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Distinct properties of amlodipine and nicardipine block of the voltage-dependent Ca^{2+} channels $Ca_v1.2$ and $Ca_v2.1$ and the mutant channels $Ca_v1.2$/Dihydropyridine insensitive and $Ca_v2.1$/Dihydropyridine sensitive

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ABSTRACT

The binding site within the L-type Ca^{2+} channel $Ca_v1.2$ for neutral dihydropyridines is well characterized. However, the contributions of the alkylamino side chains of charged dihydropyridines such as amlodipine and nicardipine to channel block are not clear. We tested the hypothesis that the distinct locations of the charged side chains on amlodipine and nicardipine would confer distinct properties of channel block by these two drugs. Using whole-cell voltage clamp, we investigated block of wild type Ca_v 2.1, wild type $Ca_v1.2$, and $Ca_v1.2$/Dihydropyridine insensitive, a mutant channel insensitive to neutral DHPs, by amlodipine and nicardipine. The potency of nicardipine and amlodipine for block of closed (stimulation frequency of 0.05 Hz) $Ca_v1.2$ channels was not different (IC_{50} values of 60 nM and 57 nM, respectively), but only nicardipine block was enhanced by increasing the stimulation frequency to 1 Hz. The frequency-dependent block of $Ca_v1.2$ by nicardipine is the result of a strong interaction of nicardipine with the inactivated state of $Ca_v1.2$. However, nicardipine block of $Ca_v1.2$/Dihydropyridine insensitive was much more potent than block by amlodipine (IC_{50} values of 2.0 μM and 26 μM, respectively). A mutant $Ca_v2.1$ channel containing the neutral DHP binding site ($Ca_v2.1$/Dihydropyridine sensitive) was more potently blocked by amlodipine (IC_{50} = 41 nM) and nicardipine (IC_{50} = 175 nM) than the parent $Ca_v2.1$ channel. These data suggest that the alkylamino group of nicardipine and amlodipine project into distinct regions of $Ca_v1.2$ such that the side chain of nicardipine, but not amlodipine, contributes to the potency of closed-channel block, and confers frequency-dependent block.

1. Introduction

L-type voltage-gated Ca^{2+} channels are key modulators of contraction of vascular smooth and cardiac muscle, secretion of peptide hormones, and gene expression (Jones, 1998). The drugs that block L-type channels fall into three distinct chemical classes: dihydropyridines such as isradipine, amlodipine, and nicardipine; phenylalkylamines such as verapamil; and benzothiazepines such as diltiazem. Each of these classes of drugs bind the $Ca_v1.2$ α_1 subunit (Hockerman et al., 1997a; Striessnig et al., 1998) and block L-type channels in a characteristic manner (Lee and Tsien, 1983).

The binding determinants within transmembrane segments IIIS5, IIIS6, and IVS6 for dihydropyridines were identified using both biochemical and mutational approaches (Hockerman et al., 1997a; Mitterdorfer et al., 1998). A $Ca_v1.2$ channel with two mutations in transmembrane segment IIIS5 ($Ca_v1.2$/dihydropyridine insensitive; Fig. 1A) was shown to be highly insensitive to neutral DHP drugs such as isradipine (Hockerman et al., 2000), nifedipine (Liu et al., 2003), and nisoldipine (Walsh et al., 2007). Conversely, several groups constructed high affinity binding sites for DHP drugs in normally insensitive $Ca_v2.3$ (Ito et al., 1997) or $Ca_v2.1$ (Hockerman et al., 1997b; Sinnegger et al., 1997) channels (termed $Ca_v2.1$/Dihydropyridine sensitive; Fig. 1B) by insertion of several L-type-specific amino acid residues.

The studies that led to the identification of the DHP binding site in L-type Ca^{2+} channels utilized high affinity, neutral drugs. The drug amlodipine (Arrowsmith et al., 1986) contains an ionizable alkylamino on the 2 position of the DHP ring (Fig. 1C). The drug nicardipine also contains an ionizable alkylamino group, but on the 5 position of the DHP ring (Fig. 1C) (Iwanami et al., 1979). The DHP pharmacophore is commonly described as a "flat boat" with the 4-aryl group in a psuedoaxial position forming the bowspirit of the boat and the DHP-ring nitrogen as the stern. Thus, the two non-equivalent sides of the molecule can be designated as "port" or "starboard", based on orientation to the 4-aryl group (Goldman and Stoltefuss, 1991). In the crystal structure of the most active form of amlodipine, S-(−), the alkylamino substituent is on the port side of the molecule (Goldmann et al., 1992). In contrast, a solution NMR structure of the most active form of nicardipine, R-(+), placed the alkylamino group on the

