The binding of dihydropyridine (PN200-110) to skeletal muscle microsomes (which were 84% sealed inside-out vesicles) was not influenced by the addition of calcium or magnesium nor by addition of their chelators (EDTA or EGTA) unless the vesicles were pre-treated with the calcium-magnesium ionophore A23187 and EDTA to remove entrapped cations. Separation of inside-out vesicles from right-side-out vesicles by wheat germ agglutinin chromatography revealed that only the right-side-out vesicles exhibited a calcium-, magnesium-, and chelator-dependent binding of PN200-110. Dihydropyridine binding to cardiac sarcolemma membranes (which were 46% inside-out) and to solubilized skeletal muscle membranes was inhibited by EDTA and could be fully restored by 10 μM calcium or 1 mM magnesium. Calcium increased PN200-110 binding to partially purified rabbit skeletal muscle calcium channels from 3.9 pmol/mg protein to 25.5 pmol/mg protein with a pK0.5 = 6.57 ± 0.059 and a Hill coefficient of 0.56 ± 0.04. Magnesium increased binding from 0.7 pmol/mg protein to 16.8 pmol/mg protein with a pK0.5 = 3.88 ± 0.085 and a Hill coefficient of 0.68 ± 0.074.

These studies suggest that calcium binding to high affinity sites or magnesium binding to low affinity sites on the extracellular side of skeletal muscle T-tubule calcium channels regulates dihydropyridine binding. Further, similar calcium and magnesium binding sites exist on the cardiac calcium channel and serve to allosterically regulate dihydropyridine binding.

Voltage-dependent calcium channels initiate and modulate many important cellular functions including muscle contraction and secretory processes (1-3). The slow inward calcium current through these channels is blocked by calcium antagonists including; dihydropyridines (nifedipine and felodipine), benzothiazepine (diltiazem), and phenylalkylamines (verapamil) (4-6). The dihydropyridines (DHP) are the most potent and most extensively studied class of calcium antagonists (5, 6). The DHP receptor exists with high density in the transverse tubules of skeletal muscle, and it has been purified (7-9) and its amino acid sequence predicted (10) from this source. Further, the purified DHP receptor reconstitutes calcium channel activity (11), and microinjection of DHP receptor complementary DNA restores slow calcium currents and excitation-contraction coupling in dysgenic mouse muscle myotubes (12). This suggests that the DHP receptor is a calcium channel and that it is essential for excitation-contraction coupling in skeletal muscle.

Several differences have been observed with DHP binding to its receptors in different tissue types. DHP blockade of calcium channel in smooth and cardiac muscle blocks calcium influx and promotes relaxation (6), but DHPs appear to have little effect on skeletal muscle (13). DHP binding to brain and cardiac microsomes is inhibited by EDTA and restored by addition of calcium or magnesium (14-16). DHP binding to skeletal muscle microsomes and T-tubules is virtually insensitive to EDTA or EGTA treatment suggesting DHP binding to skeletal muscle receptors is not dependent on divalent cations (17-19). DHP binding to solubilized and purified skeletal muscle calcium channels has been reported to be sensitive to EDTA and EGTA treatment (20-22), but thus far calcium and/or magnesium dependence of DHP binding has not been studied. The present study was undertaken to determine the effects of calcium and magnesium on the regulation of DHP binding to skeletal muscle calcium channels and to determine if the calcium binding sites which regulate DHP binding exist on the extracellular or intracellular side of the T-tubule membrane.

**EXPERIMENTAL PROCEDURES**

(+)-(3H)PN200-110 (80 μCi/tmol) was purchased from Amersham Corp. Felodipine was the generous gift of A. B. Hassle Pharmaceutical, Molndal, Sweden. WGA lectin, digitonin, A23187, N-acetylglucosamine, and PMSF were obtained from Sigma. Alfa-Gel 10 and Bradford reagents were obtained from Bio-Rad. Protein concentration was measured by the method of Bradford (27).

