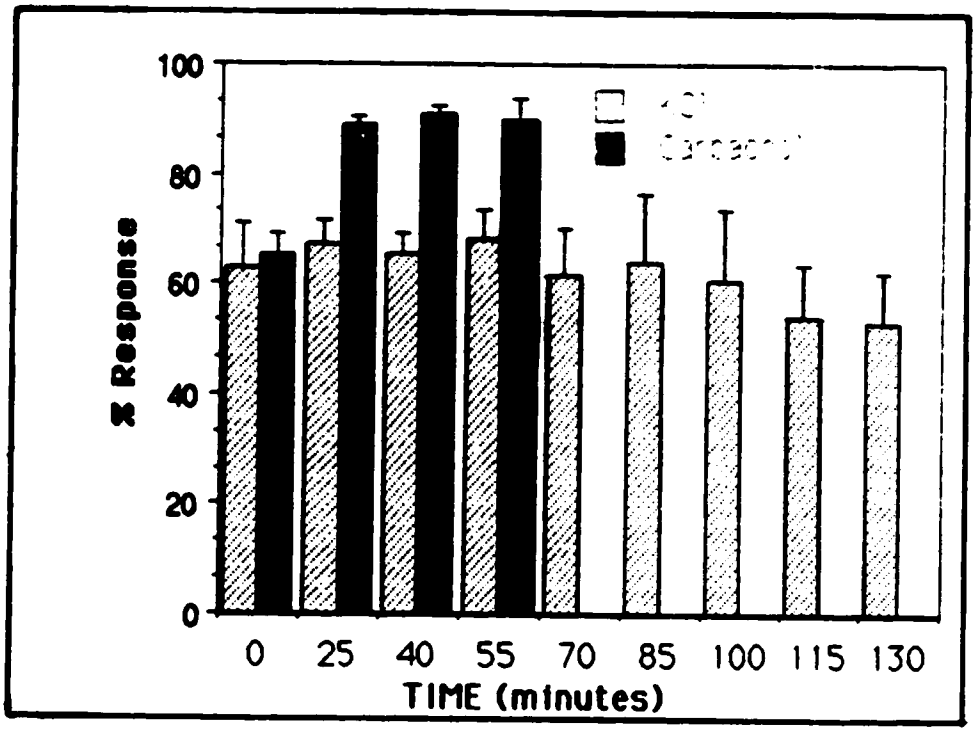
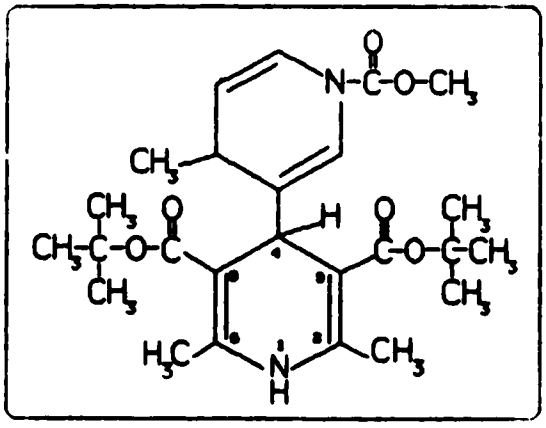


Figure 4.5 GPILSM recovery after Reek 50 in normal HPSS. Concentration used was close to the IC_{50}.



Reek 50

(Reek 47, Reek 53 and Reek 61), or a large R^3 substituent (t-butyl or phenyl), did not produce this effect.

In order to confirm the selectivity of the compounds for inhibition of DIC, further experiments were carried out in nominally Ca^{2+}-free solution. The experimental protocol was altered so that Ca^{2+} (2.0 mM) was added to the tissue bath containing the Ca^{2+}-free HPSS. The agonist (carbachol or KCl) was added to the bath before the Ca^{2+}. The rationale for this approach was that since GPILSM is primarily dependent on extracellular Ca^{2+} for contraction, inhibition of KCl-induced contraction should be greater in Ca^{2+}-free medium, if these compounds were working on voltage-sensitive Ca^{2+} channels. Since Ca^{2+} antagonists are more effective in inhibiting DIC than receptor-mediated contraction, the novel analogues would not be able to inhibit MRM contraction as effectively as they do DIC. The difference between the two modes of Ca^{2+} mobilisation is illustrated in figure 4.5. The MRM response recovered very well in the Ca^{2+}-free medium whereas the DIC remained inhibited even after eight washes (i.e. for longer than two hours after exposure to the compound - only one set of tissues followed for this period).

This exciting discovery therefore indicated the existence of different pathways of Ca^{2+} entry into the smooth muscle cells of GPILSM (voltage-dependent and receptor-mediated Ca^{2+} mobilisation), which could be selectively inhibited by some of the novel analogues. It also demonstrated the possibility that under certain conditions each pathway of Ca^{2+} mobilisation might be selectively regulated and utilised.

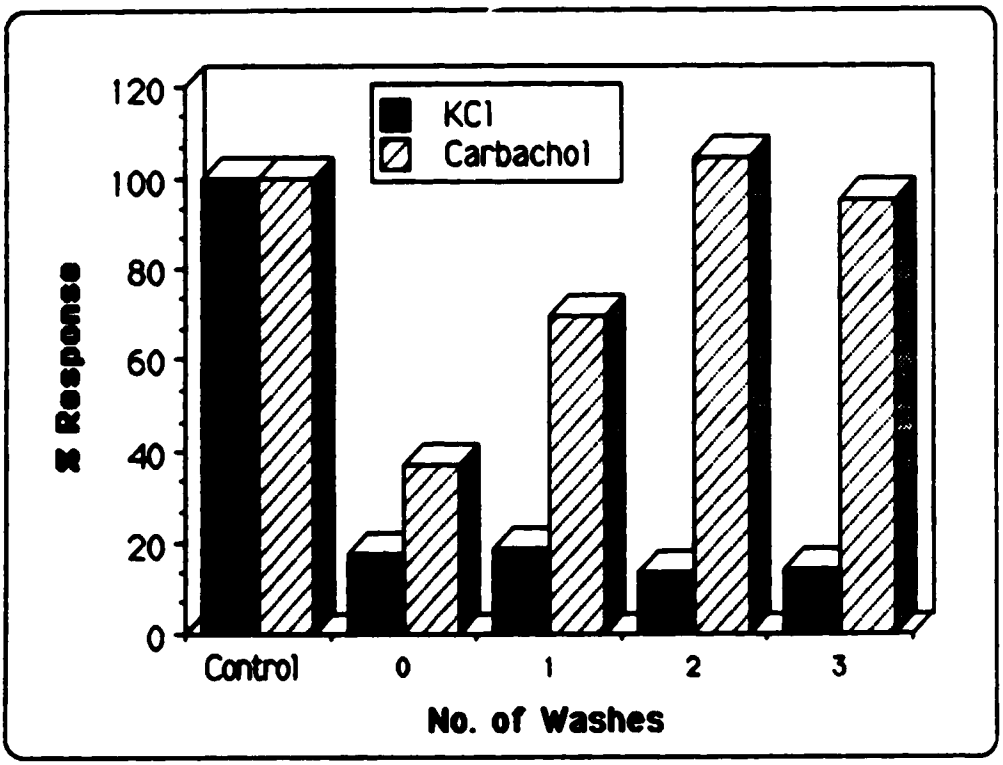


Figure 4.6 Recovery of DIC and MRM responses in nominally Ca^{2+}-free HPSS after exposure to Reek 50 (1.4×10^{-6} M).

Analogues capable of producing this differential response may be useful tools for identifying different classes or subclasses of Ca^{2+} channels, and their possible roles in the normal physiological function of the tissue or organ. As well, they could elucidate the roles played by receptor-activated and depolarisation-induced Ca^{2+} mobilisation in specific tissues.

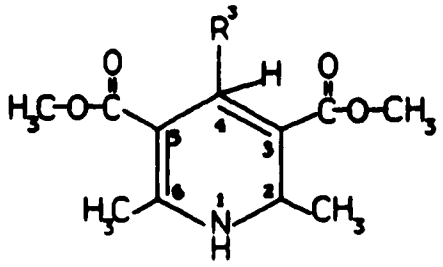
4.6.6 Ca^{2+} Antagonist effects of 1,2-DHPs with symmetric methyl diesters and heterogeneous C-4 substituents.

This group of compounds differed from nifedipine in that the 1,4-DHP ring was changed to a 1,2-DHP ring, and the 2-nitrophenyl ring was replaced with different substituents (Table 4.6).

The group as a whole was more than two orders of magnitude less active than nifedipine. All the compounds had fairly comparable activity, with the most active analogue. There were no significant differences in the activities of the compounds. Reek 73, the most active of the group was only slightly more than twice as active as the least active analogue, Reek 70. In spite of the great diversity in the C-4 substituents the group was rather unremarkable.

It is interesting to note that the C-4 substituents of Reek 69 and Reek 70 are positional isomers of the 2-nitrophenyl ring of nifedipine, differing only in the point of attachment of the C-4 pyridyl substituent to the 1,4-DHP. However these two compounds are more than one hundred times less active than nifedipine. This differentiation of the activities may be critical for the pharmacophoric conformation of the molecule, a 1,4-DHP ring being

Table 4.6



Compound	R^3	IC_{50}	+/-	SEM	n	RP
Reek 66	2-CF_3Ph	$6.73x10^{-6}$		0.78	3	1.3
Reek 67	3-CF_3Ph	$8.25x10^{-6}$		0.45	4	1.0
Reek 68	4-CF_3Ph	$7.57x10^{-6}$		0.06	3	1.1
Reek 69	3-NO_2Ph	$6.98x10^{-6}$		3.83	4	1.2
Reek 70	4-NO_2Ph	$8.52x10^{-6}$		3.81	3	1.0
Reek 71	phenyl	$4.32x10^{-6}$		3.04	3	2.0
Reek 72	methyl	$6.72x10^{-6}$		4.38	4	1.3
Reek 73	c-hexyl	$3.58x10^{-6}$		1.17	3	2.4
nifedipine.....		$1.43x10^{-8}$		0.38	18	2384

GPILSM Ca^{2+} antagonist effects of 1,2-DHPs with symmetric methyl diesters and heterogeneous C-4 substitituents. (Ph = phenyl; CF_3 = trifluoromethyl; NO_2Ph = nitrophenyl).

more conducive to producing a favourable conformation than a 1,2-DHP ring.

It is also interesting to note that the compound with an unsubstituted C-4 phenyl (Reek 71) was slightly more active than Reek 69 and Reek 70, suggesting that a nitro substituent in the ortho and meta positions of the phenyl ring may have actually decreased activity for these analogues.

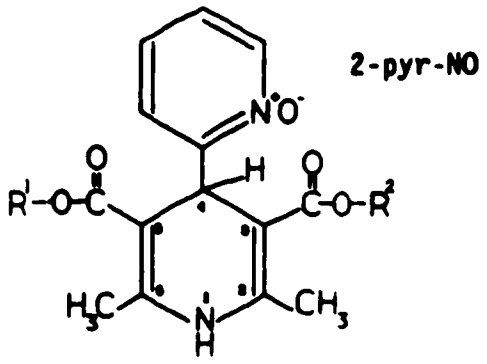
4.6.7 GPILSM Ca^{2+} antagonist activity of 1,4-DHPs with symmetric methyl, ethyl, i-propyl, i-butyl, t-butyl and asymmetric methyl, ethyl, i-propyl diesters and a C-4 1-oxido-2-pyridyl substituent instead of the 2-nitrophenyl moiety.

The analogues in this series differed from nifedipine in that the C-4 substituent was a 1-oxido-2-pyridyl (2-pyr-NO in Table 4.7) group instead of the 2-nitrophenyl moiety of nifedipine, and the C-3 and C-5 esters were symmetric or asymmetric alkyl groups as in previous sections (Table 4.7).

These compounds were not very active, being about three orders of magnitude less active than nifedipine. There was a significant difference between Reek 79 and Reek 92 ($p < 0.05$), and also between Reek 79 and Reek 93.

The first two members of the group Reek 77 and Reek 78 only decreased GPILSM contraction in response to carbachol by a maximum of 20 to 30 % and did not cause any further decrease in the response. This may be an indication that the compounds selectively inhibited specific Ca^{2+} entry pathways and hence may also provide a means of

Table 4.7



Compound	R^1	R^2	IC_{50}	+/-	SEM	n	RP
Reek 77	methyl	methyl	(>$1.70x10^{-4}$)		4 n/a		
Reek 78	ethyl	ethyl	(>$1.00x10^{-5}$)		4 n/a		
Reek 79	i-butyl	i-butyl	$3.52x10^{-6}$		0.21	3	5.1
Reek 80	i-propyl	i-propyl	$1.15x10^{-5}$		0.10	3	1.6
Reek 84	t-butyl	t-butyl	$5.65x10^{-6}$		1.09	3	3.2
Reek 92	methyl	ethyl	$1.81x10^{-5}$		0.54	3	1.0
Reek 93	methyl	i-propyl	$1.12x10^{-5}$		0.22	3	1.6
nifedipine.....			$1.43x10^{-8}$		0.38	18	2384

GPILSM Ca^{2+} antagonist activity of 1,4-DHPs with symmetric methyl, ethyl, i-propyl, i-butyl, t-butyl and asymmetric methyl, ethyl, i-propyl diesters and a C-4 1-oxido-2-pyridyl substituent.

distinguishing between different Ca^{2+} mobilisation processes.

For symmetric compounds, increasing the size of the diesters caused an increase in the activity, the order being i-butyl > t-butyl > i-propyl > ethyl $\geq$ methyl.

Asymmetry also increased potency with larger groups (i.e. a larger discrepancy in the size of ester substituents produced a greater increase), but there were only two compounds (Reek 92 and Reek 93) with asymmetric esters.

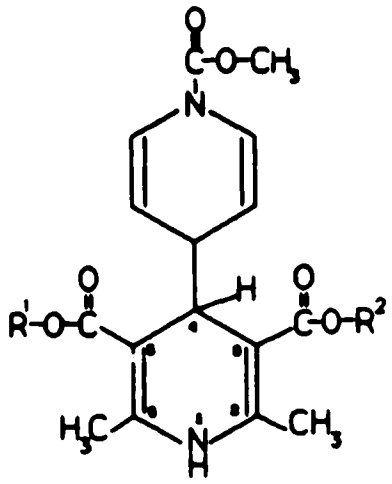
4.6.8 GPILSM Ca^{2+} antagonist activity of analogues possessing a C-4 3'- or 4'-(1'-methoxycarbonyl-1',4'-dihydropyridyl) group with symmetric methyl. ethyl, i-propyl, i-butyl, t-butyl and asymmetric methyl, i-propyl, i-butyl diesters.

The compounds in this group comprised two subgroups which differed only in the point of attachment of the C-4 1'-methoxycarbonyl-1',4'-dihydropyridyl ring; the first subgroup possessing a 4'-dihydropyridyl ring and the second possessing a 3'-dihydropyridyl ring. The ester substituents at C-3 and C-5 are listed in table 4.8 along with the IC_{50} and RP values.

These compounds were about two orders of magnitude less active than nifedipine and were generally about equipotent with compounds in which there was a 4'-substituent in the C-4 (N1-ester)-dihydropyridyl ring (Table 4.5).

Symmetric diester 1,4-DHPs possessing the C-4 (3'-dihydropyridyl) substituent were more potent than those possessing the C-4

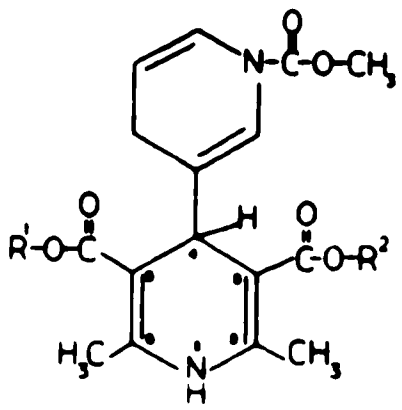
Table 4.8



Compound	R^1	R^2	IC_{50}	+/- SEM	n	RP
a).						
Reek 74	t-butyl	t-butyl	1.30×10^{-6}	0.18	4	6.6
Reek 75	methyl	methyl	3.70×10^{-6}	1.63	3	2.3
Reek 76	ethyl	ethyl	1.12×10^{-6}	0.11	3	7.7
Reek 81	i-propyl	i-propyl	4.15×10^{-6}	1.30	4	2.1
Reek 82	i-butyl	i-butyl	3.42×10^{-6}	4.18	3	2.5
Reek 83	methyl	i-butyl	8.57×10^{-6}	7.48	3	1.0
nifedipine.....			1.43×10^{-8}	0.38	18	599

GPILSM Ca^{2+} antagonist activity of analogues possessing a C-4 4'-(1'-methoxycarbonyl-1',4'-dihydropyridyl) group with symmetric methyl, ethyl, i-propyl, i-butyl, t-butyl and asymmetric methyl, i-butyl diesters.

Table 4.8



Compound	R^1	R^2	IC_{50}	+/- SEM	n	RP
b).						
Reek 85	t-butyl	t-butyl	1.58×10^{-6}	1.54	3	5.4
Reek 86	methyl	methyl	8.61×10^{-7}	1.13	3	10.0
Reek 87	i-butyl	i-butyl	7.46×10^{-7}	1.55	4	11.5
Reek 88	ethyl	ethyl	5.64×10^{-7}	3.81	3	15.2
Reek 89	i-propyl	i-propyl	1.17×10^{-6}	0.12	3	7.3
Reek 90	methyl	i-butyl	1.16×10^{-6}	0.79	3	7.4
Reek 91	methyl	i-propyl	1.02×10^{-6}	0.18	3	8.4
nifedipine.....	.....		1.43×10^{-8}	0.38	18	602

GPILSM Ca^{2+} antagonist activity of analogues possessing a C 4 3'-(1'- methoxycarbonyl-1',4'-dihydropyridyl) group with symmetric methyl, ethyl, i-propyl, i-butyl, t-butyl and asymmetric methyl, i-propyl, i-butyl diesters.

(4'-dihydropyridyl) substituent, when the dialkyl diesters were the same, except for the t-butyl diester compounds, where Reek 74 was about equipotent with Reek 85.

The order of potency for compounds possessing symmetric dialkyl diesters did not follow any consistent pattern. Compounds possessing ethyl diesters (Reek 76 and Reek 88) were the most potent in each subgroup. These were followed by compounds possessing symmetric butyl esters. For the C-4 (4'-dihydropyridyl) substituted compounds, the t-butyl diester analogue was slightly more potent than the i-butyl diester analogue (Reek 74 vs Reek 82). The order was reversed for the C-4 (3'-dihydropyridyl) susbtituted compounds, the t-butyl diester compound being weaker than its i-butyl diester analogue (Reek 85 vs Reek 87).

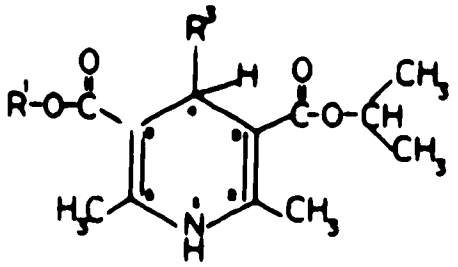
Among compounds with symmetric diesters, those possessing symmetric isopropyl diesters tended to be the least potent, except for Reek 85, which was less potent than its isopropyl diester analogue.

Asymmetry decreased activity for both C-4 3'-dihydropyridyl substituted and C-4 4'-dihydropyridyl substituted compounds.

4.6.9 Ca^{2+} antagonist activity of C-4 pyridyl 1,4-DHPs with asymmetric isopropyl arylalkyl diesters.

Modifications made to produce the analogues in Table 4.9 included incorporation of different unsubstituted arylalkyl esters at position C-5 of the 1,4-DHP ring, while maintaining an isopropyl group as the other ester; all compounds therefore possessed an isopropyl ester

Table 4.9



Compound	R^1	R^3	IC_{50}	+/-	SEM	n	RP
A(2)21	PEE	3-pyr	$1.24x10^{-7}$		0.40	3	31.0
A(2)22	PEE	2-pyr	$4.20x10^{-8}$		0.37	3	93.0
A(2)24a	pyr-MeE	3-pyr	$3.90x10^{-6}$		0.63	3	1.0
A(2)26	Benzyl-E	3-pyr	$6.21x10^{-7}$		1.78	4	6.0
A(2)27	Benzyl-E	2-pyr	$3.26x10^{-7}$		0.36	3	12.0
nifedipine.....			$1.43x10^{-8}$		0.38	18	273

GPILSM Ca^{2+} antagonist activity of C-4 pyridyl 1,4-DHPs with asymmetric isopropyl arylalkyl diesters. (PEE = phenylethyl ester; pyr-MeE = pyridylmethyl ester; Benzyl-E = benzyl ester).

at position C-3 and a 2- or 3-pyridyl substituent at the C-4 position (Table 4.9).

With the exception of the pyridylmethyl ester (pyr-MeE), this modification improved activity appreciably over that of analogues synthesised to this point. The activity of A(2)22 (IC_{50} of $4.20x10^{-8}$) was approaching that of the parent compound nifedipine. The activities of the compounds were quite different, (Range 1-93), and significant differences in the activities were demonstrated between A(2)21 and A(2)24a as well as A(2)22 and A(2)24a.

