PHARMACOLOGICAL AND CLINICAL EVALUATION OF A CALCIUM CHANNEL BLOCKER (DILTIAZEM)

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(Anaesthesiology)

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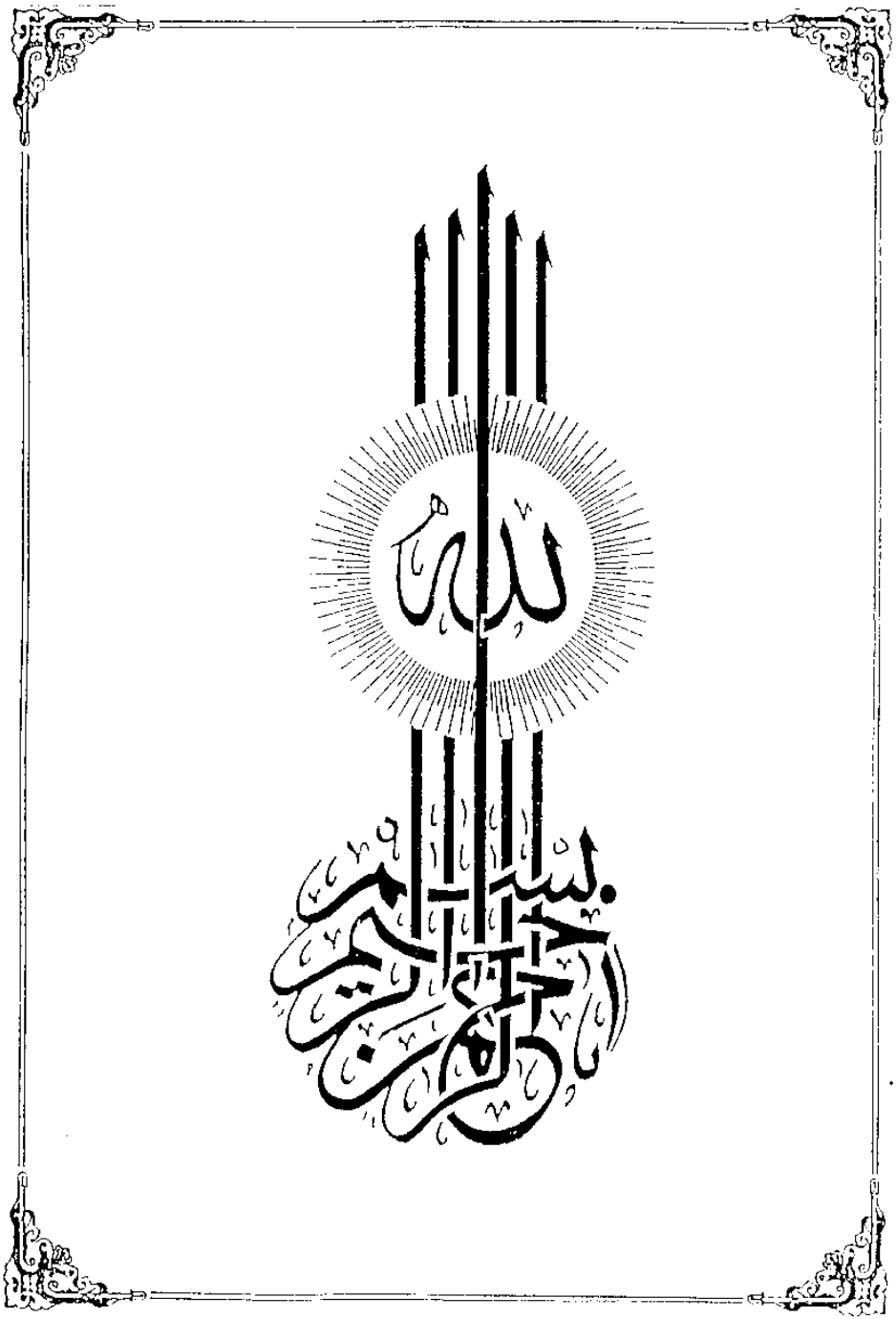

To the memory of my
father and mother

To my husband and my
little kid.