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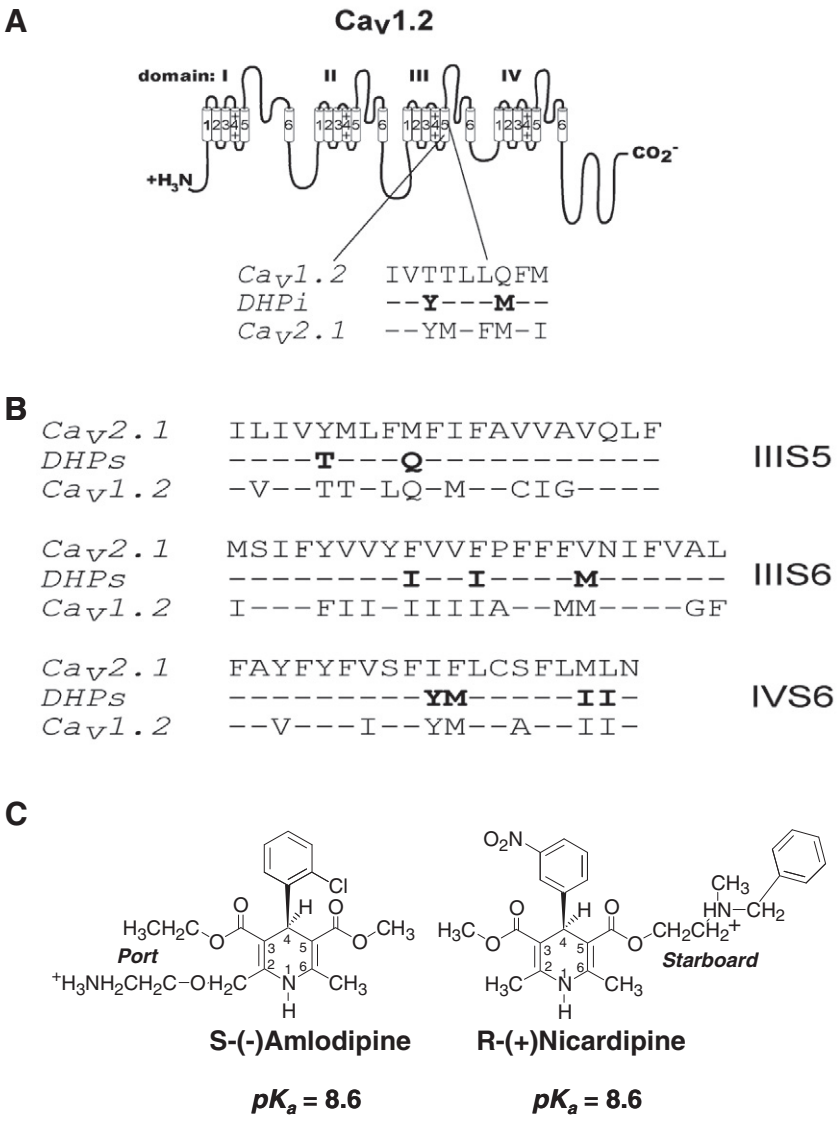

Fig. 1. Structural features of the α_1 subunit of Ca_v channels, amlodipine and nicardipine— A. The structure of amlodipine and nicardipine. Note that the ionizable alkylamino groups are on opposite sides of the DHP ring relative to the pseudoaxial 4-aryl group. B. Topology of Ca_v Channels. Shaded cylinders represent transmembrane segments (1–6) organized into four homologous domains (I–IV). The C- and N-terminal domains are intracellular. The position of transmembrane domain IIIS5 is indicated. The alignment of transmembrane domain IIIS5 amino acids residues for $Ca_v1.2$, $Ca_v1.2$/Dihydropyridine insensitive, and $Ca_v2.1$ is shown. Dashes represent identity between all three channels. Bold letters indicate the amino acids inserted into $Ca_v1.2$/Dihydropyridine insensitive. The L-type channel $Ca_v1.2$/Dihydropyridine insensitive is a double mutant that substitutes the $Ca_v2.1$ amino acids in positions 1039 and 1043 (T 1039 to Y + Q 1043 to M). $Ca_v1.2$/Dihydropyridine insensitive is highly insensitive to neutral dihydropyridines such as isradipine, nifedipine, and nisoldipine but retains normal sensitivity to the benzothiazepine diltiazem (Hockerman et al., 2000; Liu et al., 2003; Walsh et al., 2007). Fig. 1B shows the amino acid alignments of all three transmembrane domains containing the amino acid residues critical for DHP block, IIIS5, IIIS6, and IVS6, from $Ca_v2.1$ and $Ca_v1.2$. The $Ca_v1.2$-specific residues indicated were inserted into $Ca_v2.1$ to construct the mutant channel $Ca_v2.1$/Dihydropyridine sensitive. B. The amino acid sequences for transmembrane segments IIIS5, IIIS6, and IVS6 in wild type $Ca_v2.1$, $Ca_v2.1$/Dihydropyridine sensitive, and WT $Ca_v1.2$ are aligned. The nine amino acids in bold font were inserted into $Ca_v2.1$ to create $Ca_v2.1$/Dihydropyridine sensitive.

starboard side (Belciug and Ananthanarayanan, 1994). We hypothesized that the distinct positions of these charged groups in amlodipine and nicardipine could confer distinct channel blocking properties to these drugs. Therefore, we compared the ability of these two clinically relevant charged dihydropyridine drugs to block the L-type channel $Ca_v1.2$ (Snutch et al., 1991), the non-L-type channel $Ca_v2.1$ (Starr et al., 1991), and the mutant channels $Ca_v1.2$/Dihydropyridine insensitive (Hockerman et al., 2000) and $Ca_v2.1$/Dihydropyridine sensitive (Hockerman et al., 1997b). Our results suggest that nicardipine, but not amlodipine, accesses binding determinates outside of the canonical dihydropyridines site in mediating tonic and frequency-dependent block of the L-type Ca^{2+} channel $Ca_v1.2$.

2. Materials and methods

2.1. Construction of wild type and mutant Ca^{2+} channels

The $Ca_v1.2$ (Snutch et al., 1991), $Ca_v2.1$ (Starr et al., 1991), and $Ca_v1.2$/Dihydropyridine insensitive (Hockerman et al., 2000) channels were sub-cloned into the pCDNA3 expression vector (Invitrogen, Carlsbad, CA). The DHP sensitive $Ca_v2.1$ mutant, $Ca_v2.1$/Dihydropyridine sensitive (Hockerman et al., 1997b), was in the expression vector pMT-2 (Genetics Institute, Boston, MA). The desired mutations were verified, and the integrity of the clones was confirmed by cDNA sequencing and extensive restriction digest analysis.