PHARMACOLOGICAL STUDY OF THE HAEMODYNAMIC AND CARDIAC EFFECTS OF THE NEWLY INTRODUCED CALCIUM CHANNEL BLOCKERS, ISRADIPINE AND NITRENDIPINE

THESIS

Submitted In Partial Fulfilment for
M.D. Degree in "*Pharmacology*"

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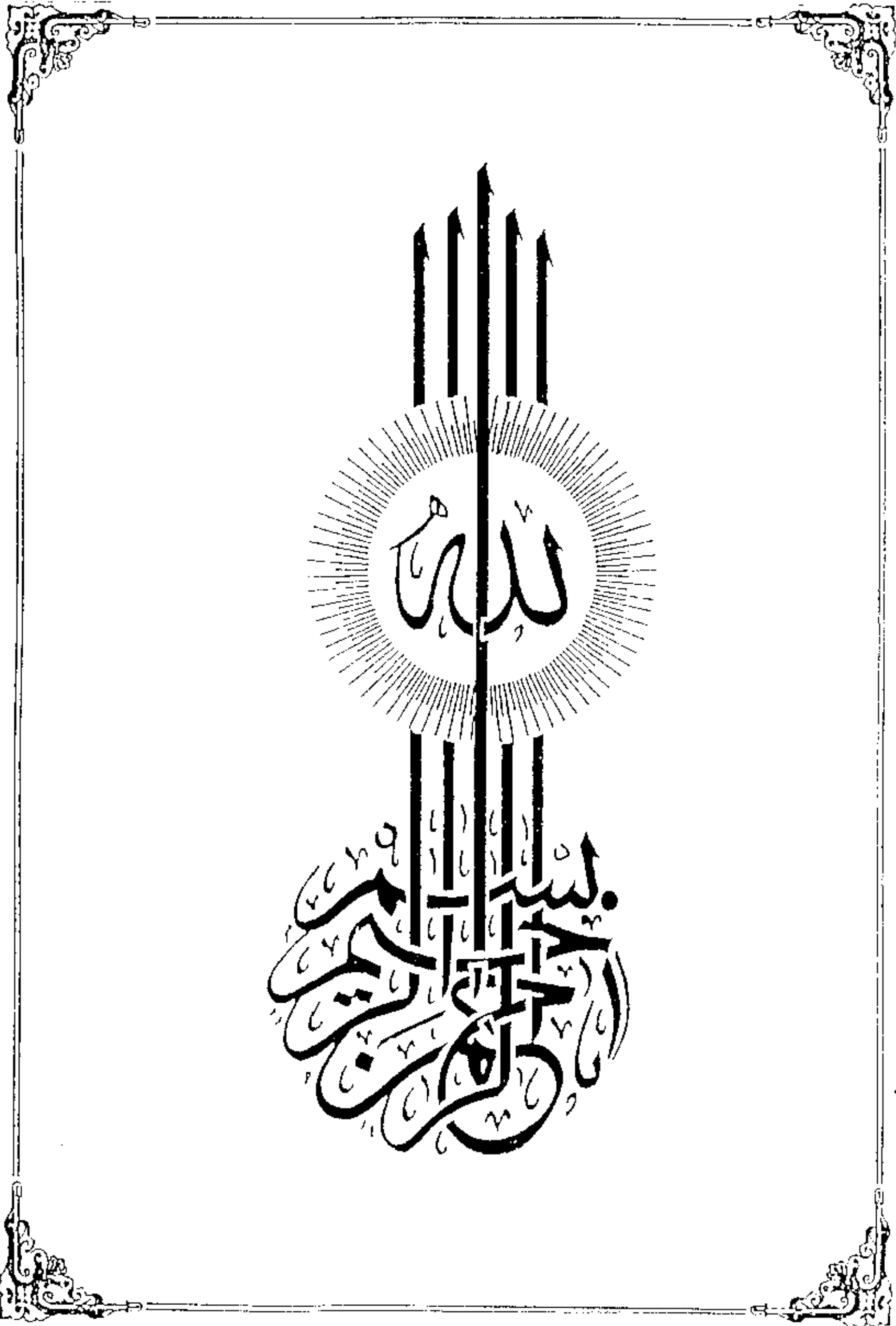

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Acknowledgment

I'm deeply indebted to prof. Dr. Salah Ahmed Abdel-Tawab, Professor of Pharmacology, Ain Shams University, who insisted to review this thesis despite his illness. His helpful comments and suggestions are greatly appreciated.

