Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 2792–2796, May 1986
Biochemistry

High-affinity antibodies to the 1,4-dihydropyridine Ca^{2+}-channel blockers

(nitrendipine/nifedipine/nisoldipine/Ca^{2+} antagonists/dihydropyridine receptor)

KEVIN P. CAMPBELL, ALAN SHARP, MOLLY STROM, AND STEVEN D. KAHL

Department of Physiology and Biophysics, The University of Iowa, Iowa City, IA 52242

Communicated by James O. Davis, November 6, 1985

ABSTRACT **Antibodies with high affinity and specificity for the 1,4-dihydropyridine Ca^{2+}-channel blockers have been produced in rabbits by immunization with dihydropyridine–protein conjugates. Anti-dihydropyridine antibodies were found to specifically bind [^{3}H]nitrendipine, [^{3}H]-nimodipine, [^{3}H]nisoldipine, and [^{3}H]PN 200-110 (all 1,4-dihydropyridine Ca^{2+}-channel blockers) with high affinity, while [^{3}H]verapamil, [^{3}H]diltiazem, and [^{3}H]trifluoperazine were not recognized. The average dissociation constant of the [^{3}H]nitrendipine–antibody complex was 0.06 (±0.02) × 10^{-9} M for an antiserum studied in detail and ranged from 0.01 to 0.24 × 10^{-9} M for all antisera. Inhibition of [^{3}H]nitrendipine binding was specific for the 1,4-dihydropyridine Ca^{2+}-channel modifiers and the concentrations required for half-maximal inhibition ranged between 0.25 and 0.90 nM. Structurally unrelated Ca^{2+}-channel blockers, calmodulin antagonists, inactive metabolites of nitrendipine, and UV-inactivated nisoldipine did not modify [^{3}H]nitrendipine binding to the anti-dihydropyridine antibodies. Dihydropyridines without a bulky substituent in the 4-position of the heterocycle were able to displace [^{3}H]nitrendipine binding, but the concentrations required for half-maximal inhibition were >800 nM. In summary, anti-dihydropyridine antibodies have been shown to have high affinity and specificity for the 1,4-dihydropyridine Ca^{2+}-channel blockers and to exhibit dihydropyridine binding properties similar to the membrane receptor for the 1,4-dihydropyridine Ca^{2+}-channel blockers.**

Ca^{2+} influx via voltage-dependent Ca^{2+} channels initiates and modulates cardiac and smooth muscle contraction (1–3). Drugs that block these voltage-dependent Ca^{2+} channels are used for the treatment of various cardiovascular disorders and are referred to as Ca^{2+}-channel blockers (4–8). Nifedipine, nitrendipine, nisoldipine, and related 1,4-dihydropyridines are the most potent class of Ca^{2+}-channel blockers, and radiolabeled dihydropyridines have been shown to bind with high affinity to membranes isolated from cardiac, skeletal, and smooth muscle (9–13). To better understand the mechanism of action of the dihydropyridine Ca^{2+}-channel blockers, investigators have undertaken the identification of the membrane receptor for these drugs in cardiac and skeletal muscle (14–16) and the isolation and purification of the membrane receptor from enriched sources (i.e., transverse tubular membrane of skeletal muscle) (17–19).

Considerable advances have been made in recent years in the structural and functional characterization of membrane receptors and ion channels by using immunological probes (20–26). Antibodies have been raised against membrane receptors or ion channels directly by immunization with purified membrane proteins or indirectly by immunization with anti-ligand antibodies to produce anti-idiotypic antibodies. Anti-idiotypic antibodies have been very useful in studying receptor structure and function, because these antibodies bind to the ligand binding site on the receptor and in some cases can modify receptor function (21). Anti-ligand antibodies (20, 21, 23, 26–29) have been used to study structure–activity relationships between the ligand and antibody and as specific molecules that can compete with the receptor for the ligand.

We report here the production of high-affinity antibodies specific for the 1,4-dihydropyridine Ca^{2+}-channel blockers by using either a bovine serum albumin–dihydropyridine conjugate or a keyhole limpet hemocyanin–dihydropyridine conjugate. The dihydropyridine–protein conjugates were prepared by using an affinity analog of nifedipine—1,4-dihydro - 2,6 - dimethyl - 4 - (2 - isothiocyanatophenyl) - 3,5-pyridinedicarboxylic acid dimethyl ester (nifedipine-isothiocyanate)—that can covalently react with free amino groups in both proteins to form a covalent dihydropyridine–protein conjugate. Anti-dihydropyridine antibodies have been found to bind [^{3}H]nitrendipine with high affinity and specificity. The dihydropyridine binding properties of the anti-dihydropyridine antibodies suggest that the antibodies resemble the membrane receptor for the 1,4-dihydropyridine Ca^{2+}-channel blockers with respect to affinity and specificity of 1,4-dihydropyridine binding.