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(Katz et al., 1982). The mechanism of change in conductance resides in the movement of changed particles (gating particles) in the cell membrane. If the gating particle is removed, the channel opens and ions start to flow. It is because of the differences in the morphology of the action potential of the ventricular muscle and sino-atrial or atrio-ventricular node, as well as differences of the threshold potential, that the existence of two distinct inward currents has been postulated in figure (2).

One current, carried by Na^{+}, is responsible for the rapid upstroke and fast impulse conduction which characterise atrial, ventricular and His- Purkinje fibres (Colatsky and Tsien, 1979). The other, carried by Ca^{+2}, is responsible for the slower upstroke and slow conduction seen in sinoatrial and atrioventricular nodes (Cranefield, 1977). The inward sodium current (I_{Na}), also called fast current is initiated when the membrane is depolarized beyond - 85 m.v.

During the plateau phase of depolarization, most sodium channels are closed. Local anaesthetics displace the inactivation curve of the sodium channels so that stronger depolarization is needed to initiate impulses. The sodium channel density per surface area ranges from about 1-2 channels u m^{-2} in cultured cardiac cells to about 16 channels u m^{-2} in isolated cells from adult heart (Cachelin et al., 1983). The calcium inward current (I_{si}), is enhanced by catecholamines and blocked by Mn^{+2} and it is distinct from the sodium current. This current underlies impulse conduction in nodal tissue and is responsible for the plateau phase of the action potential. Sodium and potassium ions can move through the calcium channels but to a very much smaller extent (approximately 1/100) than calcium itself. (I_{si}) is increased by adrenergic agonists, either because of an increase in the number of channels

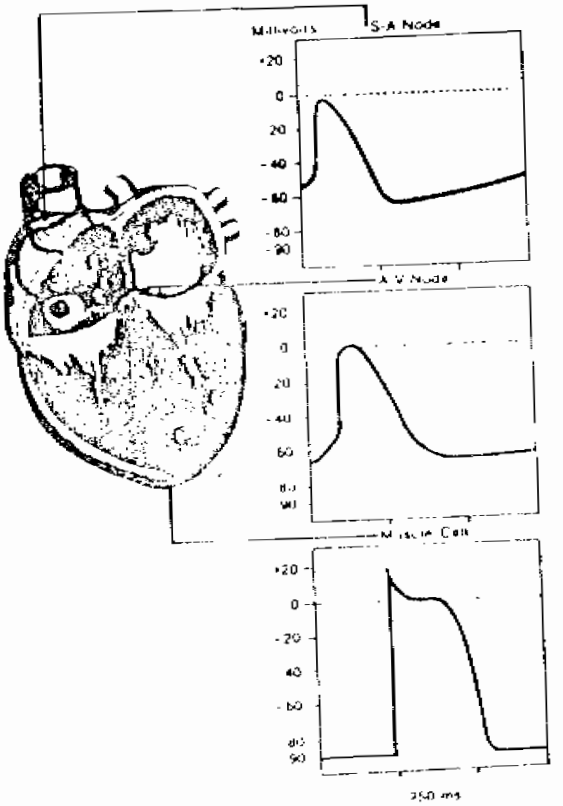

Figure (7) Cardiac Action potential differentiation (Meran, 1982)

by 0.1 m M nickel, and in certain neurons by 0.1 m M phenytoin. N- and L-channels are blocked by low concentration of cadmium (20 u M) and they are affected by the ordinary known organic calcium channel blockers (Hofmann et al., 1987). The L-type calcium channel has been identified in vitro by its three drug binding sites which are specific for dihydropyridines, phenylalkylamines and benzothiazepines. These stereospecific sites have high affinity for the responsive group. Binding to one site is regulated allosterically by the occupation of the other sites by divalent cation and by temperature (Hofmann et al., 1987). Binding of 1, 4 dihydropyridines is stimulated (at temperature >20°C) by (+)-cis diltiazem or fostedil, which act on site III.