**Skeletal Muscle Microsome Preparations**

Fresh skeletal muscle were dissected from the hind leg and backs of rabbits. Microsomes from fresh muscle were prepared as described by Moczydlowski and Latorre (23) with slight modifications. Briefly, the muscles were homogenized (Waring Blender, high speed for 30 s, twice) in an ice-cold buffer (1.3, w/v) of 0.3 M sucrose, 40 mM MOPS, pH 7.0, 0.1 mM PMSF. The homogenate was centrifuged at 10,000 × g for 30 min. The pellet was resuspended in the starting buffer and then homogenized and centrifuged a second time. The supernatants were filtered through cheesecloth and combined. Solid KCl was added to a final concentration of 0.8 M (to solubilize contractile proteins) followed by centrifugation at 100,000 × g for 30 min. This pellet was resuspended in 20 mM MOPS, pH 7.0, 0.1 mM PMSF and used as skeletal muscle microsomes in the experiments. Fresh skeletal muscle microsomes were solubilized with 2% digitonin in 20 mM MOPS, pH 7.0 and 0.1 mM PMSF on ice for 60 min. The insoluble material was
removed by centrifugation (100,000 × g) for 60 min. These digitonin extracts (supernatants) were defined as solubilized microsomes. EDTA (2 mM) was added to the solubilized microsomes, and they were applied to a WGA Affi-Gel 10 column which was equilibrated with 0.1% digitonin, 90 mM MOPS, and 0.1 mM PMSF. The partially purified DHP receptor was eluted by 0.2 M GluNAC and pooled as partially purified calcium channels. After solubilization, preparation of calcium channels was completed within 90 min. The isolated calcium channel partially purified by this rapid procedure bound PN200-110 with a B_{max} = 300 pmol/mg protein and with a K_{D} = 3.3 nM in the presence of 10 mM digitonin. Eight percent sodium dodecyl sulfate-polyacrylamide gel electrophoresis with silver stain of 10% of partially purified calcium channel revealed four proteins. The first had a molecular mass of 130 kDa under nonreducing conditions and a molecular mass of 140 kDa in the presence of 10 mM dithiothreitol (presumably α1). The second exhibited a molecular mass of 150 kDa under both reducing and nonreducing conditions (presumably α2). A 100-kDa protein represented approximately 10% of the total protein and was presumably contaminating Ca-ATPase. A 55-kDa protein stained heavily with silver (presumably β). Higher percentage gels indicated a lower molecular mass protein (31 kDa), presumably γ.

### Cardiac Sarcolemma Preparation

Cardiac sarcolemma membranes were prepared from bovine ventricular muscles by the method of Kuwamura and Kunitz (35).

#### Radioligand Binding Studies

**Binding Assay with Microsomes**—The binding of (+)-[^H]PN200-110 to microsomes was assayed as follows. Skeletal muscle microsomes (50-50 mg protein) or cardiac sarcolemmas (50-180 mg protein) were incubated in 0.5 ml of solution containing 200 mM MOPS, pH 7.0, 90 mM KCl, 2 mM EGTA (or EDTA) under different free Ca^{2+} (or Mg^{2+}) concentration with the indicated concentration of (+)-[^H]PN200-110 for 30 min at 30°C. After incubation, samples were withdrawn and filtered through a Whatman GF/C glass fiber under reduced pressure. The filter was washed with 4 ml ice-cold 50 mM MOPS, pH 7.0, and the radioactivity bound to the filter was measured using a LS 7000 Beckman liquid scintillation counter.[^H] PN200-110 binding to three types of skeletal muscle microsomes was studied. The first was defined as nontreated original microsomes. For the second type, the original skeletal muscle microsomes were suspended in 200 mM MOPS, pH 7.0, 90 mM KCl, 2 mM EDTA, 10 μM A23187 on ice for 20 min. These were defined as EDTA, A23187-treated microsomes. For the third type, A23187 (10 μM) and EDTA (2 mM) treated microsomes were centrifuged at 100,000 × g for 30 min and then resuspended in 200 mM MOPS, pH 7.0, 90 mM KCl, 2 mM EDTA, and 10 μM A23187. These were defined as A23187, EDTA-treated microsomes.

PN200-110 Binding Assay with Partially Purified Calcium Channel—This assay was carried out by a modification of the procedure of Glossman and Ferry (22). The samples (partially purified calcium channel, 2 μg of protein) were incubated with 0.5 ml of solution containing 0.1% digitonin, 1 mg/ml BSA as a carrier protein, 200 mM MOPS, pH 7.0, 90 mM KCl, 2 mM EGTA (or EDTA)), and the indicated concentration of (+)-[^H]PN200-110 at 30°C for 30 min under the different free Ca^{2+} (pMg^{2+}) conditions. Then 4 ml of ice-cold 10% polyethylene glycol, 50 mM MOPS, pH 7.0, was used to precipitate the solubilized microsomes or partially purified calcium channel onto Whatman GF/C filters. Nonspecific binding was determined in the presence of 2 μM cold felodipine.