MATERIALS AND METHODS

Preparation of Dihydropyridine–Protein Conjugates. Nifedipine-isothiocyanate was used to couple nifedipine covalently to various carrier proteins (i.e., bovine serum albumin, ovalbumin, and keyhole limpet hemocyanin). The carrier protein (10 mg) was first dissolved in 10 ml of 100 mM sodium bicarbonate (pH 9.0) and then incubated with 0.6 mg of nifedipine-isothiocyanate (in ethanol) for 24 hr at 37°C with shaking in the dark. The final concentrations of nifedipine-isothiocyanate and ethanol in the incubation medium were 168 μM and 0.65%, respectively. Incorporation of nifedipine-isothiocyanate was monitored by including [^{3}H]nifedipine-isothiocyanate (2.5 μCi/ml; 1 Ci = 37 GBq) during the formation of the conjugate. Analysis of the total counts of [^{3}H]nifedipine incorporated into bovine serum albumin showed that ≈1 mol of [^{3}H]nifedipine was incorporated per mol of bovine serum albumin.

Immunization and Antibody Preparation. New Zealand White rabbits were first bled to obtain preimmune sera and then immunized intramuscularly and subcutaneously at multiple sites along the back and legs. Rabbits received 0.5 mg of dihydropyridine conjugate in Freund's complete adjuvant on

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: nifedipine-isothiocyanate, 1,4-dihydro-2,6-dimethyl-4-(2-isothiocyanatophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester; [^{3}H]nifedipine-isothiocyanate, 1,4-dihydro-2,6-[3-*methyl*-^{3}H]-dimethyl-4-(2-isothiocyanatophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester.

day 0 followed by 0.5 mg in Freund's incomplete adjuvant every 2–3 weeks. The keyhole limpet hemocyanin–dihydropyridine conjugate was mixed with an equal volume of aluminum hydroxide and phosphate-buffered saline and stirred for 1 hr at 4°C before emulsification in an equal volume of Freund's adjuvant. Rabbits were bled after their third immunization every 2–3 weeks. Antisera were either used directly or antibodies were partially purified from antisera by ammonium sulfate precipitation at 4°C for 24 hr.

[^{3}H]Nitrendipine Binding: Protein A-Sepharose Assay. [^{3}H]Nitrendipine binding was performed in triplicate using 1.5-ml Eppendorf tubes in the dark. Each assay tube contained 150 mM NaCl, 10 mM Tris·HCl (pH 7.2), 1 nM [^{3}H]nitrendipine, 10 μl of antiserum, 50 μl of protein A-Sepharose 4B in a final vol of 1.0 ml. Samples were slowly mixed by rotation for 1.5 hr in the dark at room temperature. Protein A-Sepharose beads were collected by centrifugation in an Eppendorf centrifuge and then washed three times with 150 mM NaCl/10 mM Tris·HCl, pH 7.2. [^{3}H]Nitrendipine bound was eluted by the addition of 0.1 M glycine (pH 2.8) and measured by liquid scintillation counting.

Competitive Radioimmunoassay. The specificity and affinity of the anti-dihydropyridine antibodies was determined by a competitive radioimmunoassay. The separation of free [^{3}H]nitrendipine from bound [^{3}H]nitrendipine was achieved by the addition of dextran-coated charcoal to the assay medium. Each assay tube contained 1.0 ml of 150 mM NaCl/10 mM Tris·HCl, pH 7.2/0.1% gelatin/20 pM [^{3}H]nitrendipine, and the appropriate amount of antibody to bind 50% of the label in the absence of inhibitor. After a 1.0-hr incubation in the dark, the samples were incubated on ice for 10 min and then 0.2 ml of dextran-coated charcoal was added to each assay tube. The tubes were shaken and then incubated on ice for 12 min. The charcoal was removed by centrifugation (850 × *g*) for 8 min and [^{3}H]nitrendipine was measured in 0.8 ml of the supernatant by using a liquid scintillation counter. Controls were run without the addition of antiserum or antibody and the amount of [^{3}H]nitrendipine in the supernatant after the addition of dextran-coated charcoal was subtracted from the value obtained in the presence of antibody to yield the amount of [^{3}H]nitrendipine bound to antibody.