Certain phenylalkylamines (e.g desmethoxyverapamil, verapamil and gallopamil) inhibit (or stimulate) 1, 4 dihydropyridine binding, again by an allosteric mechanism (Glossmann et al., 1987).

Most tissues contain in addition to the high affinity, allosterically regulated sites, low affinity, high capacity binding sites for calcium channel blockers which are not located on the L-channels. The high affinity allosterically regulated sites are low in cardiac muscle and other tissues except the transverse tubules of skeletal muscles which contain a high density of these sites (Hofmann et al., 1987). The density of low affinity binding sites for 1, 4 dihydropyridines on crude membrane preparations is often very high , and also on the human red blood cell (where no L-type calcium channels have so far been reported).

Different classes of sites exist, e.g those linked to peripheral benzodiazepine receptors, to nucleoside transporters, to phosphodiesterases and to some

ion transporting structures. These sites are not stereoselective (Glossmann et al., 1987).

Receptor-operated calcium channels have not been detected by electrophysiological techniques to date, but recent evidence suggests that excitable and non-excitable cells have calcium channels which are regulated indirectly by hormone receptors (Hofmann et al., 1987). Enhanced phosphatidylinositol breakdown may have an intrinsic function in the operation of many cell surface receptor systems, especially those which raise cell surface permeability to Ca^{+2}. Subsequently it was realised that the effects of these receptors on the cytosolic Ca^{+2} concentration could be divided into two phases: an initial transient release of Ca^{+2} from an intracellular pool and a more sustained entry of Ca^{+2} at plasma membrane. (Hofmann et al., 1987)

Several recent publications suggest that agonist-stimulated Ca^{+2} entry may be intimately related to mobilization of Ca^{+2} from intracellular pools and that both are regulated by the water-soluble product of phosphatidylinositol 4, 5 biphosphate (PI P_2) hydrolysis.

Receptor-stimulated hydrolysis of PIP_2 generates two intracellular messengers: diacylglycerol which activates protein kinase and inositol 1, 4, 5 triphosphate (1, 4, 5 IP_3) which releases Ca^{+2} from intracellular pools.

Specific receptor on the endoplasmic reticulum (ER) binds to 1, 4, 5 IP_3 and opens Ca^{+2} channel in ER membrane and since that Ca^{+2} pool appears to refill from the extracellular space without a change in cytosolic Ca^{+2} concentration. It probably has some close association with the plasma membrane.

Metabolism of 1, 4, 5 IP_3 may be by either of two known pathways. One pathway begins with removal of the 5-phosphate group of 1, 4, 5 IP_3 to give inositol 1, 4 biphosphate. This probably serves only to inactivate the messenger and regenerate inositol from which PIP_2 can be resynthesized. The alternative pathway is more interesting: a kinase catalyses phosphorylation of 1, 4, 5 IP_3 to inositol 1, 3, 4, 5 tetraphosphate (1, 3, 4, 5 IP_4). The same enzyme that catalyses dephosphorylation of 1, 4, 5 IP_3 probably also catalyses dephosphorylation of 1, 3, 4, 5 IP_4 to inositol 1, 3, 4 triphosphate (1, 3, 4 IP_3) which is then further dephosphorylated eventually to inositol (Taylor, 1987).

Metabolism of inositol 1, 4, 5 triphosphate is shown in Figure (3)

Beta one adrenergic receptors activate cAMP-dependent protein kinase and increase 3 to 4 folds voltage-dependent Ca^{-2} influx, also cAMP kinase phosphorylates L-channels or a protein closely associated with L-channels. Phosphorylation decreases the closed times, increases the open time and decreases about 3 folds the number of blanks , i.e. tracings in which the channel does not open upon depolarization.