#### The Determination of Free Ca^{2+} and Mg^{2+} Concentrations

Free Ca^{2+} and Mg^{2+} concentrations were calculated as described by Johnson and Potter (24). Ca^{2+} titrations were conducted in a buffer composed of 200 mM MOPS, pH 7.0, 90 mM KCl, 2 mM EGTA. Mg^{2+} titrations were conducted in the same buffer containing 2 mM EDTA instead of 9 mM KCl. To determine the concentration of (+)-[^H]PN200-110 at 30°C for 30 min under the different free Ca^{2+} (pMg^{2+}) conditions. Then 4 ml of 0.1% dithiothreitol, 1 mg/ml BSA at 37°C, filtered on a 0.45-μm HA Millipore filter, washed twice with 4 ml of the above buffer at 4°C, and then counted.

#### Data Analysis

Hill plots were analyzed by an iterative nonlinear regression Hill plot program, and B_{max} was determined by the best fit of the data to Hill equation.

### RESULTS

**Effect of EGTA, EDTA, Calcium, and Magnesium on PN200-110 Binding to Skeletal Muscle Microsomes**—Fig. 1A shows that PN200-110 binding to skeletal muscle microsomes was not affected by the addition of 2 mM EDTA nor by the addition of calcium over the range of pCa 9.0 to pCa 3.0. When microsomes were treated with the calcium/magnesium ionophore, A23187, in the presence of 2 mM EDTA and then resuspended in a 2 mM EGTA buffer PN200-110 binding exhibited a sensitivity to calcium chelator and to calcium. Addition of EGTA reduced PN200-110 binding from 1693 fmol/mg protein to 380 fmol/mg protein. Calcium was effective in restoring binding (to 1287 fmol/mg protein) in these A23187-chelator-treated microsomes. Fig. 1B indicates that the addition of EGTA and magnesium (from pMg 9.0 to pMg 3.0) does not affect PN200-110 binding to skeletal muscle microsomes. In the presence of A23187, strong dependence on EDTA and magnesium is observed. PN200-110 binding was reduced from 1329 fmol/mg protein to 371 fmol/mg protein by EDTA and was increased from 371 fmol/mg protein to 1126 fmol/mg protein at pMg 3.0. These results suggest that skeletal muscle microsomes do not exhibit a calcium- or magnesium-dependent binding of PN200-110 unless they are first treated with ionophore and EDTA to remove entrapped divalent cations. Under these conditions both calcium and magnesium are effective in restoring PN200-110 binding. This suggests that calcium and/or magnesium are bound inside these membrane vesicles and presumably support PN200-110 binding. These divalent cations cannot be removed by EDTA or EGTA treatment of the sealed membrane but can be removed in the presence of ionophore and chelator.

**Effects of Membrane Sideines on Divalent Cation-dependent PN200-110 Binding to Skeletal Muscle Microsomes**—The T-tubule fraction of skeletal muscle microsomes, which contain the DHP binding sites, have been reported to be more than 90% inside-out, with their extracellular surface being encapsulated inside the resealed microsome (28). We have used WGA column chromatography to separate inside-out and right-side-out vesicles in our skeletal muscle microsome preparation. The right-side-out vesicles presumably bind to WGA (by virtue of their exposed glycoproteins) while the inside-out vesicles pass through the column. When skeletal microsomes (total protein 66 mg) were applied to the WGA column, 0.2 mg of microsomes bound to the column and was eluted by GluNAC. In the presence of 1.2 nM (+)-[^H]PN200-110,
PN200-110 binding to these right-side-out microsomes was $1.3 \pm 0.21$ pmol/mg protein in the presence of 2 mM EDTA. $5.6 \pm 0.42$ pmol/mg protein at pCa = 6.0, and $4.9 \pm 0.32$ pmol/mg protein at pMg = 5.0. These results indicate that those vesicles which bound to the WGA column (presumably inside-out) constituted the majority of our microsome preparation (93%) and did not exhibit DHP binding unless they were first pretreated with calcium ionophore and chelator (as in Fig. 1). These studies suggest that divalent cation binding to sites on the extracellular side of skeletal muscle membranes control DHP binding.

