Calcium channel antagonists: pharmacological considerations

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Introduction

The term 'calcium antagonists' was introduced by Fleckenstein (for reviews see Fleckenstein, 1977, 1983) to define a group of agents with specific inhibitory action against Ca^{2+} currents and Ca^{2+} mobilization in cardiac and smooth muscle. These agents, which include the clinically available verapamil and gallopamil (D600), nifedipine and diltiazem (Figure 1) have also been described by the terms 'calcium antagonists' and 'Ca²⁺ entry blocker' to indicate that they are a specific subclass of a potentially very large group of agents that modulate Ca^{2+} metabolism.

A generalized scheme of cellular Ca^{2+} metabolism is depicted in Figure 2 and represents the interrelationship between intracellular and extracellular sources of Ca^{2+} that are used in such stimulus-response systems as excitation-contraction and stimulus-secretion coupling. Depending upon the stimulus and response, varying ratios of these sources of Ca^{2+} may be employed to sustain a particular system. Accordingly, elucidation of the pathways and mechanisms of cellular Ca^{2+} metabolism is of considerable importance to definition not only of the physiological systems but also of defects that may occur in pathological states including bronchial hyperreactivity, hypertension and other vascular diseases.

The Ca^{2+} channel antagonists are believed to exert their effects primarily at the potential-dependent Ca^{2+} channel (Figure 2) where they inhibit Ca^{2+} current and the associated Ca^{2+} influx (for reviews see Fleckenstein, 1977, 1983; Weiss, 1981; Rahwan & Witiak, 1982; van Zwieten, 1982; Cauvin *et al.*, 1983; Janis & Triggle, 1983; Janis & Scriabine, 1983). However, the chemical heterogeneity of these agents indicates that they are likely to act by different mechanisms and possibly at different sites to inhibit Ca^{2+} channel function. Consistent with this, the agents are pharmacologically hetero-

geneous, nifedipine and other 1,4-dihydropyridines being significantly more effective in smooth muscle than in cardiac muscle. This pharmacological heterogeneity parallels the different clinical and potential uses of the Ca^{2+} channel antagonists (Henry, 1980, 1982; Flaim & Zelis, 1982; Katz *et al.*, 1984; Schwartz & Triggle, 1984).

Ca²⁺ channel antagonists

Evidence that the Ca^{2+} channel antagonists constitute a specific, though heterogeneous, group of agents derives from a variety of investigations at the electrophysiological, biochemical and pharmacological levels.

Electrophysiological data, primarily from cardiac tissues, indicate a dominant or preferential action of these agents on the plateau (Ca^{2+}) component of the cardiac action potential (Bayer *et al.*, 1982) and a Ca^{2+} -sensitive block of both inward and outward current flow through the Ca^{2+} channel (Lee & Tsien, 1983) consistent with the terminology of Ca^{2+} blockers. Although comparable electrophysiological data are largely lacking for smooth muscle, the similarly potent inhibitory actions of these agents against K⁺ depolarization-induced mechanical responses and the associated $^{45}\text{Ca}^{2+}$ uptake (for reviews see Cauvin *et al.*, 1983; Triggle & Swamy, 1983), and the variable activity against mechanical response and $^{45}\text{Ca}^{2+}$ uptake in a number of specific receptor-activated systems, is also supportive of antagonist action at voltage-dependent Ca^{2+} channels. (Extensive compilations of the inhibitory actions of Ca^{2+} channel antagonists in smooth muscles have been provided by Flaim (1982) and Cauvin *et al.* (1983)). These findings indicate a plasmalemmal locus of action of Ca^{2+} channel antagonists, a conclusion supported by their essential inactivity in skinned smooth and

Figure 1 Structural formulae of some Ca^{2+} channel antagonists.

cardiac muscle (Morad *et al.*, 1982; Kreye *et al.*, 1983). Additional support for the plasmalemmal locus of action is provided by radioligand binding studies (for a general review of these studies see Triggle & Janis, 1984a) which show a preferential association of specific [^3H] dihydropyridine binding with plasmalemmal fractions of smooth and cardiac muscle (Sarmiento *et al.*, 1983; Grover *et al.*, 1984).