The benzyl esters were not much different in potency although the C-4 2-pyridyl analogue A(2)27 was slightly more active than the C-4 3-pyridyl analogue A(2)26. The phenethyl ester analogues were more potent than the benzyl ester analogues, and the C-4 2-pyridyl compound (A(2)22, was three times more potent that the C-4 3-pyridyl analogue A(2)21.

The length of the alkyl chain between the aryl substituent and the 1,4-DHP ring influenced potency, since the phenylethyl-substituted compounds were more active than the benzyl-substituted compounds. It appears that the intervening ethyl group confers an optimal distance between the 1,4-DHP ring and the aryl substituent, whereas a methyl group was too short to bridge the distance between the 1,4-DHP ring and the aryl substitutent.

A(2)24a, a pyridylmethyl ester substituted compound was the least potent in the group. This compound was twelve times less potent than its benzyl ester analogue, suggesting that the type of aryl substituent also determines potency.

4.6.10 Ca^{2+} antagonist activity of C-4 pyridyl 1,4-DHPs with asymmetric isopropyl substituted phenethyl C-3/C-5 diesters.

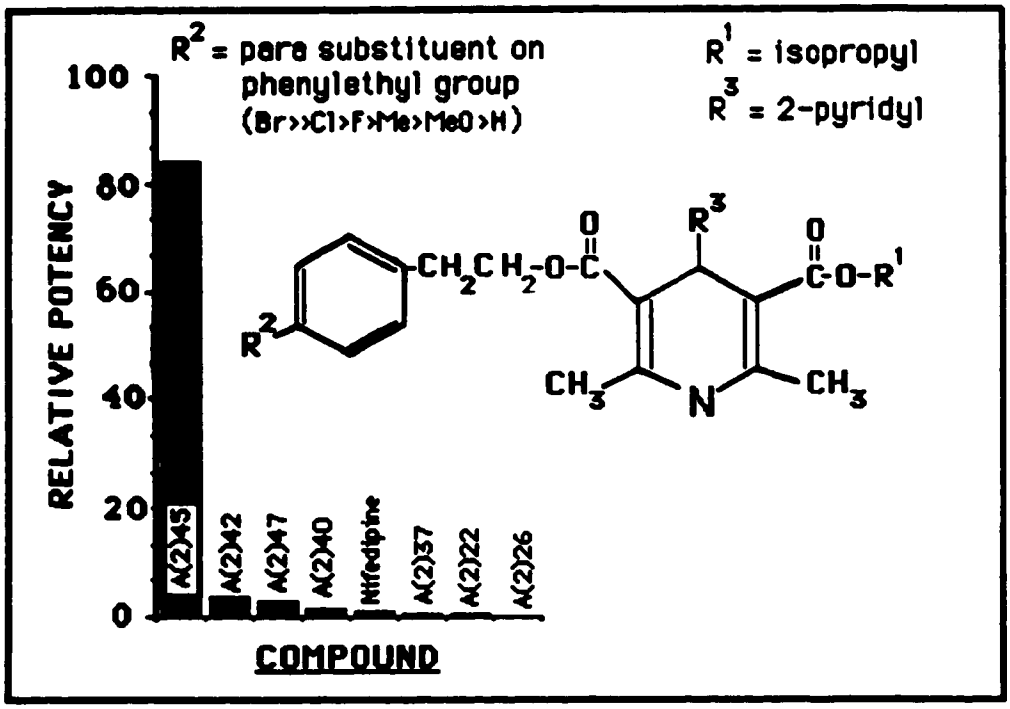
These analogues were structurally similar to those in Table 4.9, but they all possessed substituted phenethyl esters at position C-5, and 2-pyridyl or 3-pyridyl substituents at C-4, while the C-3 substituent was maintained as an isopropyl ester. The general structure of the compounds is shown in table 4.10 along with the IC_{50} and RP values of the compounds. Different substituents were placed on the para position of the phenyl group of the phenethyl ester in order to test the influence of these substituents on activity.

This modification significantly improved activity, with all compounds exhibiting activity greater than or comparable to that of nifedipine. Only one compound, A(2)37, had less activity than that of nifedipine.

Factors affecting activity appeared to be the type and size of substituent. The most effective modification increasing activity of the novel analogues was halogenation of the phenyl ring of the phenethyl ester. Bromine provided the best activity (A(2)44 and A(2)45), followed by chlorine (A(2)41 and A(2)42), then fluorine (A(2)46 and A(2)47). The activity of the p-bromo analogue, A(2)45, was at least one order of magnitude better that of the 3-pyridyl isomer, A(2)44. A(2)45 was the most potent compound tested and was eighty-four times better than the 'lead' compound nifedipine.

The 2-pyridyl isomers were two to three times more potent than

Table 4.10



Compound	R^1	R^2	R^3	IC_{50}	+/- SEM	n	RP
A(2)37	i-propyl	Me	3-pyr	3.02×10^{-8}	0.74	5	1
AK-2-38	i-propyl	Me	2-pyr	6.68×10^{-9}	0.54	4	5
A(2)41	i-propyl	Cl	3-pyr	6.42×10^{-9}	0.21	3	5
A(2)42	i-propyl	Cl	2-pyr	4.15×10^{-9}	0.05	3	7
A(2)44	i-propyl	Br	3-pyr	4.26×10^{-9}	0.25	4	7
A(2)45	i-propyl	Br	2-pyr	1.70×10^{-10}	0.25	4	178
A(2)46	i-propyl	F	3-pyr	1.80×10^{-8}	0.08	4	2
A(2)47	i-propyl	F	2-pyr	5.30×10^{-9}	0.35	4	6
A(2)40	i-propyl	MeO	2-pyr	9.52×10^{-9}	1.34	3	3
nifedipine.....				1.43×10^{-8}	0.38	18	2

GPILSM Ca^{2+} antagonist activity of C-4 pyridyl 1,4-DHPs with asymmetric isopropyl substituted phenethyl diesters.

the corresponding 3-pyridyl isomers, again demonstrating the previously observed order that for these types of analogues the 2-pyridyl isomers tend to be more potent than the 3- or 4-pyridyl analogues.

4.6.11 Selectivity effects of AK-2-38 for GPILSM over guinea pig left atrium.

The analogue AK-2-38 (fig. 4.6), possessing a C-4 2-pyridyl substituent and C-3 isopropyl C-5 p-methylphenethyl esters, was the first compound tested that had greater activity than nifedipine on GPILSM (RP of 5 vs 2 for nifedipine - table 4.10). Since this was a significant improvement in pote y by a structure that was appreciably different from the parent compound, it was decided to further test this compound on the guinea pig left atrium, to find out if it had the same spectrum of activity on this tissue as it did on GPILSM.

At low concentrations which were sufficient to inhibit contraction of GPILSM, the compound was found to exert slight positive inotropic effects on the left atrium. As the concentration was increased there was a gradual inhibition of contractility of the left atrium. Figure 4.6 shows a comparison of the dose-response effects of the compound on the two tissues. Cumulative addition of the compound to atrial tissue to a total dose of greater than 1.0×10^{-6} M could not achieve 50 % inhibition of contractility of the tissue.

This was the first demonstration of an antagonist analogue that

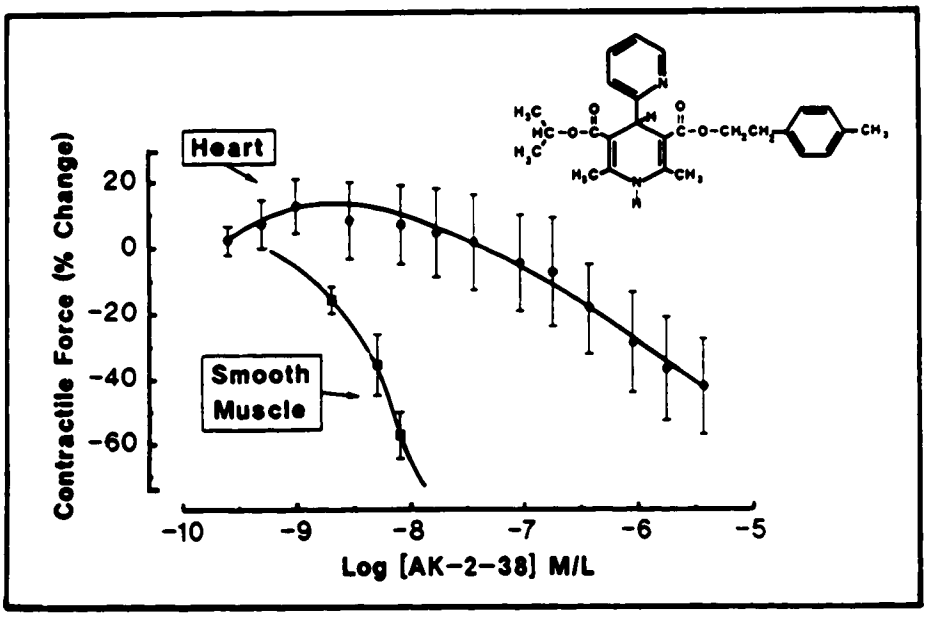


Figure 4.7 Differential effects of AK-2-38 on GPILSM and guinea pig left atrium.

had profound selectivity for smooth muscle over cardiac tissue. It was a significant discovery in the development of novel 1,4-DHP Ca^{2+} antagonists since such compounds could be a useful addition to the treatment of hypertension, particularly in those patients with compromised myocardial function.

Unfortunately there was no time to test the other analogues that were even more potent than AK-2-38. However, based on their structural similarity to this compound, it is possible that they may exhibit a similar pharmacological profile.

4.7 Discussion and conclusions

Several modifications on the 'lead' compound nifedipine were made in an attempt to improve the pharmacological profile of the 1,4-DHP Ca^{2+} antagonists. The modifications made included placing different alkyl, arylalkyl and substituted arylalkyl esters at positions C-3 and C-5 of the 1,4-DHP ring; replacing the C-4 2-nitrophenyl ring of nifedipine with 2-, 3- and 4-pyridyl or reduced pyridyl rings; and even changing the 1,4-DHP ring system to a 1,2-DHP ring.

Although many of the novel analogues were less potent than nifedipine, several had activity greater than or comparable to it. A systematic examination of the effect on potency, of changing different functional groups on the parent compound was done. The results confirmed some of the previously established SARs for this class of 1,4-DHPs, and in addition, some new SARs were discovered.

Most of the modifications did not result in an increase of

activity over that of nifedipine. However, some of the modifications provided structural clues which could be used to improve activity or to produce analogues with different pharmacological profiles. The major findings resulting from modifications covered in this chapter are summarised below.

All modifications made at position C-4 of the 1,4-DHP ring did not improve activity and did not alter the pharmacology of 1,4-DHP Ca^{2+} antagonists on GPILSM. Dagnino and coworkers (1986) reported that a C-4 pyridyl group was isosteric with a nitrophenyl substituent in the same position. Several modifications incorporating a C-4 pyridyl or reduced pyridyl group failed to improve activity, when other functional groups of the parent compound were maintained constant. Generally, for C-4 pyridyl substituents, the order of potency was 2-pyridyl > 3-pyridyl > 4-pyridyl. This order changed somewhat when a C-4 dihydropyridyl group was present, and if the substituents were large the 2-pyridyl group became less potent than the 3- and 4-pyridyl groups. Other substituents (not pyridyl) at this position also failed to improve activity (Table 4.6).

The double bonds of the 1,4-DHP ring confer two possible conformations on the ring, a chair shape or a boat shape. As shown by X-ray crystallographic analysis of different 1,4-DHPs, the boat shape is the preferred conformation assumed by both antagonist and agonist compounds (Langs and Triggle, 1985: Wynn et al., 1988). Changing the boat-shaped 1,4-DHP ring to a more planar 1,2-DHP ring (Soboleski et al., 1988) greatly decreased activity (4.6). This was to be expected since this modification resulted in the DHP nucleus becoming much more flat as shown in figure 4.7, and the C-4 aryl ring

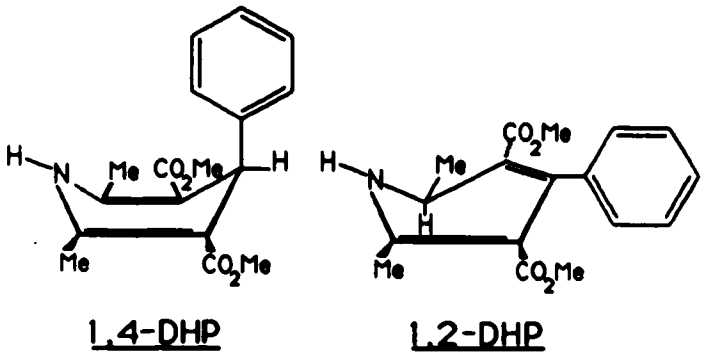


Figure 4.8 Structures of 1,4-DHP and 1,2-DHP compounds illustrating the influence of positions of double bonds on conformation.

was now coplanar to the 1,2-DHP ring. The resultant change in structure did not confer optimal binding conformation to the new analogues into which the modification was made.

The esters at position C-3 and C-5 were found to influence activity. For symmetric C-3/C-5 diesters, activity tended to increase with the increase in the size of the diesters to a point, then started to decline. This indicated that steric effects are important for molecule-receptor interactions. Asymmetry of the esters generally tended to improve activity, with increase in activity correlating to the discrepancy in the size of the diesters; however, this effect was also affected by the C-4 substituent, activity correlating to the nature and size of this moiety as well.

Compounds with C-3/C-5 isopropyl/arylalkyl ester substituents provided the first compounds that had improved activity comparable to or better than that of the parent compound nifedipine. An ethyl spacer between the aryl and ester moieties conferred more favourable

distance between the nucleus and the aryl moiety than a methyl group (phenethyl > benzyl). There were no analogues with longer intervening alkyl chains for comparison to see if the ethyl chain was optimal.

Among analogues with C-3/C-5 isopropyl/phenethyl ester substituents, a para substituent on the phenyl ring enhanced activity, with halide substituents (Br > Cl > F) providing the best improvements. Lipophilicity of the para substituents was also influential since activity increased in the order H < methoxy < methyl < F < Cl < Br. The significance of this order of increase in activity is that there probably is another site on or near the Ca^{2+} channel that interacts with the aryl moiety (hydrophobic site) to enhance channel inhibition.

A new class of the analogues able to preferentially inhibit depolarisation-induced (KCl-induced) contraction was discovered. This discovery was significant because it confirmed that at least two means of mobilising extracellular Ca^{2+}, a receptor-mediated pathway and a depolarisation-induced pathway, exist; and that the latter pathway can be preferentially blocked by this unique class of 1,4-DHPs.

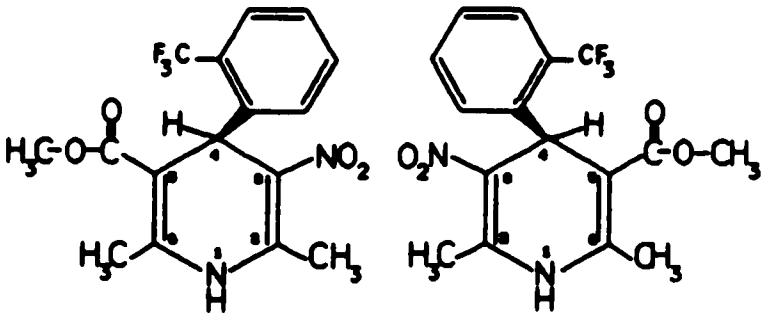
When all the results are considered collectively, it is concluded that different substitutions on the 1,4-DHP nucleus interact collectively to influence activity. This probably results from intramolecular interactions influencing the conformation of the free molecule in solution, or from molecule-receptor interactions which influence the binding interaction of the two, resulting in more or less favourable conformations depending on the substituents.

Overall, the results obtained from screening these novel analogues were in agreement with previously established SARs. Screening of the new analogues elucidated some new SARs, and provided insights into the SARs of 1,4-DHPs, fulfilling the objectives of the study. Some of these novel compounds are potential therapeutic agents, and further testing both *in vitro* and *in vivo* is required to further characterise them and to assess their therapeutic potential.

V. ANALOGUES OF BAY K 8644

5.1 Introduction.

In 1983, Schramm and coworkers reported the discovery of a 1,4-DHP compound, Bay K 8644 (fig. 5.1), that had effects diametrically opposite to those produced by nifedipine on cardiac and smooth muscles.



R(+) Bay K 8644 S(-) Bay K 8644

Figure 5.1 Bay K 8644 shown as the two enantiomeric forms. The S-(-)-isomer possesses Ca^{2+} agonist properties, the R-(+)-isomer is a Ca^{2+} antagonist.

This discovery indicated the existence of opposing Ca^{2+} channel

inhibitory and activator effects in the 1,4-DHP nucleus.

Although Ca^{2+} channel inhibitory effects led to the production of compounds used therapeutically for the treatment of a variety of cardiovascular diseases, Ca^{2+} channel activators have so far failed to yield any therapeutically useful agents. Further development of this class of 1,4-DHP compounds could, however, provide cardiotonic agents as useful as digoxin for the treatment of cardiac failure, but with a larger therapeutic index. The major limitation of the currently available compounds is that they tend to cause blood vessels to constrict at concentrations lower than or comparable to those that produce desirable cardiac activation, increasing blood pressure which could lead to disastrous cardiac failure, especially in patients whose myocardial function is already compromised.

The chiral nature of the C-4 carbon in many 1,4-DHP compounds results in at least two enantiomeric forms of such compounds. In the case of 1,4-DHP Ca^{2+} antagonists, usually one enantiomer has been found to be much more active than the other (Fossheim et al., 1982; Triggle et al., 1983; Fossheim, 1986). For the Ca^{2+} channel activators, the story is slightly different in that one enantiomer often possesses the Ca^{2+} channel activator effects, while the other is a Ca^{2+} antagonist (Schramm et al., 1984, Vaghy et al., 1987; Kamp et al., 1989). The mixed enantiomers exhibit the whole range of effects from total antagonism to full activation depending on their proportions as well as experimental conditions.

Enantiomers of Bay K 8644 (fig. 5.1), 202 791 and CGP 28 392 have been separated and have been shown to demonstrate this

dualistic activity (Hof et al., 1985; Mahmoudian and Richards, 1986; MacDonald et al., 1987; Rutledge et al., 1989). Attempts to mix the enantiomers in different proportions, with the expectation that an optimal proportion could be derived, leading to cardiac activation with little stimulatory or even producing relaxant effects on smooth muscle (Franckowiak et al., 1988; Holland et al., 1988) have been made. Such mixtures have been proposed to be therapeutically useful for the treatment of cardiac failure.

5.2 Objective.

Although the use of enantiomeric mixtures of Ca^{2+} channel activator 1,4-DHPs may offer definite possibilities with regard to the use of Ca^{2+} channel activators as cardiotonic agents, investigation of the SARs of 1,4-DHP Ca^{2+} channel activator compounds could lead to the development of compounds that would have preferential selectivity for cardiac Ca^{2+} channels, that is compounds that are pure cardiac agonists, devoid of effects on smooth muscle. With this point in mind, the goals of screening novel analogues of Bay K 8644 therefore were:

1. to screen the novel analogues for Ca^{2+} channel activator effects on guinea pig left atrium.
2. to screen the novel analogues for lack of Ca^{2+} channel activator effects on GPILSM.
3. to characterise the SARs of the new compounds.
4. to identify structural modifications that led to activity different from that of the parent compound Bay K 8644.

5. to examine ways in which the pharmacology of the novel compounds differed from that of Bay K 8644.

Since the ultimate objective was to look for compounds possessing greater selectivity for cardiac Ca^{2+} channels over smooth muscle Ca^{2+} channels, the test for Ca^{2+} activator effects on both GPILSM and left atrium was done in order to confirm selectivity for cardiac tissue over smooth muscle. Selectivity would be recognised if a compound did not cause smooth muscle contraction, or if it did so only at concentrations higher than those eliciting positive inotropic effects on the left atrium.

5.3 Evaluation of Bay K 8644 analogues on Guinea pig left atrium.

In view of the similarity of the analogues to Bay K 8644 it was assumed that they would have the same mechanism of action. The screening tests therefore served to confirm the ability of the novel compounds to elicit positive inotropic effects on the isolated guinea pig left atrium.

5.4 Novel analogues screened for Ca^{2+} activator effects.

Positions of modifications made on the Bay K 8644 nucleus to produce the novel analogues are shown in figure 5.2.

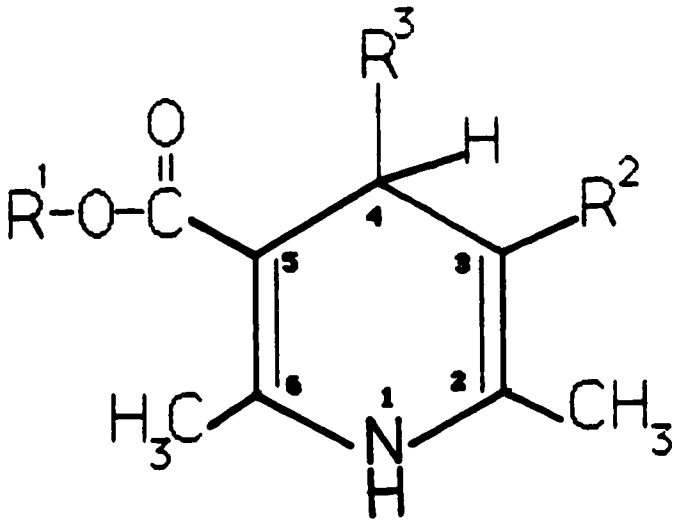


Figure 5.2 1,4-DHP nucleus showing positions R^1, R^2 and R^3, where substitutions were made to produce novel analogues.