Calcium and Magnesium Dependence of PN200-110 Binding to Cardiac Sarcolemma Membranes—Unlike skeletal muscle microsomes and T-tubules which are 90% sealed inside-out vesicles, cardiac sarcolemma membranes reseal with approximately 50% inside-out 50% outside-out orientation. Consistent with this, [3H]digoxin binding to our cardiac sarcolemma membranes indicated that 46% of these vesicles were in an inside-out configuration (data not shown). If this is the case, then PN200-110 binding to cardiac sarcolemma vesicles should be sensitive to EDTA, EGTA, calcium, and magnesium. Fig. 24 shows the calcium and magnesium dependence of PN200-110 binding to cardiac sarcolemma. Calcium increased PN200-110 binding from 100 fmol/mg protein (pCa = 9.0) to 497 fmol/mg protein (pCa = 3.0) with a $K_{Ca}$ = 6.39 \pm 0.09 and a Hill coefficient of 0.67 \pm 0.03 ($r = 0.983$). Magnesium increases PN200-110 binding from 141 fmol/mg protein (pMg = 9.0) to 390 fmol/mg protein (pMg = 2.0) with a $K_{Mg}$ = 3.86 \pm 0.12 and a Hill coefficient of 0.672 \pm 0.16 ($r = 0.990$). At pCa = 9.0 PN200-110 bound with a $B_{max}$ = 180 fmol/mg protein and $K_d$ = 0.33 nM. At pCa = 3.0, the $B_{max}$ increased to 750 fmol/mg protein while the $K_d$ was not affected ($K_d = 0.38$ nM) (Fig. 2B). Similarly at pMg = 9.0, PN200-110 bound with a $B_{max}$ = 250 fmol/mg protein and $K_d$ = 0.33 nM, while at pMg = 3.0, PN200-110 bound with a $B_{max}$ = 640 fmol/mg protein and a $K_d$ = 0.34 nM (Fig. 2C). These studies indicate that cardiac sarclemma membrane PN200-110 binding is unlike skeletal muscle microsome PN200-110 binding in that it is dependent on calcium and magnesium in the absence of calcium ionophore pretreatment. Further, calcium and magnesium bind to sites on the cardiac calcium channel and increase PN200-110 binding by increasing $B_{max}$ with little effect on $K_d$.

Calcium and Magnesium Dependence of PN200-110 Binding to Solubilized Skeletal Muscle Microsomes—Skeletal muscle microsomes were solubilized with digitonin, and the calcium and magnesium dependence of PN200-110 binding was determined (data not shown). Calcium increased PN200-110 binding from 290 fmol/mg protein to 1740 fmol/mg protein; this change occurred with an apparent $pK_{Ca}$ of 6.0 \pm 0.08 with a Hill coefficient of 0.96 \pm 0.15 ($r = 0.995$). As free magnesium is increased from pMg 6.0 to pMg 2.0, PN200-110 binding increased from 216 fmol/mg protein to 1524 fmol/mg protein. Magnesium produced this increase in PN200-110 binding with a $pK_{Mg}$ of 3.92 \pm 0.18 with a Hill coefficient of 0.7 \pm 0.18 ($r = 0.984$). These results indicate that calcium (by binding to high affinity sites) and magnesium (by binding to low affinity sites) promote PN200-110 binding to solubilized skeletal muscle microsomes.

Calcium and Magnesium Dependence of PN200-110 Binding
FIG. 2. The Ca\textsuperscript{2+} and Mg\textsuperscript{2+} dependence of PN200-110 binding to cardiac sarcolemma microsomes. PN200-110 binding as a function of calcium (C) and as a function of magnesium (O) is shown in Panel A. This data was fit with a \( pK_{Ca} = 6.39 \pm 0.09 \) and Hill coefficient of 0.674 \pm 0.03 \((r = 0.983)\) for calcium and \( pK_{Mg} = 3.86 \pm 0.12 \), Hill coefficient of \( 0.627 \pm 0.10 \) \((r = 0.990)\) for magnesium. Each point represents the mean \( \pm \) S.E. \((n = 6)\); the points without bars had a S.E. less than 2%. [\textsuperscript{3}H]PN200-110 binding as a function of calcium (C) and magnesium dependence of PN200-110 binding to partially purified calcium channel. PN200-110 binding was conduct as described under “Experimental Procedures.”