Materials. [^{3}H]Nitrendipine (88 Ci/mmol), [^{3}H]verapamil (85 Ci/mmol), [^{3}H]diltiazem (72 Ci/mmol), nifedipine-isothiocyanate, and [^{3}H]nifedipine-isothiocyanate (76.6 Ci/mmol) were obtained from New England Nuclear. Nitrendipine, nimodipine, nisoldipine, Bay k 8644, Bay m 4797, Bay n 2767, Bay o 1704, [^{3}H]nisoldipine (81.5 Ci/mmol), and [^{3}H]-nimodipine (145 Ci/mmol) were generously supplied by Miles. [^{3}H]PN 200-110 (70–85 Ci/mmol), [^{3}H]azidopine (44.6 Ci/mmol), and [^{125}I]iodipine (2000 Ci/mmol) were generously supplied by Amersham. Nifedipine, verapamil, and diltiazem were generously supplied by Pfizer (New York), Knoll (Whippany, NJ), and Marion (Kansas City, MO), respectively. UV-irradiated nisoldipine was prepared by exposing nisoldipine (1 mM) in ethanol to direct sunlight. Dextran-coated charcoal was prepared by mixing 62.5 mg of Dextran-70, 625 mg of Norit A charcoal, and 100 ml of 150 mM NaCl/10 mM Tris·HCl, pH 7.2/0.1% gelatin.

RESULTS

Binding Properties of Anti-Dihydropyridine Antibodies. The presence of anti-dihydropyridine antibodies in postimmune antiserum was tested by the protein A-Sepharose assay. Fig. 1 shows that in the presence of antiserum the amount of [^{3}H]nitrendipine bound to the antibody–protein A-Sepharose complex was >62,000 cpm. In the absence of added antiserum or in the presence of preimmune serum, the amount of [^{3}H]nitrendipine bound to the protein A-Sepharose was <100 cpm. The addition of 1.0 μM nitrendipine to the assay with antiserum reduced the [^{3}H]nitrendipine bound to 5900 cpm. Thus, the anti-dihydropyridine antibodies specifically bound >90% of the [^{3}H]nitrendipine.

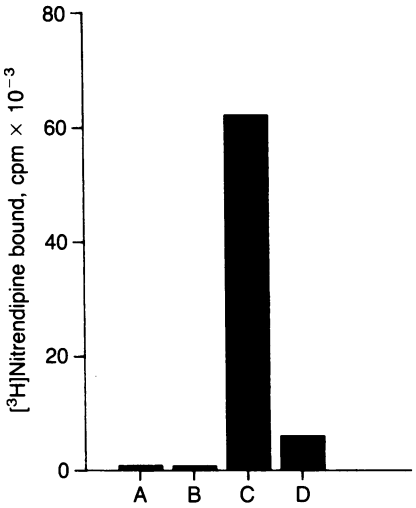

FIG. 1. [^{3}H]Nitrendipine binding to the anti-dihydropyridine antibodies: Protein A-Sepharose assay. Binding of free [^{3}H]nitrendipine to the anti-dihydropyridine antibodies was performed by using the protein A-Sepharose assay. Each assay tube contained 1.0 ml of 150 mM NaCl/10 mM Tris·HCl, pH 7.2/1.0 nM [^{3}H]nitrendipine/50 μl of protein A-Sepharose 4B beads. [^{3}H]Nitrendipine binding (solid bars) was determined in triplicate with the SD of bound [^{3}H]nitrendipine at <2%. Samples contained no antibody (A), 10 μl of preimmune sera (B), 10 μl of postimmune antisera (C), and 10 μl of postimmune antisera plus 1.0 μM nitrendipine (D).

Fig. 2 shows the binding of various radiolabeled Ca^{2+}-channel blockers or Ca^{2+} antagonists at a 1.0 nM concentration to the anti-dihydropyridine antibodies. The anti-dihydropyridine antibodies were able to bind [^{3}H]nitrendipine, [^{3}H]nimodipine, [^{3}H]nisoldipine, and [^{3}H]PN 200-110, which are all radiolabeled 1,4-dihydropyridine Ca^{2+}-channel blockers. The anti-dihydropyridine antibodies were also able to bind [^{3}H]azidopine and [^{125}I]iodipine (not shown), which