The specific modifications included the following:

1. replacement of the C-4 2-trifluoromethyl phenyl ring, with a 2-, 3-, or 4-pyridyl ring.
2. replacement of the 2-trifluoromethyl phenyl ring with a C-4 3'-1'-methoxycarbonyl-1',4'-dihydropyridyl) ring.
3. replacement of the 2-trifluoromethyl phenyl ring with a

C-4 4'-(1'-methoxycarbonyl-1',4'-dihydropyridyl) ring.

4. replacement of the methyl ester of the 1,4-DHP ring with alkyl esters, cycloalkyl esters or alkylaryl esters.
5. replacement of the methyl ester of the 1,4-DHP ring with a nitro substituent.

5.5 Results of screening novel analogues on guinea pig left atrium.

Figure 5.3a shows the dose-response curves of two standard drugs, isoproterenol, a a-adrenoceptor agonist, Bay K 8644, the prototype Ca^{2+} channel activator; and Reek 31, one of the novel analogues of Bay K 8644. All three compounds produced dose-dependent positive inotropic effects on the left atrium of the guinea pig. The EC_{50} values of isoproterenol and Bay K 8644 on the left atrium were $1.87 \pm 0.34 \times 10^{-8}$ M and $7.7 \pm 5.9 \times 10^{-7}$ M respectively. The EC_{50} value for the Bay K 8644 provided a comparative value for the Bay K 8644 analogues.

Isoproterenol exerts its effects through a-adrenoceptor activation, whereas Bay K 8644 and PN 202 791 exert their Ca^{2+} channel activator effects by prolonging the open state (duration of open time) of the L-type Ca^{2+} channel (Hof et al., 1985; MacDonald et al., 1987; Kamp et al., 1989; Rutledge et al., 1989), allowing Ca^{2+} entry into the cell. Analogues that have the same mechanism of action would be expected to exert effects qualitatively similar to those of Bay K 8644. Bay K 8644 therefore provided a good standard against which the activities of the analogues could be

measured. The isoproterenol response was used to test the viability and responsiveness of the tissue before and after exposure to the analogues.

Any analogues that behaved differently from Bay K 8644 were exhibiting different pharmacological profiles and probably had different pharmacological effects. They therefore warranted further investigation.

Except for three compounds, all the novel analogues tested produced positive inotropic effects on guinea pig left atrium that were qualitatively similar to those produced by Bay K 8644. The positive inotropic effects were antagonised by nifedipine, and addition of extracellular Ca^{2+} could partially overcome this inhibition (figure 5.3b). Although most of the analogues producing positive inotropic responses were at least an order of magnitude less active than Bay K 8644, the maximum effect produced by these compounds was generally comparable to that produced by Bay K 8644.

Analogues which did not produce positive inotropic effects included Reek 34a and Reek 35a, which antagonised contraction of the left atrium, and A(3)33, which had no discernible effect within a dose range of 2.5×10^{-8} M to 2.0×10^{-5} M.

measured. The isoproterenol response was u
and responsiveness of the tissue before
analogues.

Any analogues that behaved different
exhibiting different pharmacological prof
different pharmacological effects. They th
investigation.

Except for three compounds, all the
produced positive inotropic effects on gu
were qualitatively similar to those pro
positive inotropic effects were antagoni
addition of extracellular Ca^{2+} could
inhibition (figure 5.3b). Although most o
positive inotropic responses were at le
less active than Bay K 8644, the maximum

5.5.1 Positive inotropic effects of analogues possessing C4 4-, 3- and 2-pyridyl substituents.

The modifications made on Bay K 8644 to produce the novel analogues tested in this section included replacement of the 2-trifluoromethylphenyl ring system with a 4-, 3- or 2-pyridyl ring, and substituting the methyl ester at position C-5 with different alkyl, cycloalkyl and alkylaryl esters. The C-3 nitro group was maintained constant within this and all subsequent series tested.

As with the nifedipine analogues, criteria used to compare these analogues were also based on the structural modifications made to the parent compound, and analogues were compared with each other using the relative potencies within each group or subgroup. Again the least potent compound within each group or subgroup was always assigned an RP value of one, and the activities of the rest of the analogues were determined relative to this value. The RP value of Bay K 8644 therefore also varied from group to group.

For the first two groups (Table 5.1a and 5.1b), activity was compared to the EC_{50} values of compounds possessing C-5 methyl esters on the 1,4-DHP ring. (These analogues were not available for the present study but had been previously tested by Li-Kwong-Ken, (1986)). Li-Kwong-Ken reported that the C4 2-pyridyl compound containing a C5 methyl ester (Compd 74), however did not exhibit any activity on the left atrium in the dose range tested (1.7×10^{-7} M to 8.0×10^{-4} M) and therefore could not be used as the standard for its respective group. The relative potencies of compounds in this group were thus based on the activity of the least potent compound

5.5.1 Positive inotropic effects of anal and 2-pyridyl substituents.

The modifications made on Bay K analogues tested in this section incl 2-trifluoromethylphenyl ring system with a and substituting the methyl ester at p alkyl, cycloalkyl and alkylaryl esters. maintained constant within this and all sub

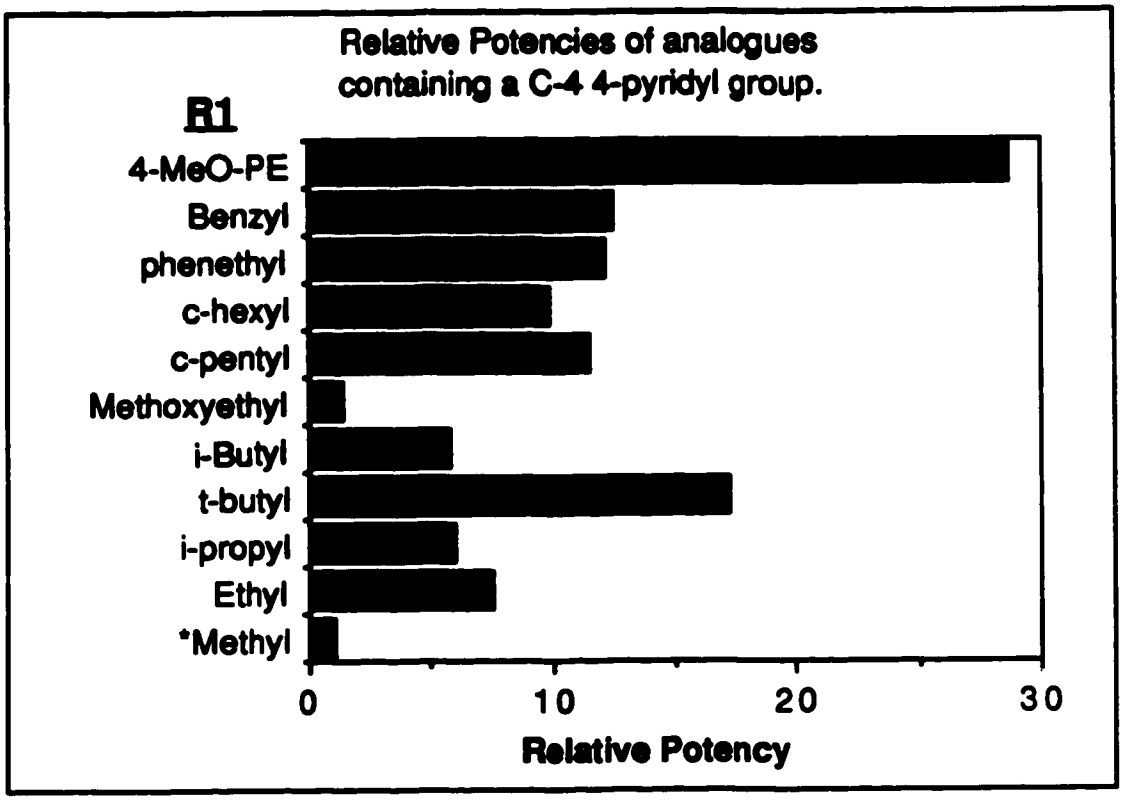
As with the nifedipine analogues, these analogues were also based on the str to the parent compound, and analogues wer using the relative potencies within each the least potent compound within each gr assigned an RP value of one, and the act

in the subgroup, Reek 24. This compound with an EC_{50} value of 1.81×10^{-3} M, was the least active of all the analogues producing a positive inotropic effect on the left atrium.

5.5.1.1 C-4 4-pyridyl analogues.

Figure 5.4 shows the basic structure of Bay K 8644 analogues possessing a C4 4-pyridyl substituent along with a graphic illustration of their relative potencies on guinea pig left atrium. Table 5.1a is a comparative summary of their positive inotropic activities. These compounds all produced positive inotropic effects on the left atrium that were qualitatively similar to those produced by Bay K 8644. As well, all the compounds elicited contraction of GPILSM at concentrations lower than or comparable to those producing positive inotropic responses on the left atrium.

The EC_{50} values and relative potencies of the analogues are listed in table 5.1a. Although there were no consistent trends with regard to the effect of the size of the C-5 ester substituents, generally there appeared a tendency for potency to increase with the size of the ester. The t-butyl ester proved to be the most potent among compounds with simple alkyl ester substituents. The i-butyl ester was comparable to the i-propyl and ethyl esters. As well, compounds with cyclic or alkylaryl esters tended to be more potent than those containing straight chain alkyl esters, and size of the ester substituent still appeared to be a determinant of potency. The most potent analogue in this group was A(3)41 with an RP value



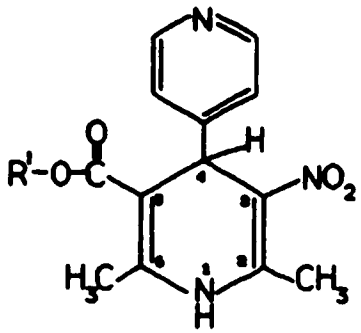


Figure 5.4 Relative Potencies of analogues possessing a C-4 4-pyridyl substituent instead of the 2-trifluoromethyl phenyl ring on guinea pig left atrium. (4-MeO-PE = 4-methoxyphenethyl).

of 28.7. This value was slightly less than half of that of Bay K 8644 (63.0).

On the whole, this group of analogues did not offer any improvement over Bay K 8644 in terms of selectivity, and did not produce any compounds which were qualitatively different with regard to pharmacology.

5.5.1.2 C-4 3-pyridyl analogues.

This subgroup of analogues differed from those in Table 5.1a in that they all possessed a C-4 3-pyridyl ring instead of a C-4 4-pyridyl ring. Their relative potencies and EC_{50} values are listed in Table 5.1b and the basic structure is given together with a graphic illustration of their relative potencies in Figure 5.5.

These analogues were very similar to Bay K 8644 and the other analogues described in section 5.5.1.1. They all elicited contractile responses on guinea pig left atrium that were qualitatively similar to those of Bay K 8644 and were all agonists on GPILSM.

Collectively there was no specific SAR trend with regard to potency. Among analogues possessing C-5 simple alkyl esters, activity was quite similar. Reek 26, with an ethyl ester was the most potent with an RP of 7.9. As for analogues possessing C-4 4-pyridyl substituents, the analogue with an i-butyl C-5 ester was also much less potent than its t-butyl counterpart. The steric size of the t-butyl ester may therefore be important for enhancing Ca^{2+} channel activator effects since smaller esters were weaker and the

of 28.7. This value was slightly less t
8644 (63.0).

On the whole, this group of analo
improvement over Bay K 8644 in terms o
produce any compounds which were qualitati
to pharmacology.

5.5.1.2 C-4 3-pyridyl analogues.

This subgroup of analogues differed f
that they all possessed a C-4 3-pyrid
4-pyridyl ring. Their relative potenc
listed in Table 5.1b and the basic struct
a graphic illustration of their relative po

These analogues were very similar t

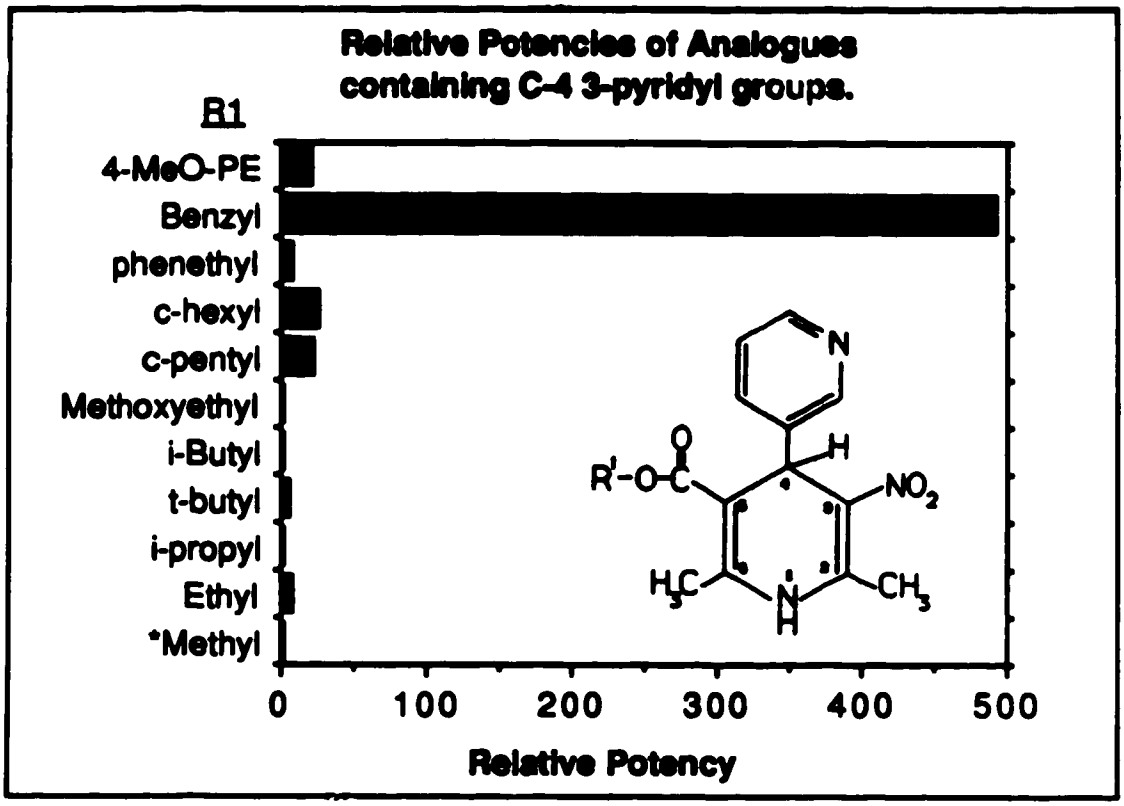
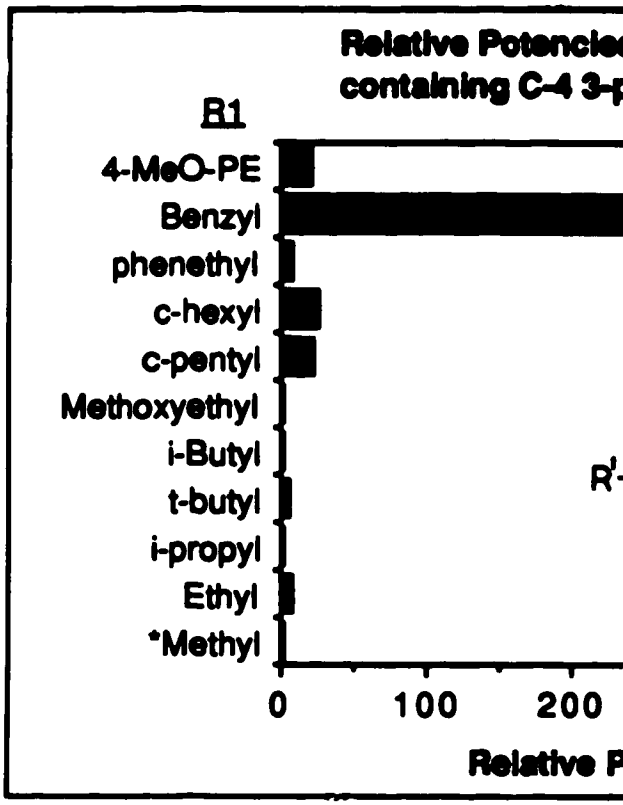
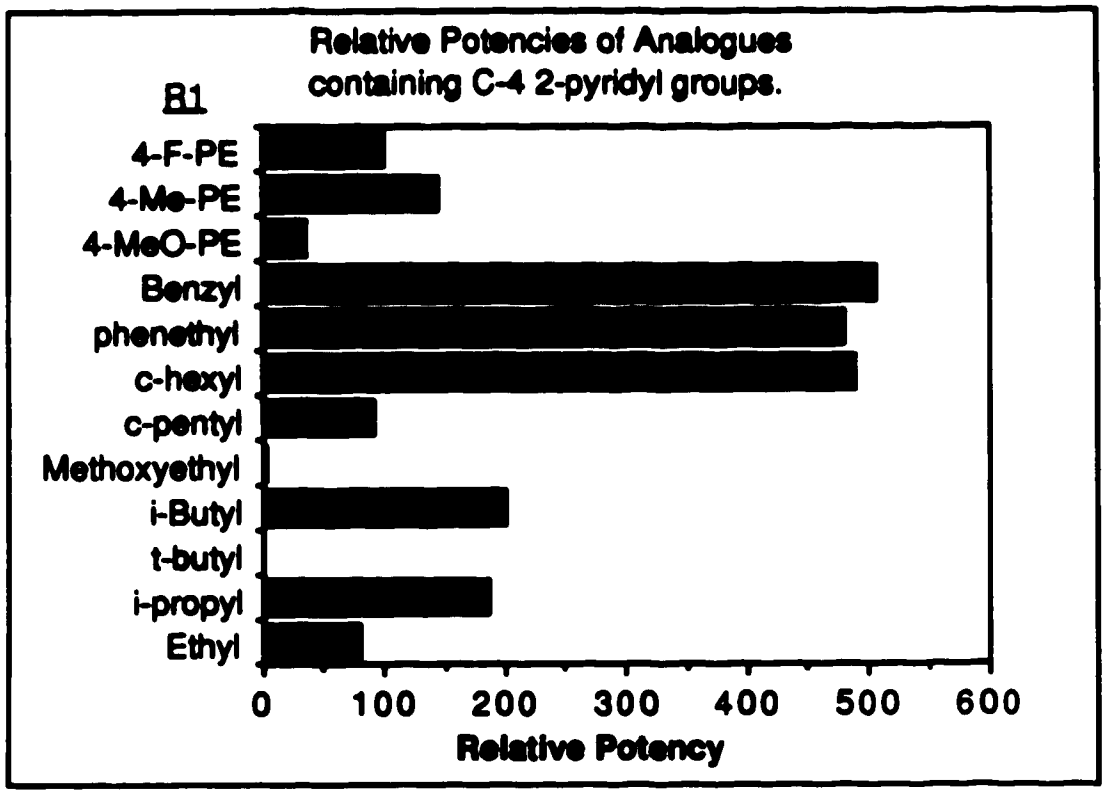


Figure 5.5 Relative Potencies of analogues possessing a C-4 3-pyridyl substituent instead of the 2-trifluoromethyl-phenyl ring on guinea pig left atrium. (4-MeO-PE = 4-methoxyphenethyl).


Relative Potencies
containing C-4 3-p
R1
4-MeO-PE
Benzyl
phenethyl
c-hexyl
c-pentyl
Methoxyethyl
i-Butyl
t-butyl
i-propyl
Ethyl
*Methyl
0
100
200
Relative P



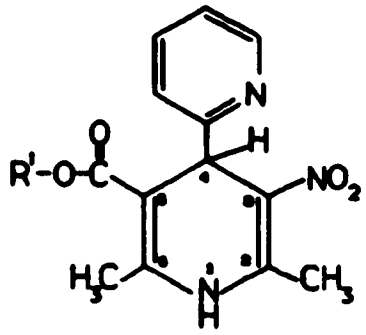


Figure 5.6 Relative Potencies of analogues possessing a C-4 2-pyridyl substituent instead of the 2-trifluoromethyl-phenyl ring on guinea pig left atrium. (4-MeO-PE = 4-methoxyphenethyl).

M). This may indicate that the dichloro substituents may have caused the molecule to exceed dimensions required for binding to the receptor site, or made the molecule too lipophilic (halide substituents enhance lipophilicity) for gaining access to the binding site.

Table 5.1c and Figure 5.6 show the RPs of this series of analogues. For compounds possessing simple alkyl esters, the most potent analogue was now one containing a C-5 i-butyl ester (*cf.* t-butyl for C-4 3- and 4-pyridyl analogues). The t-butyl analogue was now less potent, indicating that there was a difference in the interaction of this analogue with the receptor site compared to its C-4 3- and 4-pyridyl analogues. This probably came about through a shift in the orientation of the t-butyl functional group, leading to a less favourable positioning than when the C-4 group is a 3- or 4-pyridyl ring.

The C-5 4-MeO-PE was also less potent relative to the c-pentyl, c-hexyl and benzyl esters, but the general trend that simple C-5 alkyl esters were less potent than cyclic and alkylaryl esters held.

