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Importance of kinetic parameters for the tissue selectivity of calcium antagonists

Tissue selectivity is well known in the field of β-adrenoceptors and is usually related to the selective affinity of the ligand for a given subtype of β-adrenoceptor. Such a selectivity may be achieved by subtle modifications in the structure of the chemical agent. Calcium antagonists belong to various chemical families [1]. Each chemical family binds to specific sites located on the α_1 subunit of L-type Ca^{2+} channels [2]. These binding sites appear to interact allosterically, as shown for instance by the observation that diltiazem binding may be enhanced by dihydropyridines [1, 3].

The molecular diversity of L-type Ca^{2+} channels was first revealed by radioligand studies showing obvious differences between Ca^{2+} channels in skeletal muscle and other tissues. In addition, some antibodies raised against the skeletal α_1 subunit did not cross-react with the corresponding cardiac protein [4].

The molecular diversity of Ca^{2+} channels has now been demonstrated by molecular biology studies. Using cDNA probes prepared from rabbit skeletal muscle to screen cDNA libraries constructed from rabbit heart poly $(A)^+$ RNA, Mikami *et al.* [5] isolated cDNA clones from heart that allowed them to establish the total sequence of a rabbit cardiac α_1 subunit. The overall sequence homology between the cardiac and skeletal α_1 subunit is 66%. The transmembrane topology is identical in the two tissues but major differences exist in the regions that are not embedded in the membrane. For instance, four of the potential cyclic AMP-dependent phosphorylation sites identified in the skeletal muscle α_1 subunit are absent in the cardiac sequence and four new sites appear. Differences appear to exist also between smooth muscle and cardiac muscle Ca^{2+} channels. It is known that smooth muscle L-type Ca^{2+} channels are not modulated by cyclic AMP-dependent phosphorylation although five of the six potential cyclic AMP-dependent phosphorylation sites found in rabbit cardiac α_1 are also present in rat aortic α_1.

In physiological experiments, evidence has been provided that blockade of L-type Ca^{2+} channels may be related to ionization of the molecule. For instance, verapamil, which is almost entirely charged at pH 7.4, blocks Ca^{2+} channels in a manner dependent on the frequency of the stimulus. It has been proposed that ionized drugs can only gain access to their channel-associated binding sites via a hydrophilic pathway that is available only when channels are in an open state configuration [6, 7]. For drugs such as the dihydropyridines isradipine and nisoldipine, a major factor is not the frequency of the stimulus but the level of the membrane potential which influences the affinity of the binding sites for these Ca^{2+} channel ligands. This has been observed when measuring the affinity of these dihydropyridines in intact vascular preparations [8, 9].

Morel and Godfraind [8] have clamped membrane potential of rat aorta at different values with KCl and have measured K_{app} of isradipine and nisolidipine. They proposed that their observations could be interpreted assuming that binding sites coexist in two different conformations: one with high affinity, associated with inactivated Ca^{2+} channels in depolarized preparations, and the other with low affinity for the ligand, associated with resting Ca^{2+} channels; K_{app} being a function of the proportion of high and low affinity sites. Its value can be calculated from the equation:

$$1/K_{app} = L/K_L + (1 - L)/K_H \quad (1)$$

with L representing the proportion of low affinity binding sites, K_L the low affinity dissociation constant and K_H the high affinity dissociation constant. At KCl concentrations higher than 30 mM, the value of K_{app} of [^{3}H](+) isradipine in rat aorta was not markedly affected by variations in KCl concentration. K_{app} value at 100 mM KCl may be assumed to correspond to the K_D value for the high affinity binding sites. The high affinity dissociation constant measured in intact depolarized tissue was close to the K_D value found in membrane preparation [10, 11] indicating that prolonged depolarization and tissue homogenization favour a similar conformation of calcium channels. This is most likely, according to the modulated receptor models, i.e. the inactivated form of the calcium channel [6, 7].