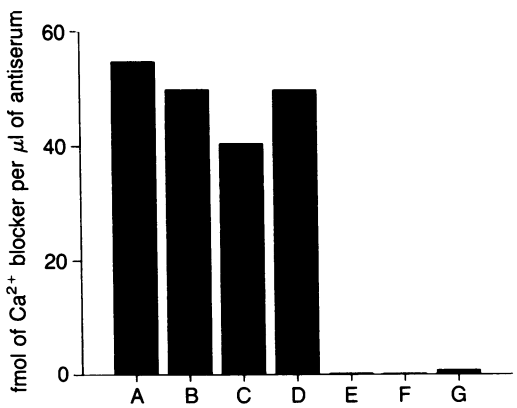

FIG. 2. Radiolabeled Ca^{2+}-channel blocker or Ca^{2+}-antagonist binding to the anti-dihydropyridine antibodies. Binding of various radiolabeled Ca^{2+}-channel blockers (A, [^{3}H]nitrendipine; B, [^{3}H]-nimodipine; C, [^{3}H]nisoldipine; D, [^{3}H]PN 200-110; E, [^{3}H]-verapamil; and F, [^{3}H]diltiazem) or Ca^{2+} antagonist (G, [^{3}H]-trifluoperazine) to the anti-dihydropyridine antibodies was measured by using the protein A-Sepharose 4B assay in the presence of radiolabeled drugs (1.0 nM). The total amount of radioligand bound (solid bars) was determined and expressed as fmol of Ca^{2+} blocker bound per μl of antiserum. SD values of the bound radioligand were <2%.

are radiolabeled 1,4-dihydropyridines that have complex side chains. [^{3}H]Verapamil and [^{3}H]diltiazem, which are radiolabeled Ca^{2+}-channel blockers, and [^{3}H]trifluoperazine, which is a radiolabeled Ca^{2+} antagonist, were not recognized by the anti-dihydropyridine antibodies.

Affinity and Specificity of Anti-Dihydropyridine Antibodies. A competitive radioimmunoassay was used to determine the specificity and affinity of the anti-dihydropyridine antibodies. Average antibody dissociation constants, antibody specificity, and concentration of specific antibodies in the antiserum were calculated according to the methods of Müller (30). The assay was performed under equilibrium conditions and a titer test was first performed to determine the antiserum or antibody dilution required for 50% of the added [^{3}H]nitrendipine to be bound in the absence of unlabeled nitrendipine. Fig. 3 shows the results of a typical competitive radioimmunoassay used for the determination of the affinity of anti-dihydropyridine antibodies for [^{3}H]nitrendipine. The average apparent dissociation constant (K_d) of the [^{3}H]-nitrendipine–antibody complex was 0.06 (±0.02) × 10^{-9} M for this antiserum, which was studied in detail. The average dissociation constant for all antisera produced ranged from 0.01 to 0.24 × 10^{-9} M.

The specificity of the anti-dihydropyridine antibodies has been examined by using various Ca^{2+}-channel modifiers and related compounds in the presence of 20 pM [^{3}H]nitrendipine (Table 1). Half-maximal inhibition (IC_{50}) of [^{3}H]nitrendipine binding by various 1,4-dihydropyridines required between 0.25 and 0.90 nM. Structurally unrelated Ca^{2+}-channel blockers, verapamil and diltiazem, did not inhibit the binding of [^{3}H]nitrendipine to the anti-dihydropyridine antibodies. Ryanodine, a possible intracellular Ca^{2+}-channel blocker, also did not inhibit [^{3}H]nitrendipine binding. Finally, divalent cation chelation with EDTA or EGTA did not inhibit [^{3}H]nitrendipine binding to the anti-dihydropyridine antibodies.

The specificity of the anti-dihydropyridine antibodies has also been examined by testing the ability of inactivated forms of the Ca^{2+}-channel blockers (31, 32) to inhibit the binding of [^{3}H]nitrendipine to the anti-dihydropyridine antibodies.

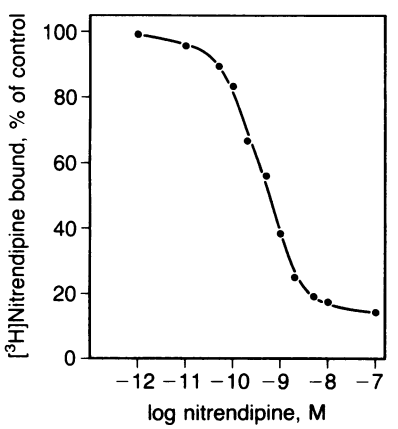

FIG. 3. Displacement of specifically bound [^{3}H]nitrendipine by unlabeled nitrendipine. A competitive radioimmunoassay for [^{3}H]nitrendipine binding was performed according to the conditions of Müller (30) to determine the average antibody dissociation constant. [^{3}H]Nitrendipine binding was measured using the dextran-coated charcoal assay in the presence of 20 pM [^{3}H]nitrendipine, a 1:5000 dilution of anti-dihydropyridine antibody, and various concentrations of unlabeled nitrendipine (1 pM to 100 nM). The data shown are from a representative experiment with the SD for each point at <2%. In this experiment, the average antibody dissociation constant and the concentration of specific anti-dihydropyridine antibodies were determined to be 0.06 nM and 0.3 mg/ml, respectively.