A decrease in activity relative to simple alkyl and cyclic esters also resulted when substituents were added onto the phenyl ring of the C-5 phenethyl ester. This decrease could have two possible causes. The first is that there is a corresponding site for the unsubstituted phenyl of the C-5 ester on or close to the 1,4-DHP site, which has fairly specific size requirements. Increasing the size of this functional moiety by adding substituents to it would therefore decrease the strength of the binding interaction with the corresponding site, and thus decrease

Table 5.1c

Compound	R^1	EC_{50} +/-	SEM	n	RP
Reek 24	t-butyl	1.81×10^{-3}	0.97	3	1.0
Reek 27	ethyl	2.28×10^{-5}	0.13	3	79.4
Reek 30	i-propyl	9.67×10^{-6}	1.27	3	187.2
Reek 33	i-butyl	9.10×10^{-6}	2.85	4	198.9
Reek 34	MeO-Et	9.70×10^{-4}	4.93	3	1.9
AMEK 3	c-pentyl	1.98×10^{-5}	0.53	3	91.4
AMEK 4	c-hexyl	3.70×10^{-6}	2.62	3	489.2
AMEK 7	PE	3.77×10^{-6}	0.95	3	480.1
A(3)21	4-F-PE	1.83×10^{-5}	0.32	3	98.9
A(3)27	4-Me-PE	1.25×10^{-5}	0.34	3	144.8
A(3)30	benzyl	3.58×10^{-6}	1.44	3	505.6
A(3)40	4-MeO-PE	5.25×10^{-5}	1.84	3	34.5
A(3)33	3,4-Cl_2-PE	no effect			

Ca^{2+} channel activator effects of C4 2-pyridyl substituted analogues of Bay K 8644 on guinea pig left atrium. (R^1 = C5 ester). EC_{50} is the concentration of compound producing 50% of the maximal contractile response due to the compound; n = no. of tests; SEM = standard error of the mean; RP = Relative Potency. MeO-Et = methoxyethyl; c = cyclo; PE = phenethyl. F = fluoro; 3,4-Cl_2-PE = 3,4-dichlorophenethyl.

activity. The second possibility is that analogues with differentelectronic properties (e.g. hydrophilic substituents) could have the opposite effect and enhance activity.

Collectively, the modifications did not provide an improvement on the potency of the parent molecule Bay K 8644, but on screening on GPILSM, it was discovered that instead of eliciting contractile responses on the tissue, these analogues inhibited contraction!. This exciting new development is discussed in detail in section 5.6.

5.5.1.4 Ca^{2+} channel activator effects of compounds with a C-4 3'-(1'-methoxycarbonyl-1',6'-dihydropyridyl) or a C-4 4'-(1'-methoxycarbonyl-1',2'-dihydropyridyl) group in place of the 2-trifluoromethyl phenyl ring of Bay K 8644.

The C-4 substituent of these compounds was no longer a simple pyridyl group as in all analogues discussed up to now, but was changed to either a 3'-(methoxycarbonyl-1',6'-dihydropyridyl) or a 4'- (methoxycarbonyl-1',6'-dihydropyridyl) group (fig. 5.7 below). The C-5 esters were just simple alkyl groups.

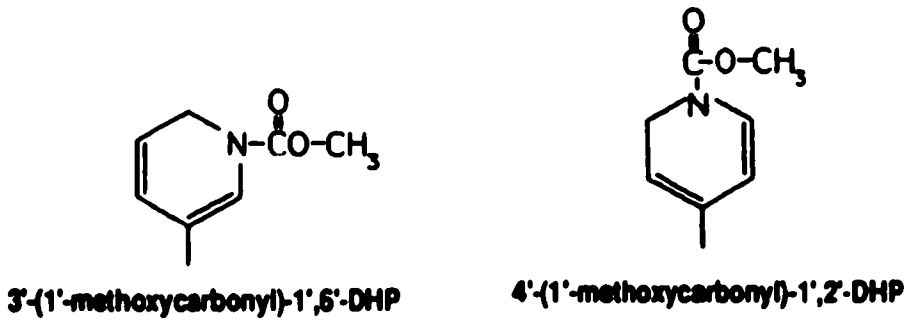


Figure 5.7 C-4 substituents of analogues.

Table 5.2 is a summary of EC_{50} values and the RP values of the analogues. The RP values are presented graphically in figure 5.8. All compounds containing a C-4 4′-(methoxycarbonyl-1′,6′-dihydro pyridyl) group, (Reek 37 to Reek 40), had approximately the same activity and were all less active than compounds containing a C-4 3′-(methoxycarbonyl-1′,6′-dihydro pyridyl) ring (Reek 41 to Reek 45). In the latter group, the order of potency of the esters was methoxyethyl > i-butyl > t-butyl > isopropyl.

Among these compounds, Reek 42 and Reek 44 were found to exert Ca^{2+} antagonist effects on GPILSM (see table 5.3). These effects were unexpected and could not be explained based on the observed structures of the compounds and the results obtained with the rest of the group. The rest of the compounds either showed minimal agonist effects or had no effect at all on smooth muscle.

Although this group of analogues showed some differences from Bay K 8644, they were either difficult to make or unstable, consequently testing was not carried through to a satisfactory degree because of inadequate supply of some of the compounds.

Table 5.2

Compound	R^1	IC_{50} +/-	SEM	n	RP
Reek 37	i-butyl	$2.31x10^{-5}$	1.08	3	1.1
Reek 38	i-propyl	$2.04x10^{-5}$	0.08	3	1.3
Reek 39	ethyl	$1.42x10^{-5}$	0.82	4	1.9
Reek 40	t-butyl	$2.65x10^{-5}$	0.63	6	1.0
Reek 41	MeO-Et	$2.53x10^{-6}$		2	10.5
Reek 42	i-butyl	$2.91x10^{-6}$	1.24	3	9.1
Reek 43	t-butyl	$3.98x10^{-6}$		2	6.7
Reek 44	ethyl	not determined			
Reek 45	i-propyl	$7.51x10^{-6}$		2	3.5

Guinea pig left atrium Ca^{2+} channel activator effects of compounds with a C-4 3'-(1'- methoxycarbonyl-1',6'-dihydropyridyl) or a C-4 4'- (1'-methoxy- carbonyl-1',2'-dihydropyridyl) group in place of the 2- trifluoromethylphenyl ring of Bay K 8644. (R^1 = C5 ester). EC_{50} is the concentration of compound producing 50% of the maximal contractile response due to the compound; n = no. of tests; SEM = standard error of the mean; RP = Relative Potency.

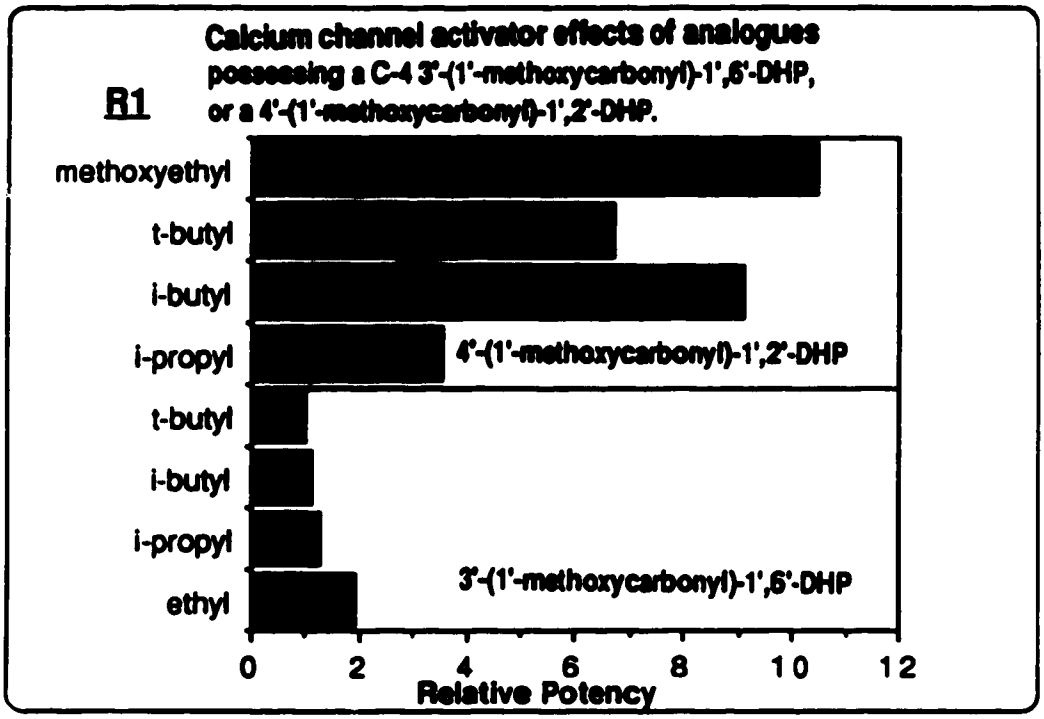


Figure 5.8 Relative Potencies of compounds with a C-4 3'-(1'-methoxycarbonyl-1',6'-DHP) or 4'-(1'-methoxycarbonyl-1',2'-DHP) group instead of 2-trifluoromethylphenyl ring of Bay K 8644. (MeO-Et = methoxyethyl).

5.6 Ca^{2+} antagonist activity of C4 2-pyridyl substituted (and other) analogues of Bay K 8644 on GPILSM.

As revealed in section 5.5.1.3, all analogues possessing a C4 2-pyridyl substituent were found to inhibit contraction of GPILSM in response to carbachol or KCl. These compounds therefore exhibited differential activity between the atrial tissue and GPILSM. This was an unexpected 'bonus', since improvement in tissue agonist specifity was what we had hoped for at the outset. The compounds described here are cardioselective Ca^{2+} channel agonists with smooth muscle antagonist activity. In contrast all compounds possessing either a C4 3- or 4-pyridyl substituent contracted GPILSM or augmented KCl contraction of rabbit bladder.

Figure 5.9 shows the augmentation of KCl-induced contraction of rabbit bladder by Bay K 8644 and Reek 28 (leftward shift of the dose-response curve), and inhibition by nifedipine (rightward shift of dose-response curve). KCl depolarises the cell membrane, leading to opening of VSCs. The open channels allow Ca^{2+} influx into the cell leading to contraction of the tissue. Since this effect is inhibited by organic Ca^{2+} antagonists, the Ca^{2+} causing the response is assumed to come from outside the cell through the opened VSCs, thus KCl (i.e. membrane depolaristion) preferentially mobilises extracellular Ca^{2+} through VSCs. Augmentation of the KCl response by Bay K 8644 and Reek 28, and its inhibition by nifedipine, would suggest that these compounds were acting at VSCs to enhance or inhibit Ca^{2+} influx respectively at these channels.

This discovery represented not only differential activity (i.e.

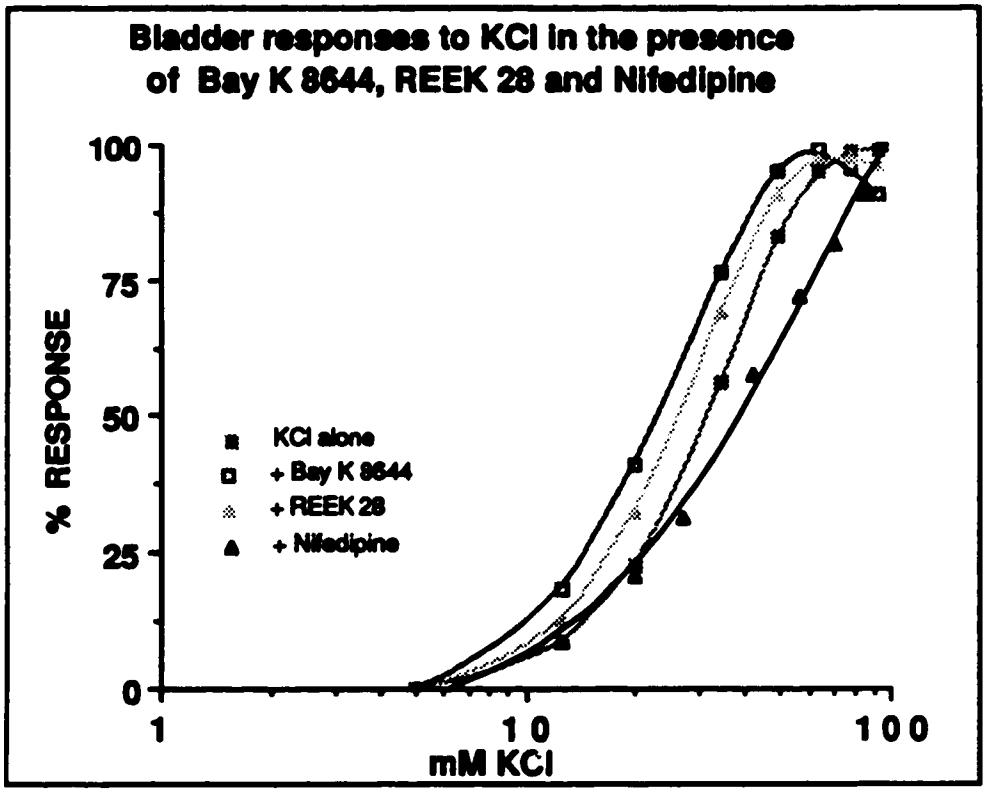


Figure 5.9 Potentiation of KCl induced responses on rabbit bladder by Bay K 8644 (1.95×10^{-7} M) and Reek 28 (4.8×10^{-6} M), and inhibition by nifedipine (1.0×10^{-8} M).

selectivity), but also the first separation of agonist and antagonist properties on the Ca^{2+} channel by the same compound. The results therefore suggested that cardiac muscle (left atrium) and smooth muscle (GPILSM), may possess different subtypes of the L-type Ca^{2+} channel. Another possible explanation of the differential effects of the compounds is that the differences in the tissues account for the differential effects between cardiac and smooth muscle. The voltage and use dependence of 1,4-DHP antagonist effects on smooth muscle (MacDonald et al., 1987; Tsien, 1987; Bolton et al., 1988; Kamp et al., 1989), are well-documented phenomena (see discussion). These novel compounds are therefore either exerting opposing effects on the same channel in different tissues, or on two different channel substypes, activating cardiac Ca^{2+} channels while inhibiting smooth muscle Ca^{2+} channels.

The implication is that such analogues could be useful in the treatment of heart failure and hypertensive heart disease. Bay K 8644 on the other hand increases cardiac contractility in the same dose range that constricts blood vessels.

A graphic presentation of the RP values of the compounds on GPILSM is given in figure 5.10 and the IC_{50} values are given in table 5.3. The RP value of nifedipine is included in the table for comparison.

The most active analogues were those possessing C-5 c-pentyl, c-hexyl and phenethyl esters. These analogues had IC_{50} values in the 10^{-7} M range and could be developed as Ca^{2+} antagonists (antihypertensive agents) with cardiac stimulatory activity at higher concentrations since IC_{50} concentrations on GPILSM were

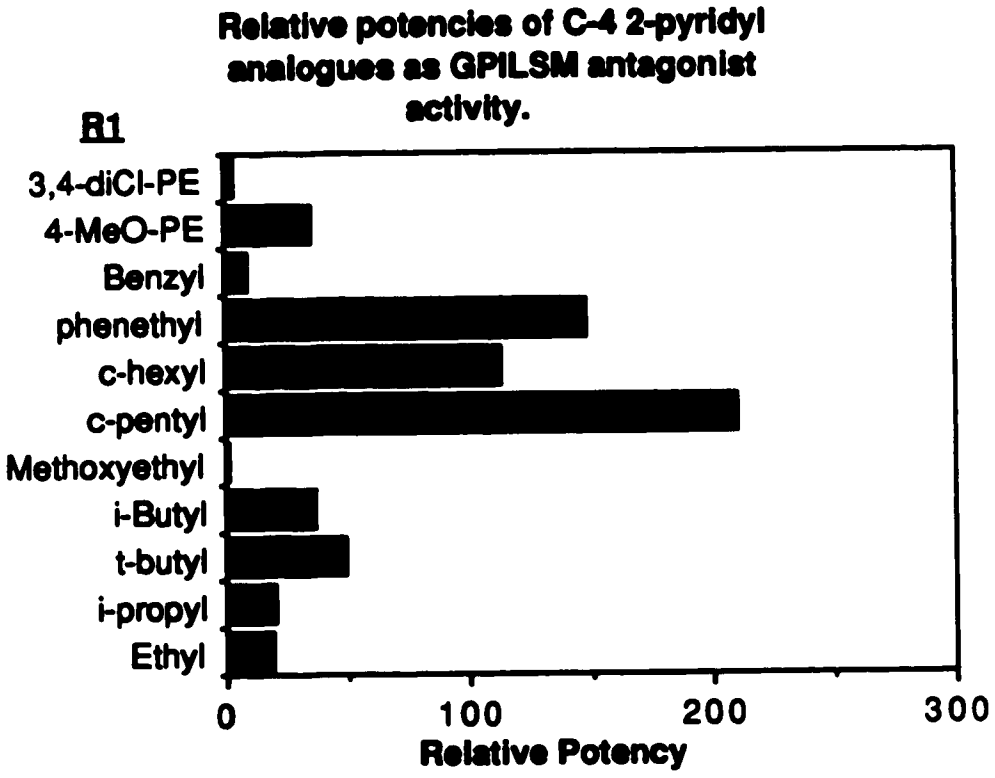
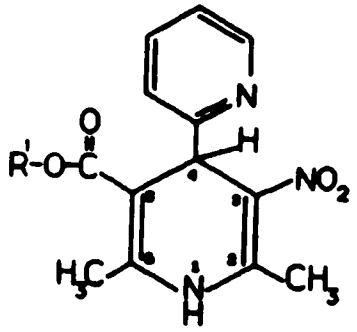


Figure 5.10 Relative potencies of C-4 2-pyridyl Bay K analogues on GPILSM (Ca^{2+} antagonist activity).

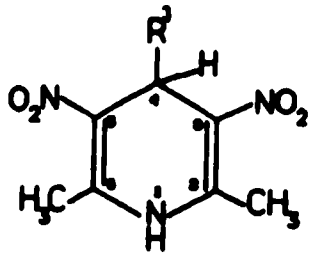
Table 5.3



Compound	R^1	IC_{50}	+/-	SEM	n	RP
a).						
Reek 24	t-butyl	$1.93x10^{-6}$		1.06	3	49.1
Reek 27	ethyl	$5.00x10^{-6}$		1.97	3	18.9
Reek 30	i-propyl	$4.87x10^{-6}$		2.06	3	19.4
Reek 33	i-butyl	$2.61x10^{-6}$		0.06	3	36.3
Reek 34	MeO-Et	$9.47x10^{-5}$		2.76	3	1.0
AMEK 3	c-pentyl	$4.50x10^{-7}$		0.48	5	210.4
AMEK 4	c-hexyl	$8.42x10^{-7}$		0.63	3	112.5
AMEK 7	PE	$6.39x10^{-7}$		2.63	3	148.2
A(3)30	benzyl	$1.09x10^{-5}$		0.34	3	8.7
A(3)33	$3,4Cl_2$-PE	$2.76x10^{-5}$		0.53	4	3.4
A(3)40	4-MeO-PE	$1.09x10^{-5}$		0.34	3	35.5
nifedipine	.....	$1.43x10^{-8}$				6622

Ca^{2+} antagonist activity of C4 2-pyridyl substituted analogues of Bay K 8644 on GPILSM. (R^1 = C5 ester). IC_{50} is the concentration of compound producing 50% inhibition of the maximal contractile response due to the carbachol; n = no. of tests; SEM = standard error; RP = Relative Potency.

Table 5.3 cont'd



Compound	R^3	IC_{50}	+/-	SEM	n	RP
b).						
Reek 34a	4-pyridyl	1.47×10^{-5}		0.41	3	6.4
Reek 35a	2-pyridyl	1.35×10^{-5}		0.01	3	7.0

Ca^{2+} antagonist activity of Bay K 8644 analogues possessing an R^1 nitro substituent on GPILSM. (R^3 = C-4 substituent). IC_{50} is the concentration of compound producing 50% inhibition of the maximal contractile response due to the carbachol; n = no. of tests; SEM = standard error; RP = Relative Potency.

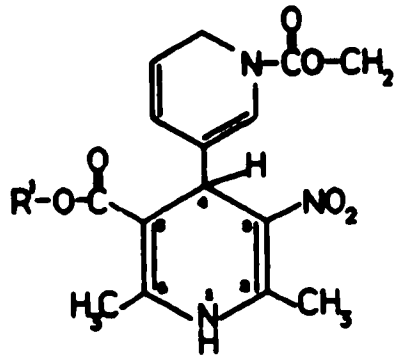
PILSM.

mpoun

due t

lativ

Table 5.3 cont'd



Compound	R^3	IC_{50}	+/-	SEM	n	RP
c).						
Reek 42	i-butyl	1.89×10^{-5}		0.17	3	5.0
Reek 44	ethyl	1.30×10^{-5}		0.09	3	7.3
nifedipine	.....	1.43×10^{-8}				6622

Ca^{2+} antagonist activity of Bay K 8644 analogues possessing a C-4 3'-(1'-methoxycarbonyl-1',6'-dihydropyridyl) substituent on GPILSM. (R^3 = C-4 substituent. IC_{50} is the concentration of compound producing 50% inhibition of the maximal contractile response due to the carbachol; n = no. of tests; SEM = standard error; RP = Relative Potency.

lower than EC_{50} concentrations on the left atrium.