I'm particularly grateful to prof. Dr. Zeinab Mohammed Labib El-Gharib, Professor of Pharmacology, Ain Shams University, for her fruitful criticism of the manuscript and her generous assistance.

I would like to thank very much Prof. Dr. Ahmed Abdel-Salam El Melegy, Professor of Pharmacology, Ain Shams University for his enthusiastic support and continuous guidance during the course of the work.

I wish also express my appreciation and thanks to Dr. Laila Allam Besheer, Lecturer of Pharmacology, Ain Shams University for her helpful comments during the practical work as well as on the manuscript.

Besides, I wish to extend my thanks to my colleagues, Dr. Alaa Soliman, Lecturer of Pharmacology, and Dr. Ahmed Abdel-Basett, Animal House Supervisor, for their assistance in the practical work.

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Errata

Page no.	Para-graph no.	Line no.	Error	Correction
3	1	8	comitate	committee
4	tab 2	5	"Tachy-arrhythmias" is in a wrong place	move ⟶ above line
5	1	2	First "the" is extra	delete it
14	2	12	relaxation	relaxant
	3	6	formula	formulae
18	2	15	Ca^{++} antagonists	nitrendipine
19	2	13	the peak	the increase in peak
20	2	3	enhances	enhanced
35	4	4	life	lives
43	col 5	last	missed "5% CO_2"	add it
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	2	last	2	2 x 10^{-10}
47	last	1	forth	fifth
57	last	8,9	last 2 lines in page are in the wrong place	move ⟶ above line
63	3	2	missed "diazepam"	add it before "was"
66	1	8	missed "a"	add it before "hook"
74	4	2	57.8	57.2
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149	last	7	contraction	contractions
		8	utilizes	utilize
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INTRODUCTION

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INTRODUCTION

Although the importance of calcium for the maintenance of cellular activity was observed by Ringer as early as 1883 its key role in intracellular functions was not conceived until 70 years later by by Kamada in Japan and Heibrunn in the United States, **(Kamada and Kinosita, 1943, and Heilbrunn and Weircinski, 1947)**. By the end of 1950s and the early 1960s the role of calcium in pharmacological processes was measured **(Evans et al., 1958, and Durbin and Jenkinson, 1961)**. In the 1960s the concept of calcium antagonism was pioneered in Europe independently in Fleckenstein and Godfraind laboratories. Inhibition of calcium entry as a mechanism of drug action was demonstrated in cardiac tissues by Fleckenstein et al. **(Fleckenstein, 1964, 1968, and 1971 $_{a,b}$, and Fleckenstein et al., 1967, 1968, and 1969 $_{a,b}$)** and in arterial wall by Godfraind and coworkers **(Godfraind et al., 1966 and 1986, Godfraind 1968, Godfraind and Polster, 1968, and Godfraind and Kaba, 1969 $_{a,b}$)**.

Hartfelder reported in 1962 that verapamil, a coronary vasodilator, had a negative inotropic and chronotropic effects that were not seen with other, apparently similar vasodilator agents, such as nitroglycerin **(Needlemann et al., 1985)**. The mechanism of action of verapamil was initially thought to be due to coronary vasodilatation and blockade of myocardial ß-adrenergic receptors. However, **Fleckenstein et al., (1967)** suggested that the mechanism of action was not related to ß-adrenergic blockade but to inhibition of excitation contraction coupling. Fleckenstein termed such agents calcium antagonists. In 1969 Rougier et al. presented substantial evidence that

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depolarization in atrial tissues was mediated by 2 inwardly directed ionic currents; the fast sodium current and the slow calcium current (**Rougier et al., 1969**). Gallopamil, -a derivative of verapamil- was subsequently shown to block the movent of calcium through the slow channels and thereby alter the plateau phase of cardiac action potential (**Kohlhardt et al., 1972**).