Table 1. Inhibition of [^{3}H]nitrendipine–antibody binding by various Ca^{2+}-channel drugs and related compounds

Compound	% inhibition at 100 nM*	IC_{50}, nM†
Dihydropyridines		
Nitrendipine	85.6 ± 0.4	0.60
Nisoldipine	85.9 ± 0.7	0.34
Nifedipine	85.9 ± 0.7	0.25
Nimodipine	82.9 ± 0.6	0.90
Bay k 8644	83.4 ± 4.4	0.60
Ca^{2+} blockers and antagonists		
Diltiazem	5.7 ± 2.2	>1000
Verapamil	2.5 ± 1.6	>1000
Trifluoperazine	<1.0	>1000
Ryanodine	<1.0	>1000
Ca^{2+} chelators		
EDTA	<1.0	>1000
EGTA	<1.0	>1000
Inactive derivatives of Ca^{2+} blockers		
Bay n 2767	<1.0	>1000
Bay m 4797	<1.0	>1000
Bay o 1704	<1.0	>1000
UV-exposed nisoldipine	<1.0	>1000
Miscellaneous		
2,6-Lutidine	<1.0	>1000
2,6-Dimethylpiperidine	<1.0	>1000
3,5-Diacetyl-1,4-dihydro-2,6-dimethylpyridine	5.1 ± 4.4	>1000
Diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridine dicarboxylate	18.9 ± 1.3	800

[^{3}H]Nitrendipine binding to anti-dihydropyridine antibodies was performed by using the dextran-coated charcoal assay. Inhibition of [^{3}H]nitrendipine binding to the dihydropyridine-specific antibodies was measured by the addition of various unlabeled drugs or reagents (1 pM to 100 μM) in the presence of 20 pM [^{3}H]nitrendipine.

*Inhibition of [^{3}H]nitrendipine binding at 100 nM unlabeled drug or reagent is the average ± SD.

†Half-maximal inhibition (IC_{50}) was determined from a plot of percentage [^{3}H]nitrendipine bound versus concentration of drug or reagent.

Fig. 4 compares the displacement of specifically bound [^{3}H]nitrendipine by unlabeled nitrendipine or Bay n 2767, an inactive pyridine metabolite of nitrendipine. Bay n 2767 did not displace [^{3}H]nitrendipine even when present in 10^6-fold excess. Bay o 1704, another inactive pyridine metabolite of nitrendipine, and Bay m 4797, an inactive lactone derivative of nitrendipine, were also ineffective in displacing [^{3}H]nitrendipine from the antibody-binding site (Table 1). The inhibition of [^{3}H]nitrendipine binding by nisoldipine or UV-inactivated nisoldipine is also shown in Fig. 4. Half-maximal inhibition of [^{3}H]nitrendipine binding required only 0.34 nM nisoldipine, whereas 1 μM UV-inactivated nisoldipine caused no inhibition of [^{3}H]nitrendipine binding.

To further examine the specificity of the anti-dihydropyridine antibodies, we have tested the effects of several pyridine and dihydropyridine compounds on [^{3}H]nitrendipine binding (Fig. 5; Table 1). 2,6-Lutidine, a pyridine, did not displace [^{3}H]nitrendipine binding. 2,6-Dimethylpyridine was also ineffective in displacing [^{3}H]nitrendipine binding. Diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridine dicarboxylate, a 1,4-dihydropyridine without a bulky substituent in the 4-position (33), was able to displace [^{3}H]nitrendipine binding, but half-maximal inhibition required 800 nM. 3,5-Diacetyl-1,4-dihydro-2,6-dimethylpyridine was also able to inhibit [^{3}H]nitrendipine binding but required >1000 nM for half-maximal inhibition.