Analogues possessing simple C-5 esters were weaker than those with cyclic esters by about one order of magnitude. The exceptions to this were the benzyl and 4-MeO-PE esters. This order was a reversal of the orders produced on the left atrium. This may indicate very specific structural differences for the interaction of these groups in binding to the 1,4-DHP receptor, suggesting that the benzyl and 4-MeO-PE groups are more favourable for cardiac Ca^{2+} channel activator effects than for smooth muscle antagonist effects.

A(3)33, the only analogue devoid of cardiac activity was found to possess smooth muscle Ca^{2+} antagonist activity, although it was not as potent as the other compounds. This analogue may have been demonstrating more structural differences between cardiac and smooth muscle Ca^{2+} channel, with the compound not being able to bind to the former, but able to bind to the latter.

Replacing the C5 ester with a nitro substituent made the compounds antagonists on both left atrium and GPILSM (Reek 34a and Reek 35a). This supported the original observation that for the activator enantiomer(s) of Bay K 8944 (and its congeners), Ca^{2+} channel activator effects depend on a nitro group at one of positions C-3 or C-5 but not both.

The C-4 pyridyl substituent is therefore a critical determinant for the separation of Ca^{2+} activator and antagonist effects. With the exception of the benzyl ester analogue, the C-4 4-pyridyl substituent provided the most potent analogues for cardiac Ca^{2+} channel activator effects, and the poorest for smooth muscle Ca^{2+} channel inhibitory effects. The converse was true for the C-4

1. Activity Ratio (AR) = $\frac{EC_{50} \text{ (left atrium)}}{EC_{50} \text{ (GPILSM)}}$

2. Selectivity Index (SI) =((1/AR) - 1)

In considering AR and SI, a compound that is equi-active on both tissues would therefore have an SI of zero; a compound with a negative SI would indicate greater selectivity for smooth muscle (GPILSM); and a positive SI would indicate a higher selectivity for cardiac tissue. An obvious advantage of this formula is that it can be used to relate activities that are in the same or opposite directions (e.g. stimulation of both cardiac and smooth muscle, or stimulation in one and inhibition in the other).

Figure 5.11 shows the SI values for compounds showing selectivity of effects between guinea pig left atrium and GPILSM. Of the initial group of Bay K analogues tested (Reek series), Reek 30, with an SI of -0.5, had provided the best differential activity.

Although AMEK 7 was the most potent of the analogues on GPILSM, A(3)30, (EC_{50} of 3.58 $\pm$ 1.44 x 10^{-6} M on left atrium and IC_{50} of 1.09 $\pm$ 0.34 on GPILSM), was by far the best compound tested. A(3)30 was the only compound with a positive index, and thus had the largest differential activity between the two tissues. An added advantage was that this compound had good activity on the left atrium (EC_{50} of 3.58 $\pm$ 1.44 x 10^{-6} M), and is therefore potentially the most useful cardiotonic agent.

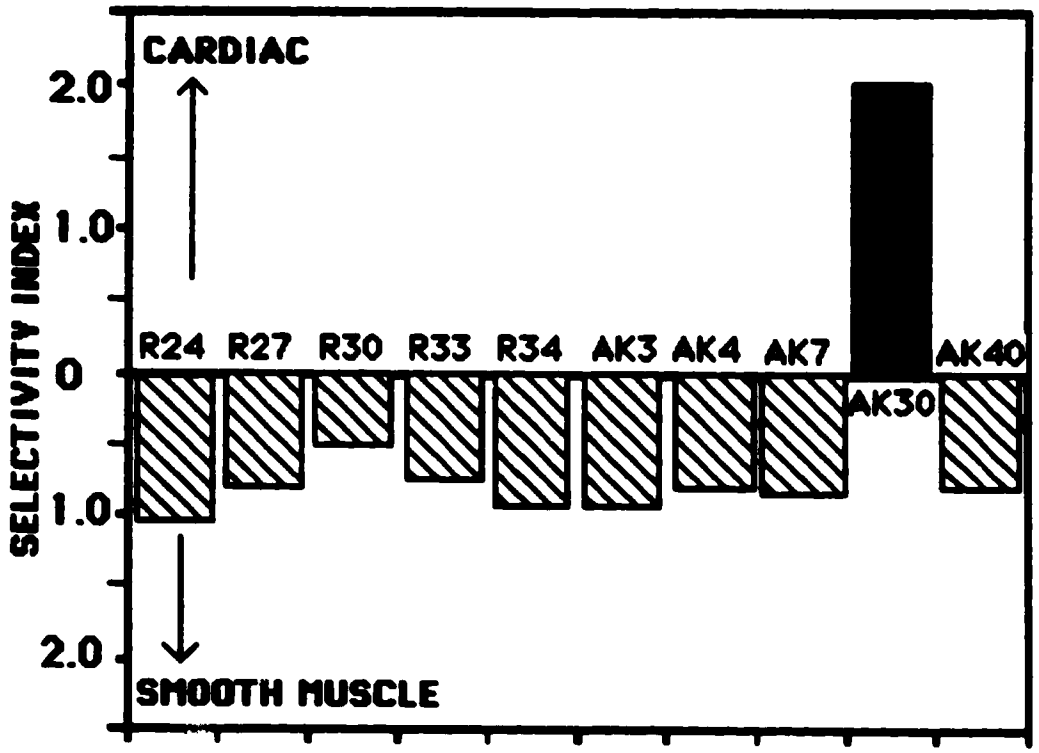
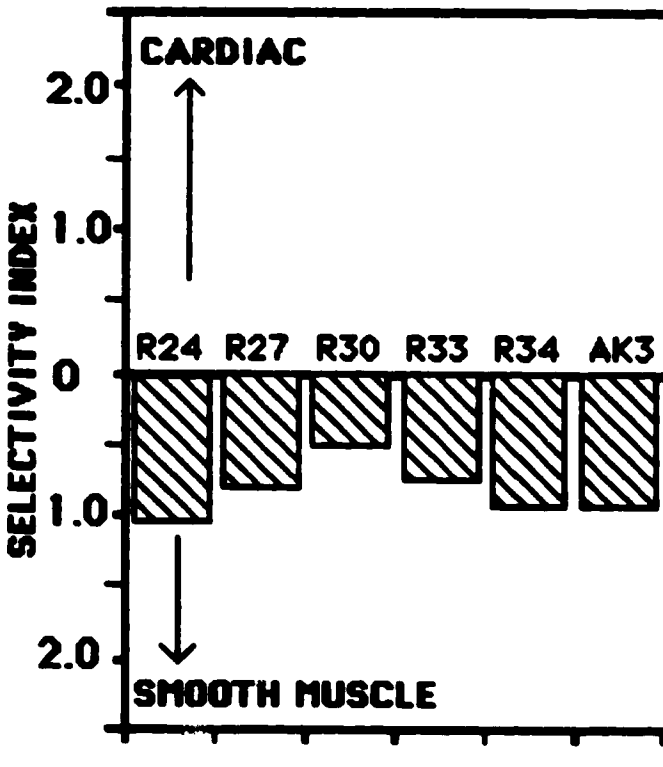


Figure 5.11 Selectivity indices of analogues possessing C-4 2-pyridyl substituents. (R = Reek; AK = AMEK or A(3)).


CARDIAC
2.0
1.0
SELECTIVITY INDEX
R24 R27 R30 R33 R34 AK3
0
1.0
2.0
SMOOTH MUSCLE

5.7 Discussion and conclusions.

Several alterations to the Bay K 8644 nucleus were evaluated in order to develop novel analogues as potential cardiotonic agents. The goal was to identify the structural alterations leading to novel analogues possessing selectivity for cardiac tissue over smooth muscle. The study led to the identification of several SARs for the 1,4-DHP Ca^{2+} channel activators.

In terms of potency, a general trend for the unsubstitued C-4 pyridyl ring was that compounds possessing a C4 4-pyridyl substituent (table 5.1a), were more potent, or equipotent with those possessing a C4 3-pyridyl substituent (table 5.1b), and were generally more potent than those possessing a C4 2-pyridyl (table 5.1c). Exceptions to this generality were compounds possessing a phenethyl ester, where the compound possessing the C4 2-pyridyl substituent was more potent than the ones possessing a C4 4-pyridyl or 3-pyridyl which were about equipotent (AMEK 7 > AMEK 8 $\geq$ AMEK 9); and compounds possessing a benzyl ester where the C-4 3-pyridyl analogue was a full order of magnitude more potent than the C-4 4-pyridyl or 2-pyridyl analogues.

For analogues possessing simple esters, potency tended to increase with the size of the ester, although the trend was not consistent. The t-butyl ester substituent appeared to promote Ca^{2+} channel activator effects when the C-4 substituent was a 4-pyridyl group but dramatically reduced activity when the C-4 substitutent was a 2-pyridyl group.

Among compounds with cyclic and arylalkyl ester substituted analogues, the c-hexyl ester did not appear to influence activity at all regardless of the nature of the C-4 pyridyl substituent. The remaining compounds followed the trend that activity declined in the order 4-pyridyl > 3-pyridyl > 2-pyridyl.

The most important discovery was that compounds possessing C-4 2-pyridyl substituents exerted smooth muscle antagonist effects. This fact was further supported by electrophysiological studies done by Dr. Susan Howlett, (Dept. of Pharmacology, University of Alberta) which definitely demonstrated that under voltage clamp conditions in whole cell current recordings, Reek 30 inhibited Ca^{2+} current in smooth muscle cells.

Several investigators have reported that for 1,4-DHP Ca^{2+} agonist compounds, usually one enantiomer possesses the Ca^{2+} channel activator effects while the other is a Ca^{2+} antagonist (Triggle, 1987; Reuter et al., 1988; Kamp et al., 1989; Rutledge et al., 1989). Since all Bay K 8644 analogues tested possessed a chiral carbon at position C-4 of the 1,4-DHP ring, they all have enantiomeric forms. It is possible that their Ca^{2+} channel activator effects are mediated by one enantiomer, and the other enantiomer is a Ca^{2+} antagonist or lacks activity altogether. If this is the case, it is interesting that analogues possessing the C-4 2-pyridyl substituents have differential effects on GPILSM and left atrium. This possibility then raises the question whether these differential effects are mediated by the same enantiomer, or whether each enantiomer is active on only one tissue leading to the production of the observed effects.

VI. *IN VIVO* Ca^{2+} CHANNEL MODULATOR EFFECTS OF SOME OF THE NOVEL ANALOGUES.

6.1 Introduction.

Although valuable pharmacodynamic information may be derived from studying isolated systems, the response of the whole organism to pharmacological manipulation may be far different from that predicted by *in vitro* observations. The tests used *in vitro* are usually limited to one, or at most, a few preparations. However, for drugs such as Ca^{2+} antagonists, which may not necessarily have a diffuse action throughout the vascular system, specificity for certain vascular beds may be missed and important, clinically relevant information may not be apparent during *in vitro* screening. The *in vivo* haemodynamic response of an animal would therefore compliment *in vitro* testing and could provide more meaningful characterisation of the novel compounds.

For Ca^{2+} antagonist compounds, this issue is made even more poignant by the fact that this class of compounds is generally much more selective for vascular tissues than myocardial tissues, and among the 1,4-DHPs themselves nicardipine and nisoldipine have been shown to have greater selectivity for coronary and cerebral vessels respectively. Furthermore, felodipine, in a single oral dose of 15 mg reduced total peripheral resistance by 40 %, with a concomitant

increase in cardiac output of 54 % and a slight increase in stroke volume (Agner et al., 1985). In the same study, forearm vascular resistance was reported to decrease by 66 %, with a 175 % increase in forearm blood flow.

Since some of the 1,4-DHP Ca^{2+} channel agonists have been shown to exhibit differential activity (smooth muscle vs cardiac tissue), further *in vivo* investigations were also carried out.

6.2 Objectives.

The principal goals of the *in vivo* experiments were:

1. to confirm the findings of *in vitro* experiments with regard to the mechanism of action of the analogues.
2. to characterise the haemodynamic effects of some of the novel analogues in order to assess their antihypertensive and/or cardiotonic effects.

6.3 *In vivo* effects of some Bay K 8644 analogues.

Three analogues of Bay K 8644 (Reek 28, 29 and 30), were tested *in vivo* in order to determine their acute haemodynamic effects. The structures and *in vitro* effects of these compounds have been discussed in chapter 5. Testing was done on anaesthetised male rabbits and on spontaneously hypertensive male (SHR) rats. The major objective of testing these analogues *in vivo* was to confirm the observed pharmacological spectrum of activities exhibited *in vitro* by analogues possessing different C-4 pyridyl rings,

increase in cardiac output of 54 % and a sl
volume (Agner et al., 1985). In the same
resistance was reported to decrease by 66 %
in forearm blood flow.

Since some of the 1,4-DHP Ca^{2+} chann
shown to exhibit differential activity (s
tissue), further in vivo investigations were a

6.2 Objectives.

The principal goals of the in vivo experiments

1. to confirm the findings of in
   regard to the mechanism of action of
2. to characterise the haemodynamic
   novel analogues in order to assess
   and/or cardiotonic effects.

6.3.1 Effects of Bay K 8644 analogues in anaesthetised rabbit.

Figure 6.1 shows the results of *in vivo* testing of the C-4 4-pyridyl analogue Reek 28. This compound caused a marked transient increase in arterial blood pressure from 130/85 to 180/130 mm Hg which lasted about five minutes. This was accompanied by a dysrhythmic heart beat and altered EKG pattern which was manifested as S-T segment elevation.

The effect of Reek 28 was qualitatively similar to that of the parent compound Bay K 8644 and Reek 29. Figure 6.2 shows the effects of Bay K 8644, Reek 28 and Reek 29 after β-blockade with propranolol (1.0 mg/kg). All three compounds produced transient increases in arterial pressure whose intensity and duration of action was dose-dependent. At the concentrations used, the effects lasted up to eight minutes.

In contrast to the effects of Bay K 8644, Reek 28 and Reek 29, the *in vivo* effects of Reek 30 were hypotensive (fig. 6.3), indicating a peripheral vasodilatory action. This was in agreement with the effects observed *in vitro* on GPILSM. A single dose of Reek 30 (0.3 mg/kg) produced an immediate decrease in blood pressure (35 mm Hg systolic and 40 mm Hg diastolic) which recovered rapidly (about 30%) within half a minute, then gradually recovered to control levels after about six minutes. This response was qualitatively similar to the effect produced by nifedipine, the prototype 1,4-DHP Ca^{2+} antagonist (fig. 6.4). Reek 30 did not produce any changes in heart rate or EKG even when the animal was not β-blocked. An inference can be made that since there was an

6.3.1 Effects of Bay K 8644 analogues in a

Figure 6.1 shows the results of in
4-pyridyl analogue Reek 28. This compound
increase in arterial blood pressure fro
which lasted about five minutes. This
dysrhythmic heart beat and altered EKG pat
as S-T segment elevation.

The effect of Reek 28 was qualitative
parent compound Bay K 8644 and Reek 2
effects of Bay K 8644, Reek 28 and Reek
propranolol (1.0 mg/kg). All three com
increases in arterial pressure whose in
action was dose-dependent. At the concent
lasted up to eight minutes.

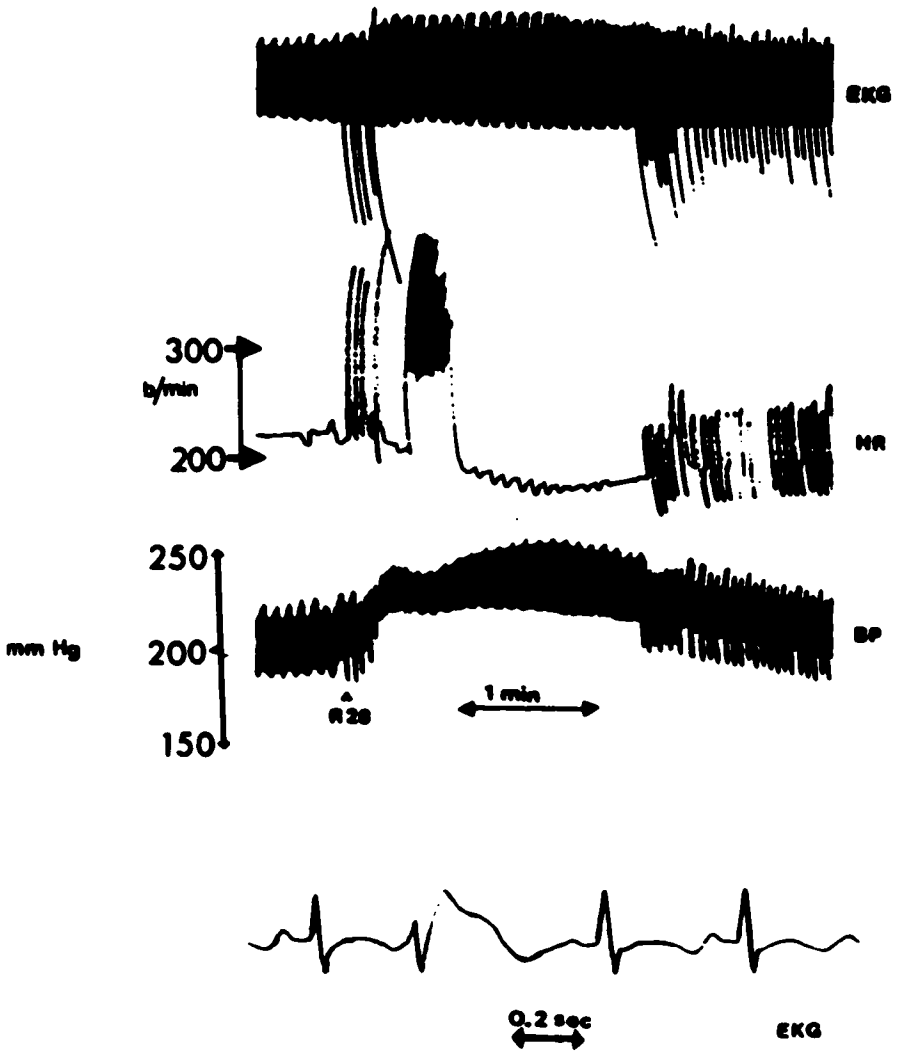


Figure 6.1 *In vivo* effects of Reek 28 (0.9 mg/kg) in the rabbit showing EKG; heart rate and blood pressure. The bottom tracing is EKG taken at a faster chart speed.

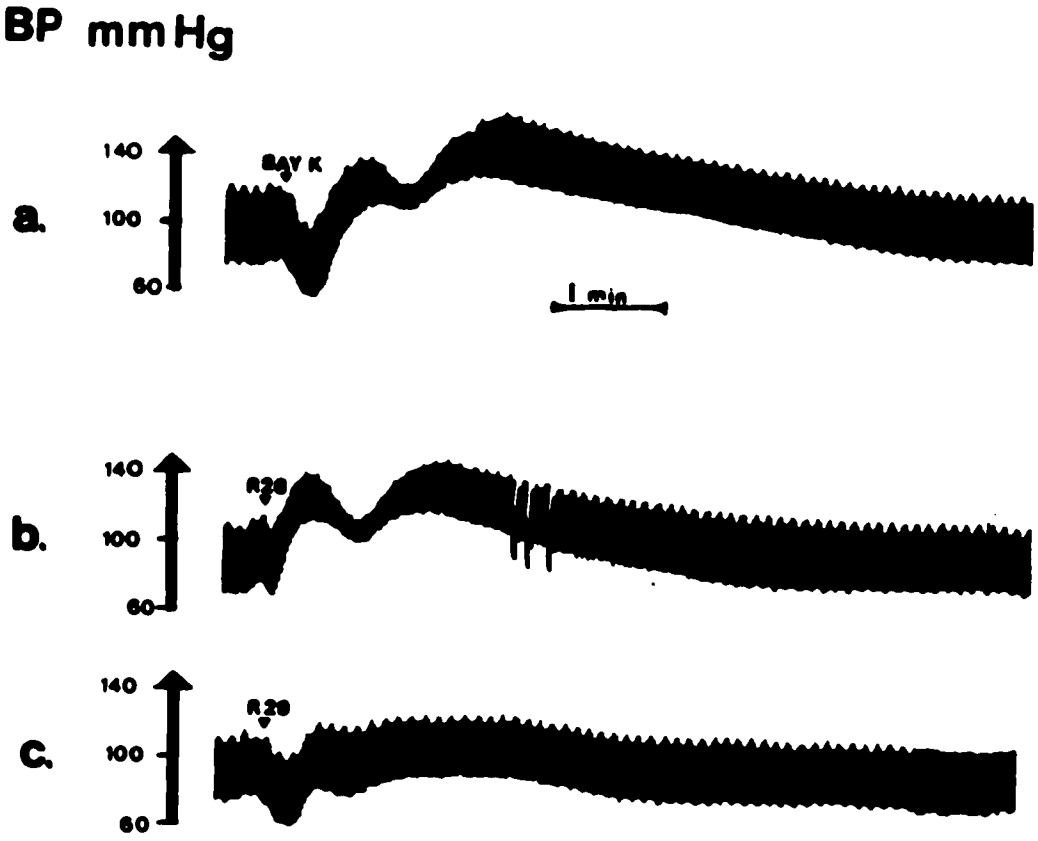


Figure 6.2 The hypertensive effects of a). Bay K 8644 - 30.0 ug/kg; b). Reek 28 - 300 ug/kg; and c). Reek 29 - 300 ug/kg; on rabbit aortic blood pressure, shown after β-blockade with propranolol 1.0 mg/kg.

increase in pulse pressure, cardiac output may have been increased through increased contractility. A decrease in arterial pressure (afterload), would lead to an increase in stroke volume, and therefore increase cardiac output. Unfortunately there were no means of measuring left ventricular pressure development to ascertain that the compound did increase contractility. As well, it is possible that the dose of compound injected was not sufficient to induce a positive inotropic effect and hence the effects seen were strictly those resulting from vasodilation.