Phosphorylation of the cardiac L-channel is stimulated in vivo by all hormones which activate adenylate cyclase. An increased current can be decreased by hormones which inhibit adenylate cyclase activity. Stimulation of ventricular receptors decreases the calcium current through the latter mechanism by activation of inhibitory GTP-binding protein G (Hofmann et al., 1987).

The ratio of 1, 4, 5 IP_3/1, 3, 4, 5 IP_4 may also be the link between angiotensin II receptor activation and the increase in a voltage-dependent calcium

bind to each other and hydrolyse adenosine triphosphate (ATP). Consequent myocardial shortening and/or tension development occurs as the filaments slide past each other due to the change in the angles of the actin-myosin cross bridges (Kraynack, 1983).

The combination of the actin binding and the increase in Ca^{+2} concentration results in marked increase in activity of ATPase enzyme sufficient for rapid hydrolysis of ATP.

For relaxation to occur, the Ca^{+2} concentration in the sarcoplasm must be returned to less than 10^{-7} molar. Dissociation of Ca^{-2} from troponin reuslts in the breakage of the actin-myosin cross links and relaxation occurs (Merin, 1982).

Although the quantitative influx of Ca^{+2} through the slow channel during depolarization appears to be insufficient to raise the sarcoplasmic concentration to that required for troponin binding and cross-bridge formation, the stimulus of the influx is necessary not only in cardiac muscle, but also in smooth muscles. The influx of Ca^{+2} appears to trigger release of an internal store (s) of Ca^{+2} which then brings the sarcoplasmic Ca^{-2} concentration to the proper level.

The internal stores in cardiac muscle are the sarcoplasmic reticulum and other stores in the inner layer of the sarcolemma and the mitochondria (Stull and Sanford, 1981).

In smooth muscle, the stimulus for contraction is not the depolarization of the cell membrane but an agonist-receptor interaction (e.g. adrenergic). Consequently, a some what different model of the membrane channel has been developed (Seefigure 4).

In this model, rather than inner and outer gates for the calcium channel, separate channels labeled "voltage sensitive" and "receptor sensitive" have been substantiated.

The voltage-sensitive channel appears to be responsible for the calcium influx through depolarization. In most experimental circumstances this is produced by high extracellular potassium concentration. In vascular smooth muscle, the calcium entry blocker drugs block this channel.

The receptor linked channel is responsible for the calcium linked influx from agonists such as norepinephrine, angiotensin..... etc. In addition, there are superficial and internal low and high affinity calcium binding sites.

The low affinity sites are associated with transmission through the voltage-dependent depolarization channel and hence are susceptible to calcium entry blocking drugs, whereas the high affinity sites interact with the receptor channel and are sensitive to the direct acting vasodilators, such as sodium nitroprusside. However, neither the calcium entry blockers nor nitroprusside appear to have any effect on Ca^{-2} binding. The effect of vasoconstrictor hormones (drugs), such as norepinephrine are mediated through the high affinity sites and may be partially and unpredictably blocked by Ca^{+2} entry blockers.