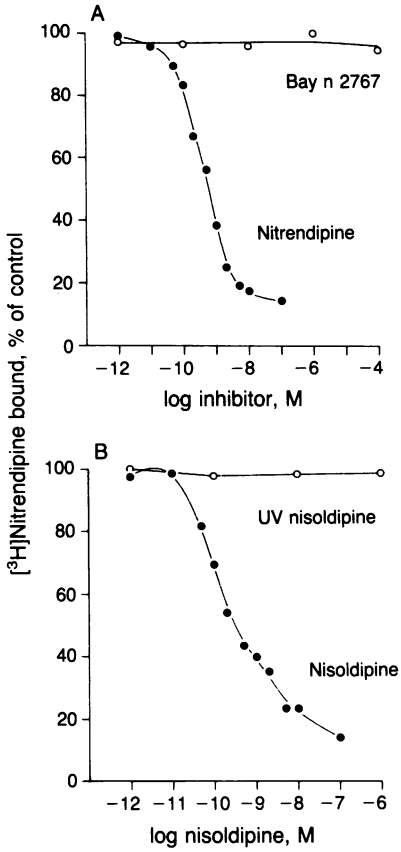

FIG. 4. Displacement of [^{3}H]nitrendipine by active and inactive forms of the dihydropyridine Ca^{2+}-channel blockers. [^{3}H]Nitrendipine binding was determined using the dextran-coated charcoal assay as described in *Materials and Methods* and Fig. 3. Inhibition of [^{3}H]nitrendipine (20 pM) binding was measured in the presence of increasing concentrations of the following: (*A*) 1 pM to 100 nM nitrendipine (●) or 1 pM to 100 μM Bay n 2767, the inactive pyridine metabolite of nitrendipine (○); (*B*) 1 pM to 100 nM nisoldipine (●) or 1 pM to 1 μM UV-inactivated nisoldipine (○).

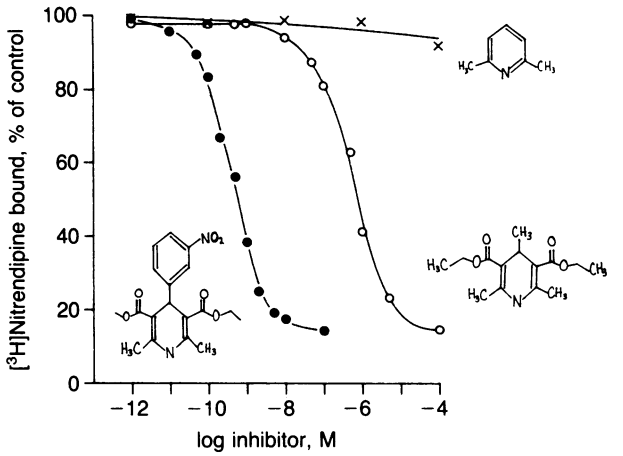

FIG. 5. Inhibition of [^{3}H]nitrendipine binding by pyridine and dihydropyridine compounds. [^{3}H]Nitrendipine binding was determined by using the dextran-coated charcoal assay as described in *Materials and Methods* and Fig. 3. Inhibition of [^{3}H]nitrendipine binding (20 pM) was measured in the presence of increasing concentrations of the following: 1 pM to 100 nM nitrendipine (●), 1 pM to 100 μM diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridine dicarboxylate (○), and 1 pM to 100 μM 2,6-lutidine (×).

DISCUSSION

The dihydropyridine binding properties of the anti-dihydropyridine antibodies and the membrane receptor for the 1,4-dihydropyridine Ca^{2+}-channel blockers have been compared with respect to affinity and specificity. The average dissociation constants of the [^{3}H]nitrendipine–antibody complex determined using the competitive radioimmunoassay ranged from 0.01 nM to 0.24 nM for all antisera studied and are essentially in the same range as that measured for the membrane receptor for the 1,4-dihydropyridine Ca^{2+}-channel blockers (9–13). The average dissociation constant calculated from kinetic data (not shown) was 0.66 nM, which is higher than that determined by the competitive radioimmunoassay but still within the range of that measured for the membrane receptor. Thus, the anti-dihydropyridine antibodies, like the membrane receptor, have high affinity for [^{3}H]nitrendipine.

The specificity of the anti-dihydropyridine antibodies—i.e., the cross-reactivity of antibodies with compounds structurally related to the hapten (nifedipine) used for immunization—has been examined using the competitive radioimmunoassay. In this assay, the competition of [^{3}H]nitrendipine and various Ca^{2+}-channel modifiers for the [^{3}H]nitrendipine antibody binding sites was measured. The specificity of the antibodies was then determined by comparing the concentration required for half-maximal inhibition of [^{3}H]nitrendipine–antibody binding. As shown in Table 1, significant cross-reactivity occurred only with other 1,4-dihydropyridines (nifedipine, nisoldipine, nimodipine). Concentrations of 1,4-dihydropyridine Ca^{2+}-channel blockers required for half-maximal inhibition of [^{3}H]nitrendipine binding ranged between 0.25 and 0.90 nM. Bay k 8644, a Ca^{2+}-channel activator (34), was also able to specifically displace [^{3}H]nitrendipine from the antibody and required 0.60 nM for half-maximal inhibition. Thus, the anti-dihydropyridine antibodies mimic the binding characteristics of the membrane receptor for the 1,4-dihydropyridines with respect to the inhibition of high-affinity [^{3}H]nitrendipine binding by the various 1,4-dihydropyridine Ca^{2+}-channel modifiers.