The *in vivo* results therefore confirmed the smooth Ca^{2+} agonist effects of analogues possessing C-4 3- and 4-pyridyl substituents as observed *in vitro*. This finding ruled out C-4 3- and 4-pyridyl Bay K 8644 analogues as potential therapeutically useful cardiotonic agents, since they retain the inherent undesirable stimulatory actions of Bay K 8644 on smooth muscle.

The experiments also confirmed the pharmacodynamic contribution of the C-4 2-pyridyl substituent to smooth muscle Ca^{2+} antagonism, as manifested by the hypotensive response. Compounds with this general structure are therefore potential therapeutically useful cardiotonic/antihypertensive agents. Although the potency of Reek 30 *in vitro* was less than that of nifedipine by more than two orders of magnitude, the difference was less (one order of magnitude) *in vivo*. Reek 30 therefore appeared to be more effective *in vivo*.

6.3.2 *In vivo* effects of Reek 28, 29 and 30 on the conscious, restrained SHR rat.

In restrained, awake SHR rats the analogues again showed pharmacodynamic responses that were similar to those produced in the anaesthetised rabbit. Bay K 8644, Reek 28 and Reek 29 all increased blood pressure in the animals on i.p. injection. The parent compound, Bay K 8644, proved to be quite toxic, causing distress and agitation of the animals at lower concentrations. Two of three animals given Bay K 8644 (2.0 - 3.0 mg/kg i.p.) died within an hour of administration of the compound. Because of this toxicity, further experiments with Bay K 8644, Reek 28 and Reek 29 were not carried out.

Reek 30 on the other hand proved to be hypotensive in this animal model. Figure 6.4 shows the effects of Bay K 8644 and Reek 30 on SHR blood pressure. These effects were dose-dependent. These results confirmed the action of Reek 30 as a vasodilator with little effect on heart rate. These effects were qualitatively similar to those of nifedipine, but the compound was about an order of magnitude less potent than nifedipine.

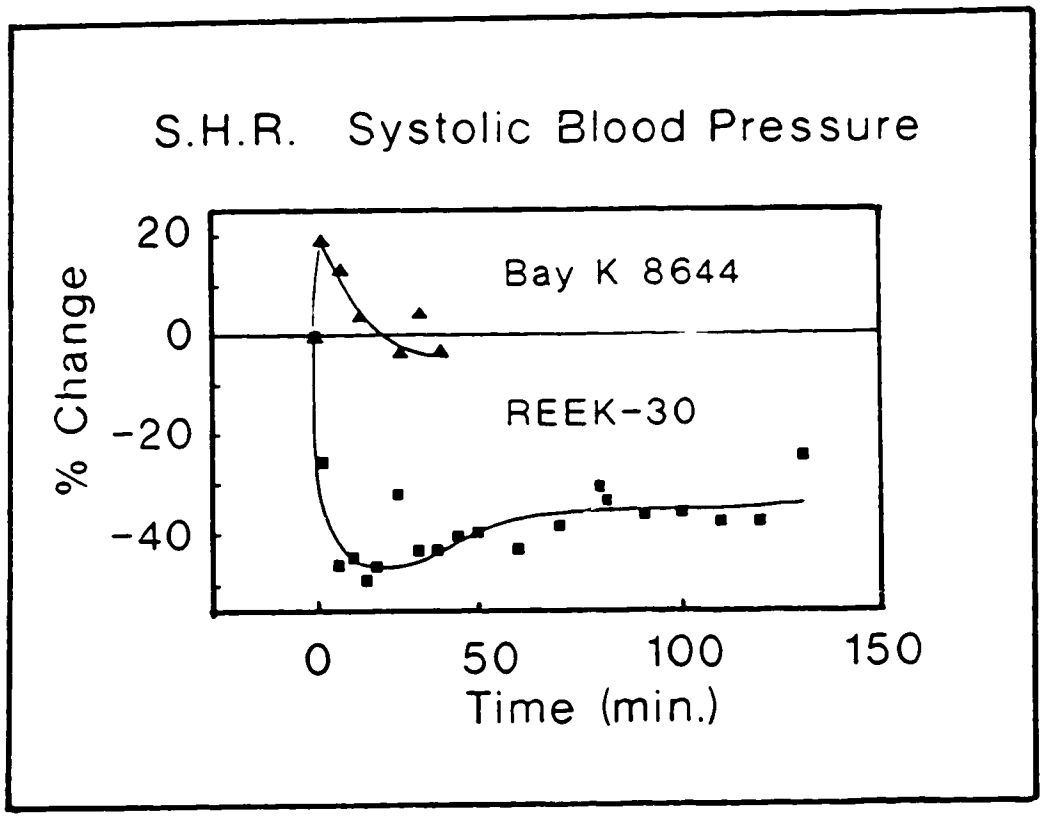


Figure 6.4 **The effects of Bay K 8644 (3.0 mg/kg) and Reek 30 (30 mg/kg) on SHR blood pressure *in vivo*.**

6.4 *In vivo* effects of the nifedipine analogue AK-2-38 in the anaethetised rabbit.

Only one analogue of nifedipine (AK-2-38) was tested *in vivo*. This compound was chosen because it was the first compound that demonstrated *in vitro* activity on GPILSM that was better than that of the parent compound, and was much more selective for smooth muscle than cardiac muscle *in vitro* (fig. 4.6 p90).

6.4.1 Effects of AK-2-38 on anaethetised rabbit.

In vivo, AK-2-38 produced dose-dependent hypotensive effects in the anaesthetised rabbit. The hypotensive effect was fairly long lasting, only recovering to control levels after a period of about twenty minutes at doses of up to 2.0×10^{-3} mg/kg. When the administered dose was greater than this, blood pressure remained decreased for longer than twenty minutes, with both intensity and duration of response being dose-dependent. Peak effects were observed at a dose of about 1.0 mg/kg. The compound did not affect the heart rate of the animal at doses of up to 1.0×10^{-1} mg/kg. At very high doses (> 1.0 mg/kg) the compound also decreased heart rate.

6.5 Discussion and Conclusions.

The results of *in vivo* testing of the novel compounds generally confirmed the findings of the *in vitro* studies. Analogues of Bay K 8644 were shown to possess the differential activities shown on

smooth muscle *in vitro*. The compounds possessing a C-4 3- or 4-pyridyl group exhibited hypertensive effects in the anaesthetised rabbit. Reek 30 on the other hand was shown to be an effective hypotensive agent. Its hypotensive effects were comparable to those of nifedipine and the difference in potency between the two compounds was reduced by about an order of magnitude, relative to that observed *in vitro*.

Unfortunately, there were no means for assessing the positive inotropic effect of the compounds *in vivo*. However, testing of compounds in the same category as Reek 30 should provide even better differential activity.

The compound AK-2-38 produced some very encouraging results in the anaesthetised rabbit, proving to be a potent hypotensive agent. However, preliminary testing in the SHR rat produced some conflicting results and further testing is required in order to assess its haemodynamic profile in the SHR rat.

VII. GENERAL DISCUSSION AND CONCLUSIONS.

7.1 Discussion and conlusions.

Close to one hundred and fifty novel analogues of nifedipine and Bay K 8644 were screened *in vitro* on either GPILSM or guinea pig left atrium in order to determine their Ca^{2+} channel modulator effects. Some of the analogues were screened on both tissues in order to discern differential or selective effects between the two tissues. As well, some experiments were done on the isolated rabbit bladder in order to ascertain the mode of action of some of the analogues.

All analogues of nifedipine were found to possess Ca^{2+} channel inhibitory effects on GPILSM. The IC_{50} values of the compounds on GPILSM ranged from of 10^{-5} to 10^{-10} M. Although the majority of these analogues did not have potency comparable to or better than that of the parent compound, screening them served to characterise their SARs, and resulted in confirmation of, as well as new additions to established SARs. A few of the analogues exhibited activity greater than that of nifedipine and therefore warrant further testing in order to establish their pharmacological profiles both *in vitro* and *in vivo*.

Substitution of the 2-nitrophenyl ring of nifedipine with several pyridyl and substituted pyridyl ring systems did not enhance

VII. GENERAL DISCUSSION AND

7.1 Discussion and conlusions.

Close to one hundred and fifty novel
Bay K 8644 were screened in vitro on
left atrium in order to determine th
effects. Some of the analogues were
order to discern differential or select
tissues. As well, some experiments were
bladder in order to ascertain the mo
analogues.

All analogues of nifedipine were fo

activity. However, when the C-4 substituent was a pyridyl ring, the order of potency was 2-pyridyl > 3-pyridyl > 4-pyridyl.

It is interesting to note that when the equivalent substituent of Bay K 8644, the 2-trifluoromethyl phenyl ring, was replaced with the same pyridyl rings, the order of potency (for Ca^{2+} agonist effects on guinea pig left atrium) was reversed to 4-pyridyl > 3-pyridyl > 2-pyridyl. However, the 2-pyridyl substituted Bay K analogues were all smooth muscle Ca^{2+} antagonists. The C-4 2-pyridyl substituent is therefore important for Ca^{2+} antagonist effects (i.e. promotes closing of the Ca^{2+} channel) whereas the DHP configuration resulting from a C-4 4-pyridyl substituent enhances Ca^{2+} channel opening.

The C-5 nitro substituent on the Bay K 8644 nucleus was found to be critical for producing Ca^{2+} channel activator effects but two nitro substituents (at both C-3 and C-5 positions) inhibit activity.

Generally, the pharmacophoric conformation of a ligand (the conformation that binds to the receptor), is influenced by several factors acting on the ligand-receptor interaction. Some of these factors are inherent in the physical nature of the compound (Zimmerman and Feldman, 1974), while others are dependent on the receptor and its immediate environment (Mathison et al., 1974; Zimmerman and Feldman, 1974; Goodman et al., 1980). Physical or structural features of the compound will determine factors such as lipophilicity, stereochemistry, flexibility of the bonds within the ligand molecule, and steric effects of different functional moieties of the molecule, while receptor-determined factors include location, accessibility of binding site, pH, perfusion, and distribution of

Several questions regarding the structural basis underlying the mechanism(s) of action of the novel analogues and DHPs in general 'beg' to be answered. The appropriate first question is "What differentiates between activator and antagonist effects in the DHP nucleus?" The fact that distinct functional moieties on the DHP nucleus impart either activator or inhibitory properties to a particular molecule, (i.e. nifedipine analogues are antagonists whereas Bay K 8644 analogues are agonists on cardiac tissues), indicate that structure is a critical determinant of effect produced by that molecule. This suggests that there are specific structural requirements for the production of activator or antagonist effects.

Two points support this hypothesis. First, most, if not all 1,4-DHP Ca^{2+} antagonists in clinical use, (i.e. compounds with optimal pharmacological profiles for use in cardiovascular disease) are C-3/C-5 diesters. Asymmetric diester analogues tested for this thesis were also the most potent antagonists when compared with other analogues on GPILSM. Second, all 1,4-DHP Ca^{2+} agonists possess one ester group at either C-3 or C-5, but not both.

Simple structural differences however, cannot sufficiently explain the activator and inhibitory effects exhibited by nifedipine and Bay K 8644. Langs and Triggle (1985) demonstrated that 1,4-DHP agonists and antagonists share several conformational features, so that the overall conformation of the agonist and antagonist molecules is very similar but is not identical. In an examination of the crystal structures of Bay K 8644, CGP 28 392 and other DHP compounds using X-ray diffraction analysis, they reported that 1,4-DHP Ca^{2+} antagonists and agonists adopt a similar conformation

where the C-4 ring substituent is perpendicularly oriented in a plane that bisects the plane of the 1,4-DHP ring.

Although many compounds among both agonist and antagonist 1,4-DHPs are enantiomers, full activator effects are only produced by compounds that are not C-3/C-5 diesters. Known descriptors of agonist activity include possession of a lactone, nitro, amide or hydrogen substituent in place of one of the esters of the 1,4-DHP antagonist ring structure (Schramm et al., 1983; Truog et al., 1984; Kongsamut et al., 1985; Gjorstrup et al., 1986; Triggle, 1987). Furthermore, structural differences do not account for the effects of the enantiomers of Bay K 8644 or 202 791, where activator effects reside in only one enantiomer, while the other is an antagonist. If enantiomers of DHP agonist compounds are sufficiently different either in conformation, or in their interaction with the 1,4-DHP binding site of the VSC, a further question arises - "Why do the enantiomers of DHP Ca^{2+} antagonist compounds not exhibit the same dualistic activities demonstrated by the DHP agonist compounds?". Enantiomers of nifedipine-like compounds (i.e. possessing a chiral centre at position C4) differ in their potencies, (-)-isomers being more potent than (+)-isomers (Towart et al., 1981), but the activity is in the same direction (i.e. inhibition of channel opening).

Towart and coworkers suggested that the orientation of the ester substituent, as well as hydrogen bonding by the amine group of the 1,4-DHP ring may be determinants of agonist/antagonist activity in these compounds.

Bonding of the ligand molecule to its complementary site on the VSC, also plays a crucial part in determining the strength of the

where the C-4 ring substituent is p
plane that bisects the plane of the 1,4-D

Although many compounds among bo
1,4-DHPs are enantiomers, full activat
by compounds that are not C-3/C-5 die
agonist activity include possession o
hydrogen substituent in place of one
antagonist ring structure (Schramm et al
Kongsamut et al., 1985; Gjorstrup et
Furthermore, structural differences do
of the enantiomers of Bay K 8644 or 202
reside in only one enantiomer, while the
enantiomers of DHP agonist compounds
either in conformation, or in their
binding site of the VSC, a further q

ligand-receptor interaction, as well as the final conformation of the VSC, to result in promotion or inhibition of channel opening.

The types of bonds that are important for ligand-receptor interactions include hydrogen bonds, dipole-dipole attraction forces, ion dipole, ionic and van der Waals' forces (Zimmerman et al., 1974). These bonds are important because they do not form permanent linkages between the ligand and the receptor, and can be influenced by several factors such as the environment of the ligand-receptor interaction. This allows for variable degrees of both strength and rate of ligand-receptor bonding, resulting in a dynamic interaction which can allow for subtle, but pharmacologically important different effects of the ligand on the receptor.

Ligand-receptor bonding interaction alone could explain differences between enantiomers, where one enantiomer could have the 'perfect' fit, therefore resulting in a potent effect; the other enantiomer would not fit the receptor site as well, resulting in reduced potency, as described for certain antagonist enantiomeric pairs (Towart et al., 1981).

A ligand should be able to assume a conformation resulting in an electronic pattern complementary to that of the receptor (Marshall and Cramer (1988). Different substituents on the 1,4-DHP ring may present similar but none-the-less distinctly different electronic patterns to the receptor, depending on the functional moieties comprising the substituents. How well these substituents complement the receptor site(s) may then lead to subtle changes in binding patterns. The resultant pharmacophore could be sufficiently altered under different conditions (e. g. at hyperpolarised vs partly

characterized by periods when the channel was unavailable and so there was no current observed. Ca^{2+} influx can therefore be assumed to occur in mode 1 and 2 only, and not mode 0. Hess and coworkers then proposed that DHP compounds tend to promote one or more modes of gating of the channel. They suggested that pure agonist effects result when mode 2 of channel gating is specifically favoured, and pure antagonist effects would arise when a ligand preferentially stabilised mode 0. This does not however explain why agonist compounds behave as activators up to a point, then become inhibit the response.

This explanation also suggested that structural differences between agonist and antagonist DHPs could lead to agonist or antagonist effects respectively, but it did not explain the different effects produced by enantiomers of Bay K 8644 and other agonist compounds.

Maan and Hosey (1987), suggested that both agonist and antagonist 1,4-DHPs bind to the same affinity state of the receptor. If that is the case then activator or inhibitory effects would be inherent in the nature of the molecule, and not in the molecule-receptor interaction, such that agonist molecules would promote transition of the channel to mode 2, and antagonist molecules would promote transition to mode 0. Although plausible, this explanation also does not explain the effects produced by agonist enantiomers.

Several investigators have examined the voltage- and use-dependence of 1,4-DHP compounds (Hess et al., 1984, Thomas et al., 1984; Williams et al., 1985; Kokubun et al., 1986; Kamp et al.,

1989). Voltage-dependence of antagonist compounds has shown that these compounds are more effective when the cell is relatively depolarised. Perhaps the most significant finding of all these studies was that of Kamp and coworkers (1987), which showed that the pure 'agonist' enantiomer (+)-202 791 exerted its greatest 'agonist' effects at hyperpolarised potentials, and when the cell was maintained at depolarised potentials the enantiomer blocked peak Ba^{2+} current (Ba^{2+} is a permeant ion through VSCs).

This observation could partially explain the effects of DHP agonist compounds which demonstrate a biphasic profile producing agonist effects at lower concentrations and antagonist effects at higher concentrations respectively.

One hypothesis proposed to explain the action of DHP antagonists suggested that repeated depolarizing pulses cause VSCs to cycle between the different states of channel gating. If affinity of a compound was much higher for inactivated channels (i.e. mode 0), significant binding would occur during depolarizing steps, in spite of the fact that little drug was bound under resting conditions (i.e. mode 1). Such a process would produce use- or frequency-dependence of compounds, where if the interval between sequential depolarizing voltage pulses occurred faster than the time it took for the drug to dissociate from the receptor, block of the channel would increase and persist. This effect would therefore be related to the drug-receptor interaction, duration of the stimulus pulse and the time between successive pulses. This explanation could suffice to account for the action of antagonists, but it does not explain the agonist action of R(+)-202 791 or S(-) Bay K 8644.

Several investigators have proposed models of VSCs containing two DHP binding sites (Thomas et al., 1984; Kokubun et al., 1986). The results of experiments by Kokubun and coworkers (1986), provided evidence for a second functional DHP site. They reported that the DHP antagonist compound ^{3}H-PN 200 110, enhanced binding of (+)-202 791 under hyperpolarized potentials. They also demonstrated evidence for positive cooperativity of the enantiomers of 202 791 on single channel activity of VSCs, leading to their postulation that there existed two distinct DHP binding sites, an activator site which could be preferentially bound by the 'agonist' enantiomer (+)-202 791, and an inhibitory site which could be preferentially bound by the 'agonist' enantiomer (+)-202 791. Their results were however contradicted by the results of Kamp and coworkers (1987), which did not detect cooperative interactions between the enantiomers of 202 791.

Several lines of evidence point to the existence of sub-populations of VSCs (Nowicky et al., 1985; Nilius et al., 1985; Smith et al., 1985). Biochemical, pharmacological and electrophysiological studies all indicate differences between VSCs of different tissues. For example, Tsien and coworkers (1987) reported that neuronal L-type VSCs were blocked by omega conotoxin, but this substance had no discernible effect on cardiac L-type VSCs. As well, β-adrenoceptor stimulated c-AMP-mediated phosphorylation has been reported to occur in cardiac L-type VSCs, leading to an increase in the number of available channels, and probability of channel opening (Bean et al., 1984; Nilius, et al., 1985). Norepinephrine however, was reported to inhibit Ca^{2+}

currents of neuronal VSCs (Dunlap and Fischbach, 1981). This information is also indicative of differences in the L-type VSCs of the two tissues.

Van Amsterdam and coworkers (1987), demonstrated differences in the rates of accessibility and recovery between a vascular and a myocardial Ca^{2+} channel to a series of Ca^{2+} antagonists. They showed that the vascular channel site of action was rapidly accessible to all drugs studied, whereas the myocardial site was not. They proposed that the myocardial site probably had a more hydrophobic character, and was consequently less accessible (i.e. intramebranous or intracellular.

Some of my work has also pointed to the possible existence of subtypes of VSCs. The effects of C-4 2-pyridyl analogues of Bay K 8644 on GPILSM and left atrium are strongly suggestive of differences between the two tissues which do not merely arise from potential differences of the two tissues. This is supported by the fact that although analogues were used as enantiomeric mixtures, C-4 3- and 4-pyridyl analogues did not show the same profiles as C-4 2-pyridyl analogues, exhibiting only agonist effects on both tissues.

The interaction of 1,4-DHP compounds with their receptor on the Ca^{2+} channel is complex and depends on many factors. The nature of the ligand molecule, as well as the molecule-receptor interaction and the conditions under which binding takes place all influence the outcome, (i.e. channel activation or inhibition). A dynamic equilibrium must therefore be achieved between all these factors, to produce either an agonist or antagonist effect. The equilibrium has

to be dynamic because different compounds are able to shift it toward either direction, resulting in one effect or the other as exhibited by most DHPs under varying conditions of membrane potential, stimulus rate and concentration of the 1,4-DHP compound.

Depolarizing stimuli preferentially mobilize extracellular Ca^{2+} (Van Breemen et al., 1986a; Van Breemen et al., 1986b), which, because of its location and pathway into the cell may lead to specific events (Rasmussen and Barrett, 1984; Rasmussen, 1986a). This Ca^{2+} enters the cells through VSCs, which are opened on membrane depolarisation. The ensuing cascade of cellular responses activated by Ca^{2+} from this pool may be different from that arising from receptor activation, which mobilizes Ca^{2+} from extracellular sources as well as intracellularly stores, depending on the tissue. The mode of mobilization may thus lead to differences in the nature of the response, depending on the source of the activator Ca^{2+} and the nature of the stimulus.