FIG. 3. The Ca\textsuperscript{2+} and Mg\textsuperscript{2+} dependence of PN200-110 binding to partially purified calcium channel. PN200-110 binding as a function of calcium (C) and magnesium (O) is shown in Panel A. Hill coefficient and \( pK_{Ca} \) for calcium were 0.36 \pm 0.040 and 6.57 \pm 0.059 \((r = 0.998)\), respectively. Hill coefficient and \( pK_{Mg} \) for Mg were 0.68 \pm 0.07 and 3.86 \pm 0.085 \((r = 0.994)\), respectively. In both the Ca\textsuperscript{2+} and Mg\textsuperscript{2+} titration, each value represents mean \( \pm \) S.E. \((n = 4)\). S.E. not shown are less than 5%. The concentrations of [\textsuperscript{3}H]PN200-110 used in the Ca\textsuperscript{2+} and Mg\textsuperscript{2+} titrations were 0.59 \pm 0.02 and 0.60 \pm 0.04 nM \((mean \pm S.D.)\), respectively, and protein was 4 \mu g/ml in each assay. Ca\textsuperscript{2+} and Mg\textsuperscript{2+} titrations and PN200-110 binding were conducted as described under “Experimental Procedures.” Panel B shows Scatchard plots of PN200-110 binding to partially purified calcium channel at pCa = 9.0 \((\bullet, K_d = 2.7 nM, B_{max} = 53 pmol/mg protein)\), pCa = 7.0 \((\Delta, K_d = 3.6 nM, B_{max} = 106 pmol/mg protein)\), and at pCa = 3.0 \((\Box, K_d = 3.3 nM, B_{max} = 232 pmol/mg protein)\). Panel C shows Scatchard plots of PN200-110 binding to partially purified calcium channel at pMg = 9.0 \((\bullet, K_d = 0.34 nM, B_{max} = 180 fmol/mg protein)\), pMg = 4.0 \((\Delta, K_d = 3.8 nM, B_{max} = 100 pmol/mg protein)\), and at pMg = 2.7 \((\Box, K_d = 3.7 nM, B_{max} = 190 pmol/mg protein)\). B/F, bound/free.

**DISCUSSION**

Dihydropyridines have been shown to bind to receptors in skeletal muscle, cardiac muscle, smooth muscle, and brain with affinities in the 0.1-3.6 nM range (5). DHP binding to brain, cardiac, and smooth muscle microsomes has been reported to be chelator, calcium, and magnesium dependent. In these tissues DHP binding is inhibited by EDTA and is half-maximally restored by 3-10 \mu M calcium and by 80-120 \mu M magnesium (16, 17). DHP binding to skeletal muscle microsomes has, however, been reported to be independent of EGTA and EDTA and independent of calcium or magnesium (17-19). On the other hand, DHP binding to solubilized microsomes from skeletal muscle is inhibited by mental chelating agents (20). Further, Flockerzi et al. (21) have shown DHP binding to purified skeletal muscle calcium channel to be inhibited by 1 mM EGTA. However, there have been no reports of the calcium and/or magnesium dependence of DHP binding to solubilized or purified DHP receptor from skeletal muscle.

Skeletal muscle T-tubules are more than 90% inside-out vesicles (28), while microsomes from cardiac and brain tissue
Calcium Binding Calcium Channels

are approximately 50% inside-out. Our skeletal muscle microsomes are predominantly (at least 84%) sealed inside-out membrane vesicles. With these vesicles a calcium-magnesium dependent binding of dihydropyridine can be observed only after pretreatment with ionophore and EDTA. This suggests that calcium or magnesium entrapped within these inside-out microsomes can facilitate high levels of PN200-110 binding and that divalent cation binding sites accessible from the extracellular side of the cell membrane regulate DHP binding. Consistent with this, when our skeletal muscle microsomes are subjected to WGA column chromatography, over 90% of the membranes do not bind WGA (inside-out) and show no EDTA or calcium-magnesium dependence of PN200-110 binding in the absence of ionophore. The small amount of microsomes which do bind to WGA (right-side-out) exhibit a strong EDTA, calcium-magnesium dependent PN200-110 binding. These results suggest that the extracellular side of skeletal muscle membrane has high affinity calcium binding sites which are responsible for regulating the calcium and magnesium dependence of DHP binding. Consistent with this, cardiac sarcolemma membranes, which were only 46% sealed inside-out vesicles, exhibited PN200-110 binding which was sensitive to EDTA, calcium, and magnesium in the absence of ionophore pretreatment (Fig. 2).