A comparison of the specificity of the anti-dihydropyridine antibodies and the membrane receptor has also been made using metabolites of nitrendipine (31), which are not active in inhibiting Ca^{2+}-channel activity or [^{3}H]nitrendipine binding to the membrane receptor (7, 31). As shown in Fig. 4 and Table 1, the inactive metabolites of nitrendipine had no cross-reactivity with the anti-dihydropyridine antibody. UV irradiation of the dihydropyridines has been shown to inactivate the dihydropyridines (32, 33, 35, 36) and UV-inactivated nisoldipine showed no cross-reactivity with the anti-dihydropyridine antibodies. Since all of the inactive forms of the dihydropyridine Ca^{2+}-channel blockers lack the 1,4-dihydropyridine ring structure, these results indicate that the anti-dihydropyridine antibodies, like the membrane receptor, are specific for the 1,4-dihydropyridine ring structure of the Ca^{2+}-channel modifiers.

We have also examined the requirement of a bulky substituent in the 4-position of the heterocycle of the 1,4-dihydropyridine Ca^{2+}-channel blockers using several pyridine and dihydropyridine compounds. As expected, only the dihydropyridine compounds had any cross-reactivity (Fig. 5 and Table 1). Diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridine dicarboxylate, a 1,4-dihydropyridine without a substituent in the 4-position, had the highest degree of cross-reactivity of all the compounds tested, but it still required >1000 times the concentration of nitrendipine for half-maximal inhibition of binding. Diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridine dicarboxylate has been shown to have some hypotensive activity (33), suggesting that it can bind to the dihydropyridine membrane receptor. Maximal hypotensive activity has only been observed with 1,4-dihydropyridine compounds with a cyclic substituent in the 4-position

(33, 35, 36). Therefore, again like the membrane receptor, the anti-dihydropyridine antibodies have a high degree of specificity for the 1,4-dihydropyridine ring structure but also require a bulky substituent in the 4-position of the heterocycle for maximal binding.

The anti-dihydropyridine antibodies and the 1,4-dihydropyridine membrane receptor have been found to differ with respect to allosteric modification of high-affinity [^{3}H]nitrendipine binding. Structurally unrelated Ca^{2+}-channel blockers (verapamil and diltiazem), which are able to allosterically regulate the binding of the 1,4-dihydropyridines to the membrane receptor (4–8), did not modify the binding of [^{3}H]nitrendipine to the antibodies. Divalent cation chelation, which is able to inhibit the binding of the 1,4-dihydropyridines to the membrane receptor (4–8), also did not modify the binding of [^{3}H]nitrendipine to the antibodies. Therefore, unlike the membrane receptor, the anti-dihydropyridine antibodies are not allosterically regulated by other Ca^{2+}-channel blockers or by divalent cation chelation.

It is evident from the data presented that the anti-dihydropyridine antibodies have a high affinity and specificity for the 1,4-dihydropyridine Ca^{2+}-channel blockers. The dihydropyridine binding characteristics of the anti-dihydropyridine antibodies compare favorably with the binding characteristics of the membrane receptor for the 1,4-dihydropyridines and suggest that these antibodies will be very useful in the production of anti-idiotypic antibodies to the dihydropyridine membrane receptor.

Drs. A. Scriabine and R. A. Janis are thanked for the generous supply of the various dihydropyridine compounds used in this research. We are grateful to L. Harter for typing this manuscript. K.P.C. is an Established Investigator of the American Heart Association and recipient of a grant-in-aid from American Heart Association and Grant NS-18814 from the National Institutes of Health. This research was also generously supported by Institute of Preclinical Pharmacology, Miles Laboratories, Inc.