Modifications made to the structure of nifedipine produced compounds that selectively inhibited DIC of GPILSM (i.e. Reek 50 and its congeners). This selective inhibition of DIC indicated that these compounds differentiated between two pathways of Ca^{2+} mobilisation, and hence suggested that there were subsclasses of the Ca^{2+} channel within GPILSM. The first pathway would be a receptor-operated channel, which is relatively insensitive to the effect of 1,4-DHP Ca^{2+} antagonists, and the second being the voltage-sensitive Ca^{2+} channel, and is sensitive to 1,4-DHPs. Godfraind and coworkers (1986) have given a detailed review of the different sources of activator Ca^{2+} and their modes of

mobilisation. Since these compounds were able to distinguish between the two mobilisation processes this strongly suggested that there exist subtypes of the channel with differential sensitivities to the compounds. In Ca^{2+}-free HPSS Reek 50 was able to inhibit DIC to a greater extent and more permanently than ROC mediated contraction (fig. 7.1). If depolarisation and receptor activation mobilise Ca^{2+} differently, leading to Ca^{2+} influx into the cell through the same Ca^{2+} channels (VSCs), compounds such as Reek 50 would not be able to differentiate between the two modes since they presumably act on the channel itself and not on the activation process. In light of earlier findings by other investigators (Meisheri et al., 1981; Haeusler, 1985) which confirmed the existence of two separate Ca^{2+} pathways in smooth muscle, corresponding to a depolarisation-activated pathway and a receptor-mediated pathway, it is highly plausible to propose that compounds differentiating between the two mobilisation processes act on different Ca^{2+} channels.

Since the 1,4-DHPs can be shown to be pharmacologically different in spite of their close structural similarities, it is possible that different compounds may exert other effects on different cells, depending on the effectors of the responses elicited.

Scott and Dolphin (1988) have demonstrated that Bay K 8644 promotes neuronal L-type Ca^{2+} currents through a pertussis toxin-sensitive G-protein activation. They suggest that in the absence of association with this G protein, antagonist ligand actions are promoted; and if that is the case, the action of the

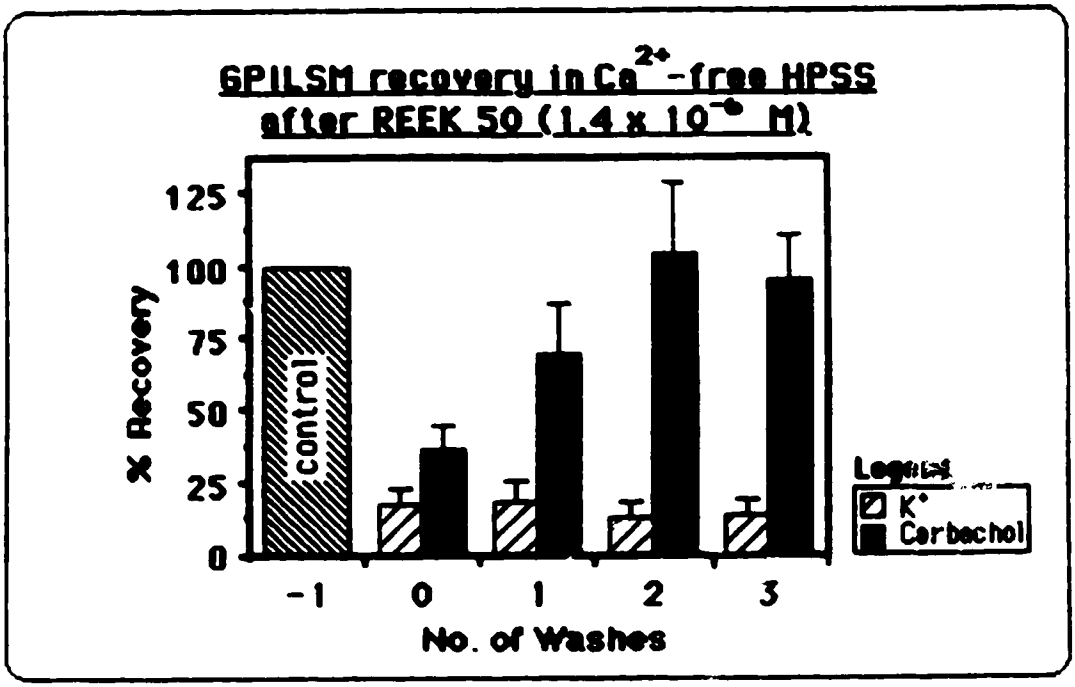


Figure 7.1 Differential effects of Reek 50 on DIC (K^+) and ROC-mediated (carbachol) contraction of GPILSM in Ca^{2+}-free HPSS.

activated channel subunit of the G-protein rather than a direct ligand effect, results in the observed response. Such an effector status (i.e. presence or absence of the G-protein) could account for the difference between GPILSM and cardiac tissues responding differently to the same ligand (C-4 2-pyridyl analogues of Bay K 8644). However, it is not sufficient to explain the fact that the positional isomers (C-4 4-pyridyl vs C-4 2-pyridyl analogues) produce different effects on GPILSM.

Further support for differences in effectors are given by Ennis and coworkers (1989), who demonstrated that the 1,4-DHP compound Wy 27569 is a combined Ca^{2+} channel blocker and thromboxane synthetase inhibitor; and the studies of Marsh and coworkers (1988), who showed that the dihydropyridine compound RS93522 possesses phosphodiesterase inhibitory activity, in addition to calcium antagonist effects on cultured myocytes.

It has been suggested that some of the effects of 1,4-DHPs may be centrally mediated (Pillai and Roth, 1986), since some of these compounds can penetrate the blood brain barrier. The haemodynamic and toxic effects observed <u>in vivo</u> may therefore be a mixture of direct effects of the compounds on peripheral target tissues, as well as indirect effects mediated centrally by the compounds. The agitation and hypertension displayed by SHR rats after the administration of Bay K 8644 and some of its analogues, suggest both central and peripheral effects of the compounds.

The general conclusion from these studies is that different functional moieties substituted onto the 1,4-DHP nucleus have a greater or lesser effect on pharmacological activity and potency.

The different functional groups interact to influence the pharmacophoric conformation of the molecule, and hence the molecule-receptor interaction, resulting in a favourable or unfavourable binding. The interaction is also influenced by tissue factors such as resting membrane potential and the nature of the stimulus (agonist) responsible for mobilizing Ca^{2+}.

7.2 Future considerations.

Although many novel analogues were tested, synthesis of more analogues in these series and new ones continues. There are therefore more analogues still to be tested and results from the testing of these compounds will provide answers to some of the unanswered questions raised in this thesis.

Several aspects of the thesis still require further investigation. Selectivity effects of some of the Bay K 8644 analogues still need to be examined. A model of cardiac failure could be used to study the positive inotropic effect of Bay K 8644 analogues. Such a study would establish whether these agents are really useful as positive inotropic agents. They could be compared and contrasted with cardiac glycosides and β-adrenoceptor agonists in order to assess their usefulness. As well, some of the more potent analogues of nifedipine need to be tested on guinea pig left atrium as well as in vivo.

Resolution of enantiomers of some of the analogues tested, particularly the C-4 2-pyridyl analogues of Bay K 8644 should receive special attention, as this could lead to resolution of the question of the existence of distinct subtypes of the VSC. It would also be a very useful exercise to study the electrophysiological effects of some of the compounds, in order to determine definitively the mode of action of the compounds.

As well, there still remains the task of further compilation of SARs of this interesting group of compounds.

BIBLIOGRAPHY

1. ABDEL-LATIF, A. A. Calcium-mobilising Receptors, Polyphosphoinositides, and the Generation of Second Messengers. Pharmacol. Rev. 38:227-271 (1986).

2. ADVENIER, C., NALINE E., RENIER, A. Effects of Bay K 8644 on contraction of the human isolated bronchus and guinea-pig isolated trachea. Brit. J. Pharmacol. 88(1):33-39 (1986)

3. AGNER, E., REHLING, M., TRAP-JENSEN, J. Haemodynamic effects of single-dose oral felodipine in normal man. Drugs 29(Supp. 2):36-40 (1985).

4. ARROWSMITH, J. E., CAMPBELL, S. F., CROSS, P. E., STUBBS, J. K., BURGES, R. A., GARDINER, D. G., BLACKBURN, K. J. long-acting dihydropyridine calcium antagonists. 1. 2-alkoxymethyl derivatives incorporating basic substituents. J. Med. Chem. 29:1696-1702 (1986).

5. BAKER, P. F. The sodium-calcium exchange system. In Calcium and the cell. Evered, D. and Whelan, J. (Eds.) Cib Foundation Symposia 122. John Wiley and Sons, Chichester, U.K. (1986).

6. BAKER, J. R. J., HAIGH, R. J., WARBURTON, G., WESTON, A. H., WILLIAMSON, I. H. M. The Measurement of Drug-Induced Calcium Changes in Vascular Smooth Muscle Using X-Ray Microanalysis of Pyroantimonate Precipitates. J. Pharmacol. Methods 18:55-67 (1987).

7. BEAN, B. P., NOWICKY, M. C., TSIEN, R. W. Beta-adrenergic modulation of calcium channels in frog heart cells. Nature 307:371-372 (1984).

8. BERRIDGE, M. J. Inositol Triphosphate and Diacylglycerol: Two interacting second messengers. Ann. Rev. Biochem. 56:158-193 (1987).

9. BIANCHI, C. P. Cell Calcium. Butterworth and Company, London, (1968).

10. BLAUSTEIN, M., NELSON, M. T. Sodium-Calcium Exchange: Its Role in the Regulation of Cell Calcium. In: Membrane Transport of Calcium. Carafoli, E. (Ed). Academic Press Inc., London, (1982).

11. BECHEM, M., HEBISCH, S., SCHRAMM, M. Ca^{2+} agonists: new, sensitive probes for Ca^{2+} channels. Trends in Pharmacol. Sci. 9:257-261 (1988).

12. BOHR, D. F., WEBB, R. C. Physiological mechanisms regulating peripheral vascular resistance. in Handbook of Hypertension Vol. 7 : Pathophysiology of hypertension. Zanchetti, A. and Tarazi, R.C. (eds). Elsevier Science Publishing Co. Inc., New York, N.Y. (1986).

13. BOLTON, T., AARONSON, P., MACKENZIE, I. Voltage-dependent calcium channel in intestinal and vascular smooth muscle cells. Ann. N. Y. Acad. Sci. 552:32-42 (1988).

14. BORG, E., VIBERG, A. Role of heating in nonivasive blood pressure measurements in rats. Acta. Physiol. Scand. 108:73-75 (1980).

15. CAUVIN, C., LOUTZENHISER, R., VAN BREEMEN, C. Mechanisms of calcium antagonist-induced vasodilation. Ann. Rev. Pharmacol. Toxicol. 23:373-396. (1983)

16. CHAMBERS, R., REZNIKOFF, P. Micrurgical Studies in Cell Physiology; 1. The action of Na, K, Ca, and Mg on the protoplasm of Amoeba proteus. J. Gen. Physiol. 8:396 (1926)

17. CHEUNG, J. Y., BONVENTRE, J. V., MARTS, C. D., LEAF, A. Calcium and Ischemic Injury. New Eng. J Med. 314#26:1670-6 (1986).

18. CHIU, A. T., BOZARTH, J. M., TIMMERMANS, P. B. Relationship between Phosphatidylinositol Turnover and Ca^{2+} Mobilization Induced by Alpha-1 Adrenoceptor Stimulation in the Rat Aorta. J. Pharmacol. Exp. Ther. 240:#1 123-7 (1987).

19. CREED, K.E. Membrane properties of the smooth muscle of the guinea pig urinary bladder. Pflug. Arch. 326:115-26 (1971).

20. COLVIN, R.A., ASHAVAID, T.F., HERBETTE, L.G. Structure-function studies of canine cardiac sarcolemmal membranes: Estimation of receptor site densities. Biochim. Biophys. Acta 807:601-608 (1985).

21. DAGNINO, L., LI-KWONG-KEN, M. C., WOLOWYK, M. W, WYNN, H., TRIGGLE, C. R., KNAUS, E. E. Synthesis and calcium channel antagonist activity of dialkyl 1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3,5-pyridinedicarboxylates. J. Med. Chem. 29: 2524-2529 (1986).

22. DAGNINO, L., LI-KWONG-KEN, M. C., WYNN, H., WOLOWYK, M. W, TRIGGLE, C. R., KNAUS, E. E. Synthesis and calcium channel antagonist activity of dialkyl 4-(dihydropyridinyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates. J. Med. Chem. 29: 640-646 (1987).

23. DAGNINO, L., LI-KWONG-KEN, M. C., WOLOWYK, M. W, TRIGGLE, C. R., KNAUS, E. E. Synthesis and calcium channel

antagonist activity of dialkyl hexahydro-1,2',6'-trimethyl[bipyridine]-3',5'-dicarboxylates. Eur. J. Med. Chem. 22: 499-503 (1987).

24. DORMANDY, J. Clinical Use of Calcium Antagonists in Peripheral Circulatory Disease. Ann. N. Y. Acad Sci. 522:611-20 (1988).

25. EBASHI, S. Calcium in the Heart. in The Calcium Antagonists - The Fleckenstein Symposium. J. Mol. Cell. Cardiol. 19(Supp II):1-18 (1987).

26. ENNIS, C., GRANGER, S. E., MIDDLEFELL, V. C., PHILPOT, M. E., SHEPPERSON, N. B. Pharmacologic effects of Wy 27569: a combined calcium channel blocker and thromboxane synthetase inhibitor. J. Cardiovasc. Pharmacol. 13:511-519 (1989).

27. FATT, P., KATZ, B. The electrical properties of crustacean muscle fibres. J. Physiol. 120:171-204 (1953).

28. FATT, P., GINSBORG, B. L. The ionic requirements for the production of action potentials in crustacean muscle fibres. J. Physiol. 142:516-543 (1958).

29. FLAMENG, W. Myocardial Protection. Ann. N. Y. Acad Sci. 522:600-10 (1988).

30. FLECKENSTEIN, A. Calcium antagonism in heart and smooth muscle: Experimental facts and therapeutic prospects. John Wiley and Sons Inc., (1983a).

31. FLECKENSTEIN, A. History of calcium antagonists. Circ. Res. 52#1:3-16 (1983b).

32. FOSSHEIM, R., SVARTENG, K., MOSTAD, A., ROMMING, C., SHEFTER, E., TRIGGLE, D. J. Crystal structures and pharmacological activity of calcium channel antagonists: 2,6-dimethyl-3,5-dicarbomethoxy-4-(unsubstituted, 3-methyl-4-methyl-, 3-nitro-, 4-nitro-, and 2,4-dinitrophenyl)-1,4-dihydropyridine. J. Med. Chem. 25:126-131 (1982).

33. FOSSHEIM, R. Crystal structure of the dihydropyridine Ca^{2+} antagonist felodipine. Dihydropyridine binding prerequisites assessed from crystalographic data. J. Med. Chem. 29:305-307 (1986).

34. FOSSET, M., LAZDUNSKI, M. Biochemical characterisation of the skeletal muscle Ca^{2+} Channel. In Structure and Physiology of the Slow Inward Calcium Channel. J. C. Venter and D. Triggle (Eds.) Alan R. Liss, Inc. New York, (1987).

35. FOVAEUS, M., ANDERSSON, K. E., BATRA, S., SJOGREN, C. Effects of Calcium, Calcium Channel Blockers and Bay K 8644 on Contractions Induced by Muscarinic Receptor Stimulation

of Isolated Bladder Muscle from Rabbit and Man. J. Urol. 137: 798-803 (1987)

36. FRANCKOWIAK, G., GROSSER, R., THOMAS, G., SCHRAMM, M., GROSS, R. Mixtures of optically active nitrodihydropyridines active on the circulatory system. United States Patent - Patent Number: 4,764,516 (1988).

37. FROHLICH, E. D. Pathophysiology of hypertension: Effects of calcium antagonists on heart and kidney. J. Cardiovasc. Pharmacol. 13(Supp. 1):S11-S17 (1989).

38. GALIZZI, J. P., FOSSET, M., LAZDUNSKI, M. Properties of receptors for the Ca^{2+} channel blocker Verapamil in transverse-tubule membranes of skeletal muscle: stereospecificity, effect of Ca^{2+} and other inorganic cations, evidence for two categories of sites and effect of nucleoside triphosphates. Eur. J. Biochem. 144:211-15 (1984).

39. GARFIELD, R. E., SOMLYO, A. P. Structure of smooth muscle. in Calcium and Contractility. A. K. Grover and E. E. Daniel (Eds). Humana Press Inc., (1985).

40. GJORSTRUP, P., HARDING, H., ISAKSSON, R., WESTERLUND, C. The enntiomers of the dihydropyridine derivative H 160/51 show opposite effects of stimutlation and inhibition. Eur. J. Pharmacol. 122:357-361 (1986).

41. GLOSSMAN, H., FERRY, D.R., GOLL, A., ROMBUSCH, M. Molecular Pharmacology of the Calcium Channel: Evidence for subtypes, multiple drug-receptor sites, channel subunits, and the development of a radioiodinated 1,4-Dihydropyridine calcium channel label, [^{125}I]Iodipine. J. Cardiovasc. Pharmacol. 6:S608-S621 (1984).

42. GODFRAIND, T., KABA, A. Blockade or reversal of the contraction induced by calcium and adrenaline in depolarized arterial smooth muscle. Br. J. Pharmacol. 36:549-560 (1969).

43. GODFRAIND, T. Mechanism of action of calcium entry blockers, Federation Proc. 40:2866-2871 (1982).

44. GODFRAIND, T., MILLER, R. C. Specificity of action of Ca entry blockers, a comparison of their actions in rat arteries and in human coronary arteries. Circ. Res. 52(Supp. 1):181-191 (1983).

45. GODFRAIND, T. Actions of nifedipine on Ca fluxes and contraction in isolated arteries. J. Pharmacol. Exp. Ther. 224:443-450 (1983).

46. GODFRAIND, T., MILLER, R., WIBO, M. Calcium antagonism and

calcium entry blockade. Pharmacol. Rev. 38#4:321-416 (1986).

47. GOLL, A., FERRY, D. R., GLOSSMAN, H. Target Size Analysis and Molecular Properties of Ca^{2+} Channels Labelled with [^{3}H]verapamil. Eur. J. Biochem. 141:177-86 (1984).

48. GOODMAN, F. R., WEISS, G. B., KARAKI, K., NAKAGAWA, H. Differential calcium movements induced by agonists in guinea-pig tracheal muscle. Eur. J. Pharmacol. 133:111-117(1987).

49. GOODMAN, A. G., MAYER, S.E., MELMON, K. L. Pharmacodynamics: Mechanisms of drug action and the relationship between drug concentration and effect. in The pharmacological basis of therapeutics. A. G. Gilman, L. S. Goodman and A. Gilman (Eds.) 6th edition MacMillan Publishing Co. Inc., (1980).

50. GRAHAM, J. E., SICHEL, F. J. M. Response of frog striated muscle to $CaCl_2$. Biol. Bull. 77:332 (1939).

51. HALLAM, T. J., RINK, T. J. Receptor-mediated Ca^{2+} entry: Diversity of function and mechanism. TIPS 10:8-10 (1989).

52. HAEUSLER, G. Contraction of vascular muscle as related to membrane potential and calcium fluxes. J. Cardiovasc. Pharmacol. 7#6:s3-s8 (1985).

53. HAGIWARA, S. Membrane Potential-Dependent Ion Channels in Cell Membrane. Phylogenetic and developmental approaches. Raven Press, New York (1983).

54. HALL, W. D. Hypertension in Blacks. In Hypertension Management: Clinical Practice and Therapeutic Dilemmas. Wollam, G. L. and Hall, W. D. (Eds.) Year Book Medical Publishers Inc., (1988)

55. HEILBRUNN, L. V. The action of calcium on protoplasm. Physiol. Zool. 13:88-94 (1940).

56. HENRY P. D. Calcium Antagonists as Antiatherogenic Agents. Ann. N. Y. Acad. Sci. 522:411-419 (1988).

57. HERMSMEYER, K. Vascular Muscle Membrane Cation Mechanisms and Total Peripheral Resistance. Hypertension 10#5:I20-I22 (1987).

58. HESS, P., LANSMAN, J., TSIEN, W. Different modes of Ca channel gating favoured by dihydropyridine Ca agonists and antagonists. Nature 311:538-544 (1984).

59. HESS, P. Elementary properties of cardiac calcium channels: a brief review. Can. J. Physiol. Pharmacol. 66:1218-1223 (1988).

60. HOF R.P., VUORELA, H.J. Assessing calcium antagonism on vascular smooth muscle: A comparison of three methods. J. Pharmacol. Methods. 9:41-52 (1983).

61. HOF, R. P., RUEGG, U. T., HOF, A., VOGEL, A. Stereo selectivity at the calcium channel: Opposite action of the enantiomers of a 1,4-dihydropyridine. J. Cardiovasc. Pharmacol. 7#4:689-693 (1985).

62. HOLLAND, D. R., WIKEL, J. H., SMALLWOOD, J. K., ZIMMERMAN, K., UTTERBACK, B. G., STEINBERG, M. I., KAUFFMAN, R, F. LY249933: A positive inotropic 1,4-dihydropyridine lacking vascular effects. APS/ASPET ABSTRACTS Cardiotonic drugs:33.1 (1988).