Specific sites of interaction of the Ca^{2+} channel antagonists are also indicated by the existence of structure-activity relationships, including stereoselectivity, for the several categories of structures which have been observed both *in vivo*

and *in vitro* (for reviews see Triggle, 1982; Mannhold *et al.*, 1982; Janis & Triggle, 1983; Triggle & Swamy, 1983; Meyer *et al.*, 1983). In a comparison of negative inotropic agents including both Ca^{2+} channel antagonists and unrelated compounds, a positive association for the latter compounds only was observed between activity and increasing hydrophobicity, suggestive of a membrane perturbant action (Rodenkirchen *et al.*, 1982). The most extensive structure-activity data are available for the 1,4-dihydropyridines and the data, derived from *in vivo* (Loev *et al.*, 1974) and *in vitro* (Rodenkirchen *et al.*, 1979; Bolger *et al.*, 1983) pharma-

Figure 2 Plasmalemmal Ca^{2+} mobilization pathways. Depicted are: 1, Ca^{2+} ATPase; 2, Na^{+} - Ca^{2+} exchange processes; 3, Ca^{2+} 'leak' pathway; 4, Ca^{2+} entry through the fast Na^{+} channel; 5, the potential-dependent (PDC) Ca^{2+} channel; 6, Ca^{2+} entry through receptor-operated channels that may be associated with specific membrane receptors A and B.

cological studies and from radioligand binding studies (Bolger *et al.*, 1983; Triggle & Janis, 1984a; Janis *et al.*, 1984), suggest the following critical interactions:

1. The presence of the 1,4-dihydropyridine ring with an unsubstituted N_1 function.
2. 2,6-Substitution in the dihydropyridine ring by lower alkyl groups.
3. Ester substituents in the 3- and 5-positions of the 1,4-dihydropyridine ring are optimal. Removal or replacement by other electron withdrawing groups usually results in reduced antagonist activity.
4. There appears to be considerable tolerance for the size of the ester group. Increases beyond the carbomethoxy groups of nifedipine as in nitrendipine, nimodipine and nisoldipine (Figure 1) may maintain activity and give rise to indications of tissue selectivity. Bis-nifedipine analogues in which two nifedipine structures are linked by a polymethylene chain maintain activity. When the ester functions are dissimilar, C-4 of the 1,4-dihydropyridine ring is chiral and stereoselectivity of antagonism is found.
5. A substituted phenyl group in the 4-position of the 1,4-dihydropyridine is apparently optimum, but the position of the substituent is critical. Ortho- and meta-substituents modify activity according to their electronic and steric effects, but para-substitution is invariably detrimental.

Although the evidence for specificity of action of the Ca^{2+} channel antagonists is very convincing, it is also apparent that non- Ca^{2+} channel mediated events can also be affected by these agents (for reviews see Triggle, 1982; Janis & Triggle, 1983; Triggle & Swamy, 1983). This is particularly true for verapamil and D600 which have been shown to affect, at concentrations ranging from slightly to significantly greater than those necessary to block Ca^{2+} channel function: Na^+ and K^+ currents, binding of drugs to a variety of neurotransmitter receptors, Ca^{2+} -ATPase from sarcoplasmic reticulum, Ca^{2+} binding to membranes and to exert local anaesthesia. In contrast, such actions are less apparent with the 1,4-dihydropyridines and they are usually observed at concentrations much greater than those necessary to block Ca^{2+} channels or which are likely to be achieved clinically. It is possible that some of these additional effects contribute to the pharmacological dissimilarities between nifedipine (and other 1,4-dihydropyridines) and verapamil, D600 and diltiazem.

Mechanisms of action of Ca^{2+} channel antagonists

The several lines of evidence briefly reviewed here leave little doubt that the Ca^{2+} channel antagonists do block voltage-dependent Ca^{2+} channel function and that this action is a major component of their pharmacological profile. However, important questions remain to be answered. These include:

1. What is the relationship between the sites of action of Ca^{2+} channel antagonists and Ca^{2+} channel structure and function?
2. What is the basis for the tissue selectivity exhibited by the Ca^{2+} channel antagonists?

Sites of action

Although electrophysiological and $^{45}\text{Ca}^{2+}$ uptake studies with the Ca^{2+} channel antagonists are consistent with Ca^{2+} channel blockade, it is also clear that their mechanism of action is not simply to act as 'channel plugs' nor to compete directly with Ca^{2+} at a channel binding site. (In contrast it is quite probable that this represents a major mechanism of action for the inorganic M^{2+} and M^{3+} species (Hagiwara & Byerly, 1981; Tsien, 1983)).