Previous studies could not demonstrate a chelator, calcium, or magnesium dependence of DHP binding to skeletal muscle microsomes (Refs. 17-19 and see Ref. 34) presumably because these sealed inside-out vesicles have their extracellular face (and divalent cations) entrapped within the resealed membrane vesicles and their intracellular face exposed to buffer. Our studies suggest that the differences reported for the calcium and magnesium dependence of DHP binding between skeletal muscle microsomes and cardiac, brain, or smooth muscle microsomes may result only from differences in the sidedness of the membrane vesicles.

Recently Schilling (30) has reported a similar calcium and magnesium dependence of DHP binding to cardiac sarcolemma, but he concluded that calcium and/or magnesium binding sites on the intracellular side of cardiac sarcolemma microsomes regulate DHP binding. This conclusion was reached because EDTA reduced ouabain binding to a similar extent as it reduced DHP binding in cardiac sarcolemma vesicles which were 27% inside-out, 44% right-side-out, and 29% leaky. Conclusions based on such correlations may be inaccurate, especially when a high percentage of the vesicles is leaky and roughly equal percentages are inside-out and right-side-out. We feel that conclusions on the sidedness of the calcium binding sites which regulate DHP binding can be more accurately determined in skeletal muscle microsomes which are nearly 90% sealed vesicles in the inside-out orientation. Further, separation of the inside-out vesicles from the right-side-out vesicles using wheat germ agglutinin chromatography has allowed us to conclusively state that calcium binding sites accessible to the extracellular space control DHP binding to skeletal muscle membranes.

In the solubilized or partially purified form, channel nans from skeletal muscle exhibit calcium and magnesium dependent DHP binding which is similar to that shown for calcium channels from heart, brain, and smooth muscle (compare Figs. 2 and 3). This suggests that high affinity calcium binding sites on the extracellular side of the membrane which regulate hydrophobic ligand binding to the channel may represent a fundamental characteristic of voltage dependent calcium channels. There may be several classes of calcium binding sites on the calcium channel. Hess and Tsien (29) and Almers and McCleskey (30) have suggested that there are at least two high affinity calcium binding sites on the channel and that calcium binding to one of these sites (on the extracellular side of the channel) provides electrostatic repulsion of calcium bound to another calcium binding site (presumably deeper within the channel) to facilitate ion permeation through a multi-ion single file pore. Kostyuk et al. (31) have postulated high affinity calcium-magnesium (pK_{Ca} = 4.2) or calcium-magnesium (pK_{CM} = 3.9) binding sites on the extracellular side of the calcium channel which controls the structure and ion selectivity of the channel. It is possible that these extracellular high affinity calcium binding sites which regulate DHP binding are the same calcium binding sites postulated by Kostyuk et al. (31) to control the ion selectivity of the calcium channel or by Hess and Tsien (29) to facilitate electrostatic repulsion of calcium through a multi-ion single file pore.

Consistent with this, DHP binding to skeletal muscle microsomes occurs with high affinity (pK_{Ca} = 6.5) over a broad range of calcium (3 log units) and with negative cooperativity, indicating that several classes of high affinity calcium sites may be present on the calcium channels. Magnesium also increases DHP binding to partially purified calcium channels, although it is approximately 500-fold less effective than calcium (pK_{CM} = 3.9). Further, in both heart and skeletal muscle, saturating calcium is capable of supporting 10-20% more PN200-110 binding than saturating magnesium (Figs. 2 and 3). This suggests that some component of DHP binding may be calcium-specific. Our studies indicate that similar high affinity calcium binding sites exist on skeletal muscle and cardiac muscle calcium channels and that these sites allosterically regulate DHP binding. It is likely that calcium (or higher concentrations of magnesium) result in the conversion from a low affinity DHP binding state (that might not be detected under the conditions of the binding assay) to a high affinity DHP binding state. Similar conclusions have been drawn from DHP binding to brain, heart, and smooth muscle calcium channels (15-17). Since calcium binding enhances dihydropyridine binding in all of these tissues, then by reciprocity, DHP binding should be sensitive to calcium binding. If this is the case, then dihydropyridines could perhaps block calcium channels by increasing calcium (or magnesium) affinity and slowing calcium flux through the ion pore as we (32) and Glossmann et al. (17) have previously suggested. If DHP increases the calcium affinity of the calcium channel it is possible that this could result in calcium binding so tightly that it could not pass through a multi-ion single file ion pore.

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