Meanwhile, **Godfraind and Polster (1968)** showed that some drugs acting as polyvalent antagonists on vascular smooth muscles have the property to inhibit at the same concentration the vasoconstriction evoked by various agents including norepinephrine, angiotensin II, antidiuretic hormone and other spasmogens. Among the drugs were the diphenyl piperazines, cinnairizine and lidoflazine. They proposed that they may interfere with common mechanism activated by various agents to produce the smooth muscle contraction. A hypothesis was that calcium translocation was the common mechanism. According to the indirect evidence available at that time, the calcium activating the contractile mechanism could have been translocated either from the outside to the inside of the cell or within the cell from an intracellular store (**Godfraind and Polster, 1968**).

This concept of calcium antagonism was later applied to other drugs including dihydropyridines (DHPs), diltiazem and flunarizine (**Van Nueten, 1969, Fleckenstein et al., 1972, Kohlhardt et al., 1972, Vater et al., 1972, Fleckenstein, 1977, Kohlhardt and Fleckenstein, 1977, Taira, 1982, and Godfraind, 1982 and 1985**).

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In 1985, the World Health Organization appointed a committee of experts to evaluate the classification of drugs used to interfere with the handling of calcium by tissue cells **(Vanhoutte, 1987)**. The committee proposed reclassification of these agents, along functional lines, taken into consideration their effect in a number of tests (Table 1). The classification as could be expected is, a balanced amalgamation of the chemical and pharmacological classifications. The comitate also proposed a classification derived from those clinical characteristics already recognized for the calcium antagonists (Table 2). Inevitably certain disorders and drug classes have been relatively more investigated in the few years that clinical use of these agents has been possible. With time, significant further evaluation of this classification can be expected to take place.

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TABLE 1: TESTING OF CALCIUM ANTAGONISTS (Godfraind, 1987)

DIRECT EVIDENCE FOR BLOCKADE OF CA^{++} ENTRY

1. ELECTROPHYSIOLOGICAL TESTS;
   - a) Myocardium; microelectrodes, patch clamp technique.
   - b) SAN and AVN; Ca^{++}-dependent bioelectric pacemaker activity.
2. PHARMACOLOGICAL TESTS;
   - a) In-vitro; vascular smooth muscles: myogenic activity, high K^{+}, vasoconstrictor agonists, comparison blood vessels and heart.
   - b) In-vivo; vasodilator properties in various beds, antihypertensive effects in animal models, effects on cardiac functions, cardiac electrophysiology.
3. BIOCHEMICAL TESTS:
   - a) Displacement radiological binding to ca^{++} channels.
   - b) Inhibition of radioactive calcium uptake.

EFFECTS IN PATHOPHYSIOLOGICAL MODELS

1. PROTECTION AGAINST HYPOXIC INSULTS;
   - a) Heart; isolated myocytes, perfused heart, in-vivo.
   - b) Brain.
   - c) Other organs.
2. HEMORRHEOLOGIC EFFECTS:
   Improvement of filterability of hypoxic blood.
3. PLATELET FUNCTION.
4. LONG-TERM EFFECTS ON BLOOD VESSELS.

Table: 2 CLINICAL USES OF CALCIUM ANTAGONISTS (Godfraind, 1987)

Level of proof	Clinical condition
Good	CARDIOVASCULAR: Exertional angina, Angina at rest; Unstable angina; Prinzmetal angina. Paroxysmal Supraventricular Tachy-arrhythmias Atrial fibrillation and flutter. Hypertension. NEUROLOGIC: Prophylaxis of migraine. Vertigo.
Reasonable	Hypertrophic cardiomyopathy Valvular incompetence, heart failure. Subarachnoid haemorrhage. Cardioplegia. Raynaud's phenomenon.
Under examination	Protect against myocardial ischemia. Infarct size reduction. Ischaemic ventricular arrhythmias. Primary pulmonary hypertension. Protection against cerebral anoxia. Epilepsy. Transient ischaemic attacks. Leg ischaemia, Dysmenorrhoea

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Cytoplasmic Ca^{++} comes mainly from 2 sources, the extracellular compartment the and the sarcoplasmic reticulum. Ca^{++} entry from the extracellular compartment occurs through regulated Ca^{++} channels or non-regulated Ca^{++} leak **(Loutzenhiser et al., 1984)**. The channel route can be blocked by organic Ca^{++} antagonists while the non regulated leak can be partially blocked by inorganic Ca^{++} antagonists e.g. (Co^{++}, Mn^{++}, and La^{+++}). Release of Ca^{++} from the sarcoplasmic reticulum occurs through 3 mechanisms: Ca^{++}-induced Ca^{++}-release, inositol triphosphate (IP_3)- activated Ca^{++}-release and non-regulated Ca^{++} leak **(Johns et al., 1987)**.