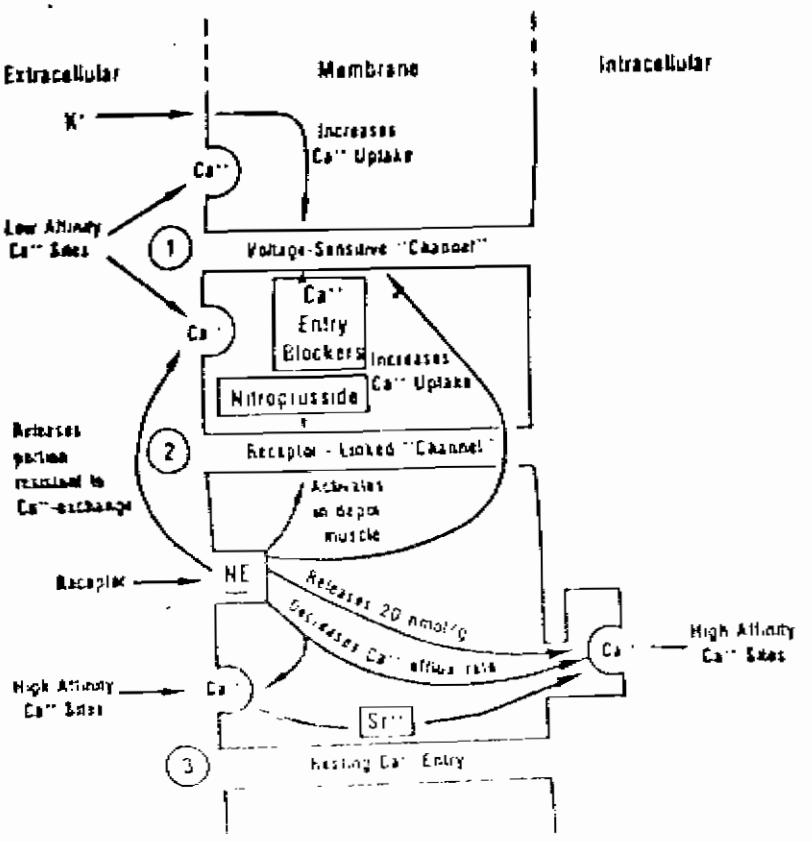

Figure (4) A model of the calcium entry mechanisms in vascular smooth muscle (Meisheri, 1982)

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The other main calcium antagonists, diltiazem, verapamil and diclofuriine, are chemically separate, although these compounds are also basic and the three compounds have very similar lipophilicity (Spedding, 1985).

In 1985 the World Health Organization (WHO) has reported classification in the hope that it may clarify when they can be used and may facilitate the exchange of experimental and clinical data in this rapidly growing field of therapeutics.

The WHO pharmacological classification depends on the fact that the only satisfactory way of classification will be to subdivide the agents according to their interaction with specific receptors and their cellular mechanism of action. Indeed, in terms of the relation between chemical structure and activity of the individual compounds, it was found that the former is not necessarily predictive of the latter. Hence it was proposed to reclassify those agents, along functional lines, taking into consideration their effect in a number of tests. These include direct demonstration of blockade of the entry of Ca^{+2} with electrophysiological (myocardial tissues), pharmacological (myocardium and vascular smooth muscles, haemodynamic and antihypertensive effects) and biochemical (radioligand-binding studies and uptake of radioactive Ca^{+2}) techniques.

Once the Ca^{+2} antagonistic properties of a drug are established, its effects should be investigated in a variety of pathophysiological models involving exaggerated entry of Ca^{+2} (in particular, hypoxic insults).

When taking into account their effect in these different bioassays of interference with Ca^{2} movements, they felt that the existing drugs can be subdivided into six types table (1).

The proposed WHO clinical classification table (2) was derived from information already available on the use of Ca^{+2} antagonists as therapeutic agents. Although certain disorders and drug classes have inevitably been relatively more investigated in the last few years that clinical use of these agents has been possible. It was predicted that, with time significant further evolution of this classification will occur.

They realized that individual calcium antagonists manifest other clinically important differences, e.g. their pharmacokinetic behaviour and their propensity for side-effects. However pharmacokinetic differences are not sufficiently distinct to make classification on that basis alone and side effects occur too infrequently (less than 15 percent of patients treated) to be a useful basis for clinical classification (Vanhoutte and Paoletti, 1987).

Dihydropyridines [^{3}H] such as nitrendipine and nimodipine bind to membrane sites; the binding is saturable, of high affinity and is displaced by other dihydropyridines in a competitive fashion. The potency of competing dihydropyridines reflects their potency as Ca^{+2} antagonists but all the compounds have an inappropriately high affinity in the brain, skeletal muscle and heart muscle membrane preparations compared with their slight pharmacological effects in these tissues.