The effects of the Ca^{2+} channel antagonists on Ca^{2+} currents in cardiac tissue are both frequency (use)- and voltage-dependent (Bayer *et al.*, 1975; Ehara & Kaufman, 1978; McDonald *et al.*, 1980; Pelzer *et al.*, 1982; Kass, 1982; Lee & Tsien, 1983; Tung & Morad, 1982; Kanaya *et al.*, 1983; Sanguinetti & Kass, 1984a,b), consistent with a preferential binding to other than a resting state of the channel. The extent to which verapamil/D600 and diltiazem on the one hand and nifedipine and 1,4-dihydropyridines on the other hand are qualitatively or quantitatively different in these respects awaits resolution. Frequency-dependence of action is more readily observed with verapamil/D600 and diltiazem than with the 1,4-dihydropyridines (Kass, 1982; Lee & Tsien, 1983), but verapamil/D600 and nitrendipine do enhance the decay rate of peak inward current (Lee & Tsien, 1983) and voltage-dependent antagonistic activity, increasing with decreasing membrane potential, is observed with verapamil, diltiazem and 1,4-dihydropyridines (Kanaya *et al.*, 1983; Sanguinetti & Kass, 1984a,b). Thus state-dependent block appears to be a component of the action of the several structural classes of antagonist, but the extent to which preferential binding occurs to activated or inactivated states and the effects of drugs on the kinetics of channel interconversion may well be structure-dependent.

The existence of 1,4-dihydropyridines of great structural similarity to nifedipine and other antagonists, but that serve as activator molecules (Figure 3; Schramm *et al.*, 1983) is further evidence that the 1,4-dihydropyridines do not serve as channel 'plugs'. Additionally, the actions of BAY K 8644 are voltage-dependent, activator properties being apparent at modest levels of membrane depolarization but antagonist activity dominating with increasing depolarization (Sanguinetti & Kass, 1984a; Hess *et al.*, 1984; Cohen & Chung, 1984). The properties of these activator molecules are potentially of great therapeutic importance. Furthermore, their existence suggests that the voltage-dependent 1,4-dihydropyridine binding site is located close to the permeation machinery of the Ca^{2+} channel.

lies in the sequence smooth muscle > cardiac muscle >> brain. However, more refined binding studies together with an increased understanding of coupling processes involved in Ca^{2+} channel activation may help to resolve this situation. It should be noted that binding studies, carried out largely on membrane fragments, may measure channel states of little physiological relevance (for discussion see Triggle, 1984; Triggle & Janis, 1984a,b).

5. State-dependent channel blockade. The extent to which the Ca^{2+} channel antagonists exhibit differential state blockade may be of particular significance to the definition of tissue selectivity. Thus, apparently greater frequency dependence of antagonism exhibited by verapamil/D600 and diltiazem may provide a partial basis for the more effective use of these agents over the 1,4-dihydropyridines in the control of cardiac arrhythmias. These effects are likely to be structure-dependent. Thus the voltage-dependent block of Ca^{2+} current by the 1,4-dihydropyridine derivative nicardipine (pK_a , 7.0) is intermediate between that of nisoldipine ($\text{pK}_a < 3.0$) and verapamil (pK_a , 8.7) (Sanguinetti & Kass, 1984a).
6. Antagonist-activator profile of drug. The discovery of 1,4-dihydropyridines with activator

properties indicates that a spectrum of such structures will exist with properties ranging from pure antagonist to pure agonist. Intermediate molecules, including known activators and antagonists, functioning as partial agonists may exhibit tissue-dependent ratios of agonist and antagonist activity.

7. Pathological state of tissue. Changes in Ca^{2+} mobilization pathways associated with altered tissue reactivity may also contribute to selectivity patterns. Thus, in hypertension there are several indications of altered cellular Ca^{2+} handling (Daniel & Kwan, 1981; Lipe & Moulds, 1983) and the Ca^{2+} channel antagonists have been reported to be more effective vasodilators in hypertensive states, both animal and human (Robinson *et al.*, 1982; Pedersen, 1983). Of related interest is the enhanced sensitivity of cardiac tissue to verapamil and nifedipine in the acidotic state (Briscoe & Smith, 1982). This may be of significance to the cardioprotective actions of these agents during ischaemia.

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Discussion

Church You have spoken about differences in sensitivity from tissue to tissue. Have you examined sensitivity differences in the presence of different stimuli? For example, the response to acetylcholine in dogs is not blocked by these drugs, but the response to histamine may be.

Triggie We have clearly only tackled one aspect by standardising the stimulus (potassium) and looked at the range of sensitivities of two tissues to the same group of drugs. However, there is a breadth of sensitivity which depends on the tissue, the stimulus, the concentration of stimulus and even the component of the stimulus.

B. Richardson Was the order of antagonistic

potency the same in guinea pig trachea and guinea pig bladder?

Triggie Yes, there was an entire shift of the regression line.

B. Richardson Does this imply that there is some physiochemical mechanism underlying sensitivity differences?

Triggie I doubt it, because we used three different structures in these experiments: (a) verapamil, diltiazem and D600 which are all protonated at physiological pH, (b) nifedipine which is also protonated at physiological pH, as well as (c) several neutral dihydropyridines.