63. HOWLETT, S.E., GORDON, T. ^{3}H-Nitrendipine Binding to Hamster Cardiac Muscle Homogenate. Proc. West. Pharmacol. Soc. 29:485-488 (1986).

64. HWANG, K.S., VAN BREEMEN, C. Effects of the Ca^{++} Agonist Bay K 8644 on ^{45}Ca Influx and Net Ca^{++} uptake into rabbit aortic Smooth Muscle. Eur. J. Pharmacol. 116:299-305 (1985).

65. HURWITZ, L., MCGUFFEE, L. J., LITTLE, S. A., BLUMBERG, H. Evidence for two distinct types of potassium-activated calcium channels in an intestinal smooth muscle.

66. IVES, N., KING, J.W., CHERNOW, B., ROTH, B.L. Bay K 8644, a Calcium Channel Agonist, Reverses Hypotension in Endotoxin-shocked Rats. Eur. J. Pharmacol. 130(3):169-175 (1986).

67. JANIS, R. A., TRIGGLE, D. J. New developments in Ca^{2+} antagonists. J. Med. Chem. 26:775-785 (1983).

68. JOHNS, A., LEIJTEN, P., YAMAMOTO, H., HWANG, K., VAN BREEMEN, C. Calcium Regulation on Vascular Smooth Muscle Contractility. Am. J. Cardiol. 59:18A-23A (1987).

69. JOHNSON, G. J., DUNLOP, P. C., LEIS, L. A., FROM, A. H. L. Dihydropyridine agonist Bay K 8644 inhibits platelet activation by competitive antagonism of thromboxane A_2-Prostaglandin H_2 Receptor. Circ. Res. 62:494-505 (1988).

70. KAMP, T. J., SANGUINETTI, M. C., MILLER, R. J. Voltage- and use-dependent modulation of cardiac calcium channels by the dihydropyridine (+)-202-791. Circ. Res. 64#2:338-351 (1989).

[illegible], W. P. Role of blood pressure in cardiovascular morbidity and mortality. Prog. Cardiovasc. Dis. 17:5-24 (1974).

72. KANNEL, W. B. Risk factors in hypertension. J. Cardiovasc. Pharmacol. 13(Supp. 1):S4-S10 (1989).

73. KANNEL, W. B., GORDON, T., SCHWARTZ, M. J. Systolic versus diastolic blood pressure and risk of coronary heart disease: The Framingham Study. Am. J. Cardiol. 27:335-346 (1971).

74. KARAKI, H. Use of Tension measurements to delineate the mode of action of vasodilators. J. Pharmacol. Methods 18:1-21 (1987)

75. KOKUBUN, S., PROD'HOM, B., BECKER, C., PORZIG, H., REUTER, H. Studies on Ca channels in intact cardiac cells: Voltage-dependent effects and cooperative interaction of dihydropyridine enantiomers. Mol. Pharmacol. 32:571-584 (1986).

76. KONGSAMUT, S., KAMP, T. J., MILLER, R. J., SANGUINETTI, M. C. Calcium channel agonist and antagonist effects of the stereoisomer of the dihydropyridine 202-791. Biochem. Biophys. Res. Comm. 130:141-148 (1985).

77. LARAGH, J. H., BUHLER, F. R., KHOBANIAN, A., HANSSON, L., ZACHARIAH, P. K. Calcium antagonists in hypertension: Update 1986. J. Cardiovasc. Pharmacol. 9(Supp. 4):S1-S3 (1987).

78. LI-KWONG-KEN, M.C. Pharmacological evaluation of novel calcium channel antagonists and agonists. Master's degree thesis. University of Alberta, Edmonton, Alberta, Canada. (1986).

79. LOEV, B., GOODMAN, M. M., SNADER, K. M., TEDESCHE, R., MACKO, E. "Hantzsch-type" dihydropyridine hypotensive agents. J. Med. Chem. 17:956-65. (1974).

80. LOUTZENHISER, R., RUEGG, U., HOF, A., HOF, R. P. Studies on the mechanism of action of the vasoconstrictive dihydropyridine CGP 28 392. Eur. J. Pharmacol. 105:229-237 (1984).

81. LOUTZENHISER, R., LEYTEN, P., SAIDA, K., VAN BREEMEN, C. Calcium compartments and mobilisation during contraction of smooth muscle. in Calcium and Contractility. A. K. Grover and E. E. Daniel (Eds). Humana Press Inc., (1985).

82. LOWRY, O. H., ROSEBROUGH, N. J., FARR, L. A., RANDALL, R.J. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275(1951).

83. MAAN, A.C., HOSEY, M. M. Analysis of the properties of calcium-channel activators and inhibitors to dihydropyridine receptors in chick heart membranes. Circ. Res. 61:379-388 (1987).

84. MACDONALD, J., MILJKOVIC, Z., HEISLER, S. Opposing actions of the enantiomers of BAY-K-8644 on calcium currents and ACTH secretion in clonal pituitary corticotrophs. Can. J. Physiol. Pharmacol. 65:2409-2414 (1987).

85. MACGREGOR, G. A. Sodium is more important than calcium in essential hypertension. Hypertension. 7:628-637 (1985).

86. MAHMOUDIAN, M., RICHARDS, W. G. A conformational distinction between dihydropyridine calcium agonists and antagonists. J. Chem. Soc., Chem. Commun., 739-741 (1986).

87. MARSH, J. D., DIONNE, M. A., CHIU, M., SMITH, T. W. A dihydropyridine calcium channel blocker with phosphodiesterase inhibitory activity: Effects on cultured vascular smooth muscle and cultured heart cells. J. Mol. Cell Cardiol. 20:1141-1150 (1988)

88. MARSHALL, G. R., CRAMER, R. D. Three-dimentional structure -activity relationships. Trends in Pharmacol. Sci. 9:285-289 (1988).

89. MATHISON, I. W., SOLOMONS, W. E., MORGAN, P. H., TIDWELL, R. R. Structural features and pharmacologic activity. in Principles of medicinal chemistry. W. O. Foye (Ed.) Lea and Febiger, Philadelphia (1974).

90. MATSUDA, S., KUROKAWA, K., HIGUCHI, K., IMAMURA, N., HAKATA, H., UEDA, M. A New Blood Pressure Measuring Apparatus Equipped with a Microcomputer System for Conscious Rats. J. Pharmacol. Methods 17:361-376 (1987).

91. MCCARRON, D. A. Is calcium more important than sodium in the pathogenesis of essential hypertension? Hypertension. 7:607-627 (1985).

92. MELDOLESI, J., POZZAN, T. Pathways of Calcium Influx at the Plasma Membrane: Voltage-, Receptor- and Second Messenger-operated Channels. Exp. Cell Res. 171:271-283 (1987).

93. MELDOLESI, J., VOLPE, P., POZZAN, T. Trends in Neuro. Sci. 11:453-457 (1988).

94. MEISHERI, K. D., HWANG, O., VAN BREEMEN, C. Evidence for two separate Ca^{2+} pathways in smooth muscle plasmalemma. J. Membrane Biol. 59:19-25 (1981).

95. MULTIPLE RISK FACTOR INTERVENTION TRIAL RESEARCH GROUP: Baseline rest electrocardiographic abnormalities, antihyprtensive treatment, and mortality in the Multiple Risk Factor Intervention Trial. Am. J. Cardiol. 55:1-15 (1985).

96. NOWYCKY, M. C., FOX, A. P., TSIEN, R. W. Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature 316:440-443 (1985).

97. NILIUS, B., HESS, P., LANSMAN, J. B., TSIEN, R. W. A novel type of cardiac calcium channel in ventricular cells. Nature 316:443-446 (1985).

98. PILLAI, N. P., Roth, D. H. Opiate receptor mediated hyperthemic responses in rat following Ca^{++} channel antagonists. Pharmacol. Biochem. Behav. 25(3):555-560 (1986).

99. PUTNEY, J. W. Jr. Calcium-mobilizing receptors. Trends in Pharmacol. Sci. 8:481-486 (1987).

100. RABKIN, S. W., MATHEWSON, F. A. L., TATE, R. B. Predicting risk of ischemic heart disease and cerebrovascular disease from systolic and diastolic blood pressures. Ann. Int. Med. 88:342-345 (1978).

101. RANG, H. P. Stimulant Actions of Volatile Anaesthetics on Smooth Muscle. Br. J. Pharmacol. 27:256-375 1964.

102. RASMUSSEN, H., BARRETT, P. W. Calcium Messenger System: An Integrated View. Physiol. Rev. 64#3:939-984 (1984).

103. RASMUSSEN, H. The Calcium Messenger System. (First of Two Parts) New Eng. J. Med. 314:1094-1101 (1986a).

104. RASMUSSEN, H. The Calcium Messenger System. (Second of Two Parts) New Eng. J. Med. 314:1164-70 (1986b).

105. RASMUSSEN, H., TAKUWA, Y., PARK, S. Protein Kinase C in the Regulation of Smooth Muscle Contraction. FASEB J. 1:177 85 (1987).

106. REUTER, H. Voltage-dependent mechanisms for raising intracellular free calcium concentration: calcium channels. In Calcium and the Cell. Ciba Foundation Symposium: 122. London, 1986.

107. REUTER, H., PORZIG, H., KOKUBUN, S., PROD'HOM, B. Calcium Channels in the Heart. Properties and modulation by dihydropyridine enantiomers. Ann. N. Y. Acad. Sci. 522:16-24 (1988).

108. RINGER, S. Concerning the influence exerted by each of the constituents of blood on the contraction of the ventricle. J. Physiol., Lond. 3:380 (1882).

109. RINGER, S. A further contribution regarding the influence of different constituents of the blood on contraction of the heart. J. Physiol., Lond. 4:29 (1883a).

110. RINGER, S. A third contribution regarding the influence of the inorganic constituents of the blood on ventricular contraction. J. Physiol., Lond. 4:222 (1883b).

111. RINGER, S. Further experiments regarding the influence of small quantities of lime, potassium and other salts on muscular tissue. J. Physiol., Lond. 7:291 (1886).

112. RODENKIRCHEN, R., BAYER, RT., STEINER, R., BOSSERT., F., MEYER, H., MOLLER, E. Structure-activity studies on nifedipine in isolated cardiac muscle. Naunyn. Schmied. Arch. Pharmacol.310:69-78 (1979).

113. ROGG, H., CRISCIONE, L., TRUOGG, A., Meier, M. In vitro studies of the calcium entry activators YC-170, CGP 28 392 and Bay K 8644. J. Cardiovasc. Pharmacol. 7(Supp 6):S31-37 (1985).

114. ROSE, G. A. Hypertension in the community. in Handbook of Hypertension Vol. 6 - Epidemiology of hypertension. Bulpitt, C.J. (ed). Elsevier Science Publishing Co. Inc., New York, N.Y. (1985).

115. RUTLEDGE, A., FERRANTE, J., LUCHOWSKI, E., TRIGGLE, D. J. S-(-)-Bay K 8644, [5-methyl-^{3}H]-, 70 to 87 Ci/mmol, NET-1002. Biotech Update 4#2:20-15 (1989).

116. SAGE, S. O., RINK, T. J. The kinetics of changes in intracellular calcium concentration in fura-2-loaded human platelets. J. Biol. Chem. 262:16364-16369 (1987).

117. SAIDA, K., VAN BREEMEN, C. Mechanism of Ca^{++} Antagonist-Induced Vasodilation (Intracellular Actions). Circ. Res. 53#2:137-142 (1983).

118. SCHNEEWEISS, S. SCHETTLER, G. In Cardiovascular Drug Therapy in the Elderly. Martinus Nijhoff Publishing, Boston, (1988).

119. SCHRAMM, M., THOMAS, G., TOWART, R., FRANCKOWIAK, G. Novel Dihydropyridines with Positive Inotropic Action through Activation of Ca^{++} channels. Nature 303:535-537 (1983a).

120. SCHRAMM, M., THOMAS, G., TOWART, R., FRANCKOWIAK, G.. Activation of Calcium Channels by Novel 1,4-dihydropyridines. (A new mechanism for positive inotropics or smooth muscle stimulants) Arzneim. Forsch. Drug Res. 33(11) #9:1268-72 (1983b).

121. SCHWARTZ, A., MCKENNA, E., VAGHY, P. L. Receptors for calcium antagonists. Am. J. Cardiol. 62:3g-6g (1988).

122. SCHWARTZ, A., GRUPP, I. L., GRUPP, G., WILLIAMS, J. S., VAGHY, P. L. Effects of dihydropyridine calcium channel modulators in the heart: Pharmacological and radioligand

binding correlations. Biochem. Biophys. Res. Comm. 125:387-394 (1984).

123. SCOTT, R. H., DOLPHIN, A. C. The agonist effect of Bay K 8644 on neuronal calcium channel currents is promoted by G-protein activation. Neurosci. Lett. 89:170-175 (1988).

124. SILVER, P. J. Pharmacological modulation of phosphorylation mechanisms in heart and vasculature. Drug Dev. Res. 12:94-107 (1988).

125. SMITH, J. S., CORONADO, R., MEISSNER, G. Sarcoplasmic reticulum contains adenine-nucleotide activated calcium channels. Nature 316:446-450 (1985).

126. SOBOLESKI, D. A., LI-KWONG-KEN, M. C., WYNN, H., TRIGGLE, C. R., WOLOWYK, M. W., KNAUS, E. E. Synthesis and calcium channel antagonist activity of nifedipine analogues containing 1,2-dihydropyridyl in place o fthe 1,4-dihydropyridyl moiety. Drug Des. Del. 2:177-189 (1988).

127. SOMLYO, A. P., JORGENSEN, A., KOBAYASHI, S., HIMPENS, B., KITAZAWA, T., SHUMAN, H., SOMLYO, A. V. Intracellular calcium distribution in cardiac and vascular smooth muscle. J. Mol. Cell. Cardiol. 20:(Supp IV) S32 (1988).

128. SPEDDING, M., BERG, C. Interactions between a "calcium channel agonist", Bay K 8644, and calcium antagonists differentiate calcium antagonist subgroups in K^+-depolarized smooth muscle. Naun.-Schmeid. Arch. Pharmacol. 328:69-75 (1984).

129. SPERELAKIS, N. Role of Ca^{++} in contraction, and wide variety of effects of calcium antagonists. In Calcium Antagonists: Mechanisms of action on cardiac and vascular smooth muscle. Sperelakis, N. (Ed.) Martinus Nijhoff Publishing, Boston, (1983).

130. STOCLET, J., BOULANGER-SAUNIER, C., LASSEGUE, B., LUGNIER, C. Cyclic nucleotides in heart and smooth muscle cells. Ann. N. Y. Acad. Sci. 522:106-115 (1988).

131. SU, C. M., SWAMY, V. C., TRIGGLE, D. J.. Calcium channel activation in vascular smooth muscle by Bay K 8644. Can. J. Physiol and Pharmacol. 62:1401-1410 (1984).

132. THOMAS, G., GROB, R., SCHRAMM, M. Calcium channel modulation: Ability to inhibit or promote calcium influx resides in the same dihydropyridine molecule. J. Cardiovasc. Pharmacol. 6:1170-1176 (1984).

133. TORWART, R., WEHINGER, E., MEYER, H. Effects of unsymmetrical ester substituted 1,4-dihydropyridine derivatives and their optical isomers on contraction of

smooth muscle. Naunyn. Schmeid. Arch. Pharmacol. 317:183-185 (1981).

134. TRIGGLE, D. J., SWAMY, V.C. Pharmacology of agents that affect calcium. Ches 78:(Supp) 174-179 (1980).

135. TRIGGLE, D. J., SWAMY, V. C. Calcium Antagonists: Some chemical-pharmacologic aspects. Circ. Res. 52:(Supp 1) I17-I28 (1983).

136. TRIGGLE, D. J. Ca^{2+} channels and 1,4-Dihydropyridine receptors. J. Cardiovasc. Pharmacol. 9(Suppl. 4):S4-S9 (1987).

137. TRUOG, A. G., BRAUNER, H., CRISCIONE, T., KUHNIS, H., MEIER, M., ROGG, H. CGP 28392, a dihydropyridine Ca-entry stimulator. in Calcium in Biological systems. Rubin, R. P., Weiss, G. B. and Putney, J. W. (eds). Plenum Press, New York. (1984).

138. TSIEN, R. W. Calcium channels in excitable cell membranes. Ann. Rev. Physiol. 45:341-58 (1983).

139. TSIEN, R. Calcium currents in heart cells and neurons. In: Neuromodulation. Kaczmarek, L.K. and Levitan, B. (eds) Oxford University Press. (1987).

140. TSIEN, R. W., FOX, A. P., HESS, P., MCLESKEY, E. W., NILIUS, B., NOWYCKY, M. C., ROSENBERG, R. L. Multiple types of calcium channels in excitable cells. In: Neuromodulation. Kaczmarek, L.K. and Levitan, B. (eds) Oxford University Press. (1987).

141. VAGHY, P., WILLIAMS, J. S., SCHWARTZ, A. Receptor pharmacology of calcium entry blocking agents. Am. J. Cardiol. 59:9A-17A (1987).

142. VAGHY, P. L., MCKENNA, E., ITAGAKI, K., SCHWARTZ, A. Mechanism of Action of Calcium Channel Modulator Drugs. Identification of a unique, labile, drug-binding polypeptide in a purified calcium channel preparation. Ann. N. Y. Acad. Sci. 522:176-186 (1988a).

143. VAGHY, P. L., ITAGAKI, K., MIWA, K., MCKENNA, E., SCHWARTZ, A. Mechanism of Action of Calcium Channel Modulator Drugs. Identification of a unique, labile, drug-binding polypeptide in a purified calcium channel preparation. Ann. N. Y. Acad. Sci. 522:176-186 (1988b).

144. VAN AMSTERDAM, F. T., ZAAGSMA, J. Stereoisomers of calcium antagonists discriminate between coronary vascular and myocardial sites. Naunyn. Schmied. Arch. Pharmacol. 337:213-219 (1988).

145. VAN BREEMEN, C., CAUVIN, C., LEIJTEN, P., LODGE, N., SAIDA, K. Regulation of cytoplasmic Ca^{++} in vascular smooth muscle. Prog. Clin. Biol. Res. 219:157-68 (1986a).

146. VAN BREEMEN, C., LEIJTEN, P., YAMAMOTO, H., AARONSON, P., CAUVIN, C. Calcium activation of vascular smooth muscle. Hypertension 8(Supp. II):II-89-II-95 (1986b).

147. VANHOUTTE, P. M. The Expert Committee of the World Health Organisation on Classification of Calcium Antagonists: The Viewpoint of the Rapporteur. Am. J. Cardiol. 59:3A-7A (1987.)

148. VAN ROSSUM, J. M. Cumulative Dose-Response Curves: II. Technique for the making of dose-response curves in Isolated organs and the evaluation of their parameters. Arch. Int. Pharmacodyn. 143:299-330 (1963).

149. VAN ZWIETEN, P.A.: Calcium antagonists - Terminology, classification and comparison. Arzneim.-Forsch./Drug Res. 35(I):298-301 (1985).

150. WALSH, K. B., BEGENISICH, T. B., KASS, R. S. ß-Adrenergic Modulation in the Heart: Independent regulation of K and Ca channels. Pflug. Arch. Eur. J. Pharmacol. 411:232-4 (1988).

151. WEISHAAR, R. E., KOBYLARZ-SINGER, D. C., QUADE, M. M., KAPLAN, H. R. Role of Cyclic AMP in Regulating Cardiac Muscle Contractility: Novel pharmacological approaches to modulating cyclic AMP degradation by phosphodiesterase. Drug Dev. Res. 12:119-29 (1988).

152. WEISS, G. B., PANG, I. H., GOODMAN, F. R. Relationship between $^{45}Ca^{++}$ Movements, Different Calcium Components and Responses to Acetylcholine and Potassium in tracheal Smooth Muscle. J. Pharmacol. and Exper. Ther. 233:389-94 (1985).

153. WILLIAMS, J. S., GRUPP, I. L., GRUPP, G., VAGHY, P. L., DUMONT, L., SCHWARTZ, A., YATANI, A., HAMILTON, S., BROWN, A. M. Profile of the oppositely acting enantiomers of the dihydropyridine 202-791 in cardiac preparations: receptor binding, electrophysiological and pharmacological studies. Biochem. Biophys. Res. Comm. 131:13-21 (1985).

154. WILLIAMSON, J. R. Role of Inositol Lipid Breakdown in the Generation of Intracellular Signals. Hypertension 8:140-156 (1986).

155. YAMORI, Y. Development of the spontaneously hypertensive rat (SHR) and of various spontaneous rat models, and their implications. in Handbook of Hypertension Vol 4: Experimental and Genetic Models of Hypertension. W. de Jong (Ed.) Elsevier Science Publishers B. V. Amsterdam (1984)

156. YENN, T. T., PEARSON, D. V., POWELL, C.E., Kirschner, G.L. Thermal stress elevates the systolic blood pressure of Spontaneously Hypertensive Rats. Life Sci. 22:359-62 (1978).

157. ZIMMERMAN, J. J., FELDMAN, S. Physical-chemical properties and biologic activity. in Principles of medicinal chemistry. W. O. Foye (Ed.) Lea and Febiger, Philadelphia (1974).

158. ZSCHAUER, A., VAN BREEMEN, C., BUHLER, F. R., NELSON, M. T. Calcium channels in thrombin-activated human platelet membrane. Nature 334:703-706 (1988).