When the contraction of various vessels in response to depolarization was studied in the presence of nisoldpine or isradipine, we observed that the inhibition of contraction was not immediate but reached a steady-state after a duration of depolarization dependent upon the concentration of the drug [10]. Furthermore, the kinetics of inhibition during depolarization was similar to the kinetics of binding to plasma membranes isolated from the rat aorta [12]. This suggests that the association of the dihydropyridines on their functional receptors does occur during the time of tissue depolarization. This process appears to be an important determinant of the tissue selectivity of a drug such as nisoldipine. Indeed, although there are obvious molecular differences between the α_1 subunit of Ca^{2+} channels from vascular and cardiac tissues, there is no indication that the affinity of nisoldipine is much different from these two isoforms. However, the concentration of nisoldipine required to reduce by 50% the contraction of human myocardium *in vitro* is 1500-fold higher than the concentration producing 50% inhibition of the contraction of isolated human coronary artery [13]. Such a large difference may be explained if it is assumed that, at active concentrations, drug binding to the channels of polarized cells is negligible and that drug binding does occur on inactivated channels, according to pseudo-first order association kinetics such as:

$$B = B_E\,(1\text{-}\exp\,[-k_1\,L_T\,B_{max}t\,B_E]) \quad (2)$$

where B_E is drug binding at equilibrium, k_1 is the association rate constant and L_T is the total drug concentration. The assumption that binding to resting channels is negligible is in agreement with a K_L estimate of 1.3 μM in polarized calf Purkinje fibers [6, 14], compared with a K_H value of 58 pM [8]. Triggle and Janis [15] have reported that k_1 for nisoldipine in rat heart membranes at 37° was equal to 0.67 nM min. We have observed that the K_i value of nisoldipine in human heart membranes is similar to its K_i value in rat heart membrane. It is, therefore, likely that the k_1 values are similar. Figure 1, illustrating the simulated time-course of binding of nisoldipine to Ca^{2+} channels in depolarized tissue, was drawn on the basis of Eqn 2. The time-scale of the left panel corresponds to systolic depolarization times in human myocardium (about 0.2 sec) and in the right panel to the time required to reach steady-state tonic contraction in arteries. At nisoldipine concentrations that are effective on coronary artery (0.2 nM), the time spent by Ca^{2+} channels in their high-affinity conformation is much too short for significant drug binding to occur. Calculation shows that, when used at a concentration of 300 nM, the IC_{50} in electrically paced ventricular muscle, nisoldipine should occupy about 50% of the channels by the end of the systolic contraction. The vascular selectivity reported for cinnarizine and flunarizine [16] may also be described by such a model since they too show a high affinity for inactivated channels [9].

The rather low vascular selectivity of phenylalkylamines

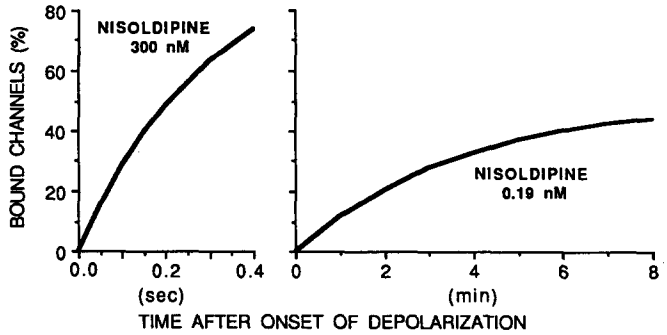

Fig. 1. Estimated time-course of binding of nisoldipine to Ca^{2+} channels in depolarized tissue.

is in agreement with this model since their affinity for Ca^{2+} channels does not appear to be enhanced by depolarization, an observation also made with diltiazem [17]. Also in the group of dihydropyridines, voltage-dependency may be absent, as for instance with nifedipine which shows a much lower vascular selectivity than nisoldipine [11].

In summary, the vascular selectivity of Ca^{2+} antagonists, that show a much higher affinity for inactivated than for resting Ca^{2+} channels, may be accounted for both by the proportion of inactivated Ca^{2+} channels and by the rate of association of drug with inactivated Ca^{2+} channels.

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