1. Hagiwara, S. & Bylerly, L. (1981) *Annu. Rev. Neurosci.* **4**, 69–125.
2. Tsien, R. W. (1983) *Annu. Rev. Physiol.* **4**, 341–358.
3. Reuter, H. (1983) *Nature (London)* **301**, 569–574.
4. Triggle, D. J. (1981) in *New Perspective on Calcium Antagonists*, ed. Weiss, G. B. (Am. Physiol. Soc., Bethesda, MD), pp. 1–18.
5. Fleckenstein, A. (1983) *Calcium Antagonism in Heart and Smooth Muscle* (Wiley, New York).
6. Janis, R. A. & Scriabine, A. (1983) *Biochem. Pharmacol.* **32**, 3499–3507.
7. Janis, R. A. & Triggle, D. J. (1983) *J. Med. Chem.* **26**, 775–785.
8. Glossmann, H., Ferry, D. R. & Goll, A. (1984) in *Proceedings of the International Union of Pharmacology Ninth International Congress of Pharmacology (London)*, eds. Paton, W., Mitchell, J. & Turner, P. (Macmillan, London), Vol. 2, pp. 329–335.
9. Bellemann, P., Ferry, D., Lubbecke, F. & Glossmann, H. (1981) *Arzneim.-Forsch.* **31**, 2064–2067.
10. Williams, L. T. & Tremble, P. (1982) *J. Clin. Invest.* **70**, 209–212.
11. Bolger, G. T., Sengo, P., Klockowski, R., Luchowski, E., Siegel, H., Janis, R. A., Triggle, A. M. & Triggle, D. J. (1983) *J. Pharmacol. Exp. Ther.* **225**, 291–309.
12. Gould, R. J., Murphy, K. M. & Snyder, S. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3656–3660.
13. Janis, R. A. & Triggle, D. J. (1984) *Drug. Dev. Res.* **4**, 257–274.
14. Venter, J. C., Fraser, C. M., Schaber, J. S., Jung, C. Y., Bolger, G. & Triggle, D. J. (1983) *J. Biol. Chem.* **258**, 9344–9348.
15. Campbell, K. P., Lipshutz, G. M. & Denney, G. H. (1984) *J. Biol. Chem.* **259**, 5384–5387.
16. Ferry, D. R., Rombusch, M., Goll, A. & Glossmann, H. (1984) *FEBS Lett.* **169**, 112–118.
17. Glossmann, H. & Ferry, D. R. (1983) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **323**, 279–291.
18. Curtis, B. M. & Catterall, W. A. (1984) *Biochemistry* **23**, 2113–2118.
19. Borsotto, M., Barhanin, J., Norman, R. I. & Lazdunski, M. (1984) *Biochem. Biophys. Res. Commun.* **122**, 1357–1366.
20. Caron, M. G., Srinivasan, Y., Snyderman, R. & Lefkowitz, R. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2263–2267.
21. Schreiber, A. B., Couraud, P. O., Andre, C., Vray, B. & Strosberg, A. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7385–7389.
22. Moore, H.-P. H., Fritz, L. C., Raftery, M. A. & Brockes, J. P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1673–1677.
23. Wassermann, N. H., Penn, A. S., Freimuth, P. I., Treptow, N., Wentzel, S., Cleveland, W. L. & Erlanger, B. F. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4810–4814.
24. Costa, M. R. C. & Catterall, W. A. (1984) *J. Biol. Chem.* **259**, 8210–8218.
25. Venter, J. C., Berzofsky, J. A., Lindstrom, J., Jacobs, S., Fraser, C. M., Kohn, L. D., Schneider, W. J., Greene, G. L., Strosberg, A. D. & Erlanger, B. F. (1984) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **43**, 2352–2539.
26. Strosberg, A. D., Chamat, S., Guillet, J.-G., Schmutz, A., Durieu, O., Delavier, C. & Hoebeke, J. (1984) in *Monoclonal and Anti-Idiotypic Antibodies: Probes for Receptor Structure and Function*, Receptor Biochemistry and Methodology, eds. Venter, J. C., Fraser, C. M. & Lindstrom, J. (Liss, New York), Vol. 4, pp. 151–162.
27. Smith, T. W., Butler, V. P., Jr., & Haber, E. (1970) *Biochemistry* **9**, 331–337.
28. Smith, T. W. (1972) *J. Clin. Invest.* **51**, 1583–1593.
29. Hoebeke, J., Vauquelin, G. & Strosberg, A. D. (1978) *Biochem. Pharmacol.* **27**, 1527–1532.
30. Müller, R. (1983) *Methods Enzymol.* **92**, 589–601.
31. Janis, R. A., Krol, G. J., Noe, A. J. & Pan, M. (1983) *J. Clin. Pharmacol.* **23**, 266–273.
32. Schlossmann, V. K. (1972) *Arzneim.-Forsch. (Drug Res.)* **22**, 60–62.
33. Loev, B., Goodman, M. M., Snader, K. M., Tedeschi, R. & Macko, E. (1974) *J. Med. Chem.* **17**, 956–965.
34. Schramm, M., Thomas, G., Towart, R. & Franckowiak, G. (1983) *Nature (London)* **303**, 535–537.
35. Prous, J., Blancafort, P., Castaner, J., Serradell, M. N. & Mealy, N. (1981) *Drugs Future* **6**, 427–440.
36. Mannhold, R., Rodenkirchen, R. & Bayer, R. (1982) *Prog. Pharmacol.* **5**, 27–52.