There are 2 types of calcium channels **(Hofmann et al., 1987_a)**:

* Voltage-operated calcium channels (VOCs) which are key component of all excitable cells. When a propagated wave of depolarization approaches the channel, a reduction in membrane potential causes the activation gate to open permitting calcium to enter the cell. The gate closes when the interior of the cell becomes electronegative again.

* Receptor- operated calcium channels (ROCs) which are present in both excitable and non excitable cells and are regulated by hormone receptors. Activation of adrenergic β_1-receptors in the heart for example, causes activation of c-AMP. This results in phosphorylation of non recruited channels with change in their molecular configuration. This increases the chance that the channel will be activated during depolarization i.e. becomes recruited.

VOCs can be further subdivided into 3 types; T-(transient) channels, N-(neuronal) channels and L-(long- lasting) channels.

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The N-channels are apparently present only in neuronal cells whereas T- and L-channels have been identified in wide variety of cells e.g. neuronal, cardiac, skeletal, smooth muscle and neurosecretory cells.

The T-channels are activated from quiet negative membrane potential, carry a small transient current and are fastly inactivated while the L-channels are activated from a higher voltage, carry a larger current and are slowly inactivated (Hofmann et al., 1987_b).

The T- and N-channels -unlike the L-channels- are largely insensitive to calcium antagonists. The L-channels are peculiar in that they have at least four binding sites for calcium antagonists. The binding of the drug to the channel is critically dependent on the state of the channel and hence on membrane potential.

The cardiac L-type calcium channels can be regulated by hormones (Reuter, 1984, and Tsien et al., 1986). All hormones which activate adenylate cyclase increase the activity of cardiac L-channels while hormones which inhibit it decrease the activity of the channel. Stimulation of β_1-adrenergic receptors activates c-AMP-dependent phosphorylation and increases the possibility that the channel is available for voltage-dependent opening. Stimulation of muscarinic receptors - on the other hand- results in dephosphorylation of the calcium channel and thereby decrease the open state possibility (Hofmann et al, 1987_a). This also applies to L-channels excised from a neurosecretory line (Armstrong and Eckert, 1987). On the contrary the smooth muscle and the neuronal L-channels are not affected by c-AMP dependent

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phosphorylation (Hofmann et al., 1987_a).

The ROCs are regulated by the water soluble products of phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis. Receptor hydrolysis of PIP_2 generates 2 intracellular messengers: diacylglycerol and 1,4,5 IP_3 (Berridge and Irvine, 1984, Downes and Michell, 1985, and Taylor 1987). 1,4,5 IP_3 binds to specific receptors on the endoplasmic reticulum and opens its calcium channels thus increasing cystosolic Ca^{++} concentration. Phosphorylation of 1,4,5 IP_3 followed by dephosphorylation -2 reactions that are catalyzed by a kinase activated by the increase of Ca^{++} concentration- results in formation of 1,3,4 IP_3. When this latter accumulates it becomes more important than 1,4,5 IP_3 in controlling endoplasmic reticulum calcium channels. Emptying the endoplasmic reticulum pool of calcium is itself the signal that leads to refilling from the extracellular space. Both 1,4,5 IP_3 and 1,3,4 IP_3 are recycled to resynthesize the membrane PIP_2 (Taylor, 1987). Diacylglycerol activates protein kinase C (PKC) by increasing its sensitivity to Ca^{++} (Löfdahl and Barnes, 1986).

Ca^{++}-induced Ca^{++} release occurs from caffeine releasable store by Ca^{++} concentration >= $2X10^{-6}$ M and is potentiated by c-AMP (e.g. after norepinephrine stimulation). Actually, this effect of c-AMP is overshadowed by internalization of Ca^{++} inside the sarcoplasmic reticulum and the mitochondria as well as extrusion of Ca^{++} outside the cell by Ca^{++} pump and Na^{+}-Ca^{++} exchange (Kumar 1978, Müeller and van Breemen, 1979, Scheid et al., 1979, and Saida and van Breemen, 1984). Ca^{++}-induced Ca^{++} release is absent during K^{+}-induced depolarization during which

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